The 9th Princess Chulabhorn International Science Congress

THE CHALLENGES OF ONE HEALTH: THE ROLES OF BIOSCIENCES AND CHEMISTRY

PROGRAM AND ABSTRACTS

December 15 - 18, 2024

Shangri-La Hotel, Bangkok, Thailand

Organized by

Chulabhorn Research Institute

The 9th Princess Chulabhorn International Science Congress

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THE CHALLENGES OF ONE HEALTH: THE ROLES OF BIOSCIENCES AND CHEMISTRY

organized to commemorate

the Sixth Cycle (72 years) Birthday Anniversary of
His Majesty King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua

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Congress Theme

THE CHALLENGES OF ONE HEALTH: THE ROLES OF BIOSCIENCES AND CHEMISTRY

Chairperson of Organizing Committee:

Professor Dr. HRH Princess Chulabhorn

The Princess Chulabhorn International Science Congress (PC) was initiated by Professor Dr. Her Royal Highness Princess Chulabhorn Mahidol, the youngest sister of His Majesty King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua (King Rama X), to provide a forum for the exchange of the latest information and the most recent advances in research among the international scientific community. Through this program, international congresses on selected topics in science and technology are typically organized every 4 to 5 years, the first congress being held in 1987 on "Natural Products" to celebrate the 60th Birthday Anniversary of His Late Majesty King Bhumibol Adulyadej the Great (King Rama IX).

The 9th Princess Chulabhorn International Science Congress (PC IX) is being organizing **to commemorate the Sixth Cycle (72 years) Birthday Anniversary of His Majesty King Maha Vajiralongkorn**, an auspicious occasion for the Thai people to celebrate and pay tribute to His Majesty. This marks a time of national joy and jubilation, which all scientists are invited to share.

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COMMITTEE

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Professor Dr. HRH Princess Chulabhorn	**Chairperson**
Khunying Mathuros Ruchirawat	Vice Chairperson
Rajata Rajatanavin	Vice Chairperson
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Cate Dewey (Canada)
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Michelle Embry (U.S.A.)
Tariq Enver (U.K.)
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Suthat Fucharoen (Thailand)
George Fu Gao (P.R. China)
Julie L. Gerberding (U.S.A.)
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Adam Kamradt-Scott (U.S.A.)
Ho Jeong Kwon (Republic of Korea)
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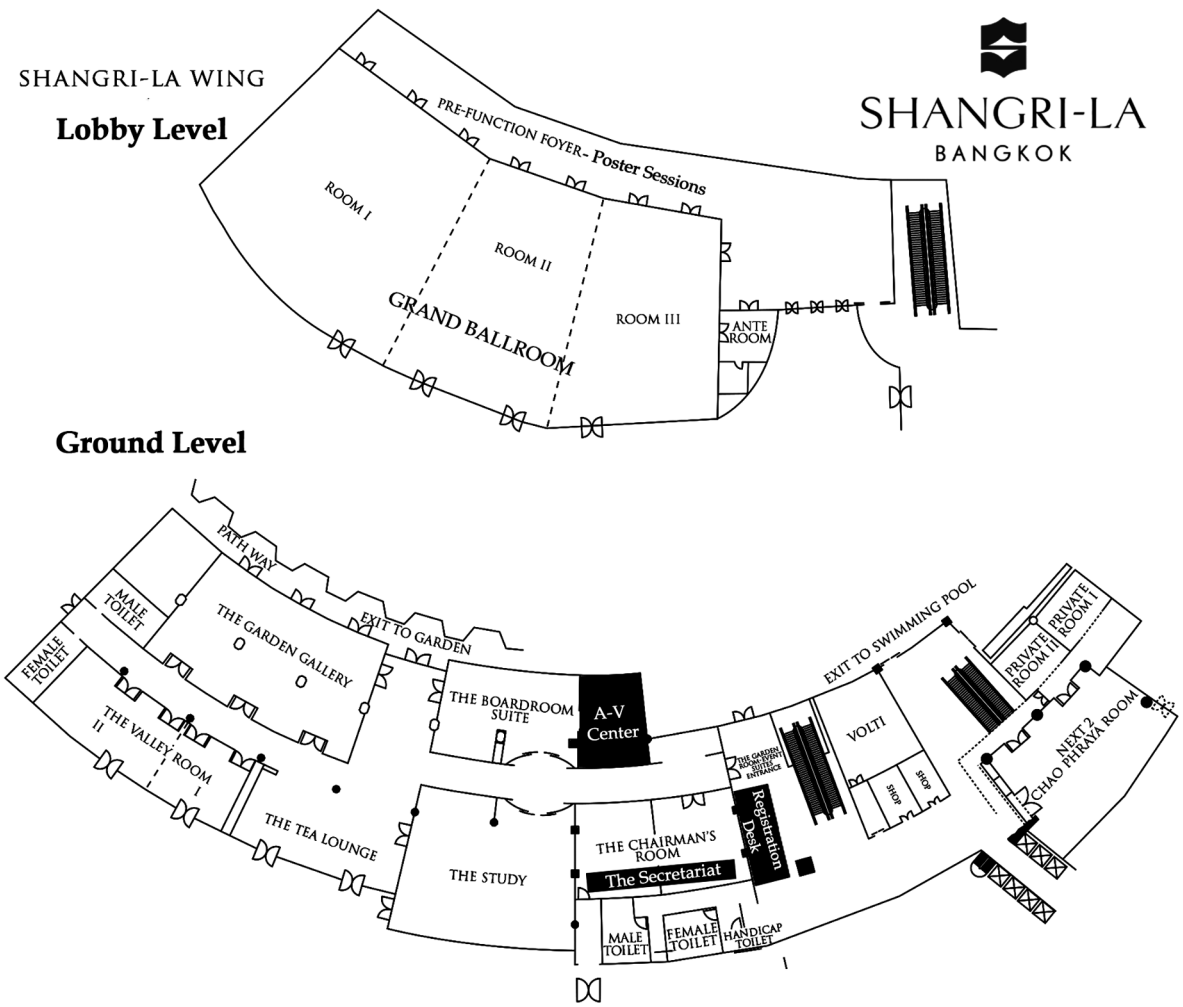
Worasuda Yoongthong (Thailand)
Maged Younes (U.K.)
Ari Zimran (Israel)

THE CONGRESS CENTER MAP



GENERAL INFORMATION

REGISTRATION:

Location: Registration takes place in ***the Garden Room-Event Suites Entrance*** on the Ground Floor of the Shangri-La Hotel.

On-site registration hours:

Sunday, December 15 from **10:00 to 15:00.**

Monday to Tuesday, December 16 - 17 from **08:00 to 17:00.**

Full registration fee includes admission to all congress sessions, the program and abstract book, and lunches.

Accompanying person's fee is only for social activities (including the opening ceremony) and accompanying guests' program. It does not admit individuals to scientific sessions.

IDENTIFICATION BADGES:

Name badges are issued to all registered participants and accompanying guests.

These are entry badges and should be worn at all times.

A replacement badge can be obtained at the ***Registration Desk*** with proper identification at a cost of 50 baht/badge.

LUNCH:

For all participants, lunch is arranged in the room indicated on the meal vouchers, starting on Monday, December 16 to Wednesday, December 18 during lunch breaks.

Meal vouchers will be required.

SECRETARIAT OFFICE:

Location: The Chairman's Room on the Ground Floor of the Shangri-La Hotel

Hours: Sunday, December 15 to Wednesday, December 18 from **08:00 to 17:30.**

SCIENTIFIC PROGRAM INFORMATION

INSTRUCTIONS FOR PRESENTERS:

Guidelines for the Preparation of Presentations:

All presentations must be in **MIcrosoft Powerpoint format**. No other formats will be supported.

If you have prepared your presentation using an earlier version of PowerPoint or if you are a Macintosh user, it is highly recommended that you test your presentation on a Windows platform to ensure the accurate conversion to Windows format with Microsoft PowerPoint 2019.

To minimize setup delays, the Congress Staff at ***the A-V Center*** will collect all files and load them to a laptop in the room in which the session will be held. All speakers must bring their updated presentations as Microsoft PowerPoint 2019 files to ***the A-V Center*** **at least 24 hours** before their scheduled presentations. Please bring your PowerPoint presentation as a backup file to ***the A-V Center*** on a USB Memory Device.

Audio-Visual Center (A-V Center):

Location: ***The Boardroom Suite*** on the Ground Floor of the Shangri-La Hotel.

Hours: Sunday, December 15 from **10:00 to 15:00**

Monday, December 16 to Wednesday, December 18 from **08:00 to 17:30.**

SCIENTIFIC PROGRAM INFORMATION

POSTER SESSIONS:

Location: The Ballroom Foyer on the Lobby level of the Shangri-La Hotel.

List of Poster Presentations:

Code	Topic	Poster No.	Group	Board No.
PA	Diseases of Public Health Concerns (Genetic Diseases, Cancer)	PA-01 to PA-42	1	001 - 042
PB	Drug Development for Prevention and Treatment	PB-01 to PB-30	1	042 - 072
PC	Antimicrobial Resistance	PC-01 to PC-26	2	001 - 026
PD	Climate Change and Communicable Diseases	PD-01 to PD-11	2	027 - 037
PE	Environmental Health Issues	PE-01 to PE-22	2	038 - 059
PF	Food Safety and Security	PF-01 to PF-07	2	060 - 066

Hours:

Group 1: Poster Number PA-01 to PB-30, Board Number 001 - 072

Posters should be set up for display on Monday, December 16 from 08:00.

Display - Monday, December 16, 08:00 to Tuesday, December 17, 10:00.

Discussion - Monday, December 16 from 08:30 to 09:00 and 12:00 to 13:00.

The display must be removed by 10:00, on Tuesday, December 17, 2024.

Group 2: Poster Number PC-01 to PF-07, Board Number 001 - 066

Posters should be set up for display on Tuesday, December 17 from 11:00.

Display - Tuesday, December 17 from 11:00 to Wednesday, December 18, 18:00.

Discussion - Tuesday, December 17 from 12:30 to 13:00 and 16:30 to 17:00.

The display must be removed by 18:00, on Wednesday, December 18, 2024.

The Poster Board is 1.0 m. (width) x 2.5 m. (height). The poster size should not be larger than 0.9 m. (width) x 1.2 m. (height).

Please check the **Poster Number and Board Number** from the list of Poster Presentations in the Poster Session of the “Program and Abstracts” book. **The Board Number** refers to the location that does not change throughout the Congress and will be posted at the top of the poster boards by the organizers. Posters can be mounted with double-sided foam mounting tape, which will be available on site.

Staff will be available in the morning of Monday, December 16 to Wednesday, November 18, and at all times during the day (08:00 to 17:00) to assist presenters.

The Princess Chulabhorn Gold Medal Award of Appreciation

Professor Dr. Her Royal Highness Princess Chulabhorn has instituted the **"Princess Chulabhorn Gold Medal Award of Appreciation"** in recognition of persons or organizations that have provided outstanding academic contributions for the development and prestige of the Chulabhorn Research Institute.

Recipients of the Princess Chulabhorn Gold Medal Award of Appreciation 2024

(in alphabetical order)

Professor Martin van den Berg (The Netherlands)

Dr. Norbert Frank (Germany)

Dr. Curtis C. Harris (U.S.A.)

Professor Leonard Ritter (Canada)

Dr. Xin Wei Wang (U.S.A.)

Thanphuying **Putrie Viravaidya** (Thailand)

OPENING CEREMONY

Sunday, December 15, 2024

Time	*Grand Ballroom, Shangri-La Hotel*
16:45	- Guests to be seated in the Grand Ballroom
17:00	- Arrival of His Majesty King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua and Her Majesty Queen Suthida Bajrasudhabimalalakshana
17:05	- Report by Professor Dr. H.R.H. Princess Chulabhorn, Chairperson of the National Organizing Committee
17:10	- Opening Address by His Majesty King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua - Video Presentation in honor of the 6th cycle (72 years) birthday of King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua - Video Presentation in honor of His Late Majesty King Bhumibol Adulyadej the Great

SCIENTIFIC PROGRAM

PC IX - PROGRAM OVERVIEW

Sunday, December 15, 2024	Monday, December 16, 2024	Tuesday, December 17, 2024	Wednesday, December 18, 2024
REGISTRATION **10:00 – 15:00**	**09:00** *Grand Ballroom* **KEYNOTE LECTURE:** From Environmental Exposures to Disease Manifestation: The Lasting Impacts of Arsenic ***Professor Dr. HRH Princess Chulabhorn*** *(Chulabhorn Research Institute, Thailand)* **SPECIAL REMARKS:** ***Tedros Adhanom Ghebreyesus*** *(WHO HQ, Geneva)*	**09:00** *Grand Ballroom* **PLENARY LECTURES:** **PL3:** Drug Development and Regulatory Sciences: Turning Crises into Opportunities — A Valuable Perspective ***Ram Sasisekharan*** *(U.S.A.)* **PL4:** The Two Sides of Precision Medicine: Proteomic Enablement of Biomarkers and Therapeutics ***Jennifer van Eyk*** *(U.S.A.)* **PL5:** Programmable Therapeutics for Genetic Diseases ***John M. Essigmann*** *(U.S.A.)* **PL6:** The Lessons Learned from COVID-19 ***George F. Gao*** *(P.R. China)*	**09:00 SYMPOSIA:** **S10:** Protein Changes in Disease (S-39 to S-43) **S11:** Relationships between Genetic Mutation and the Environment in Cancer and Infectious Diseases (S-44 to S-47) ***SPECIAL PLATFORM SESSION ON CANCERS*** *(sO-5 to sO-8)* **S12:** Role of Chemical and Biological Sciences for Discovery of Modern Drugs II (S-48 to S-51) ***FREE COMMUNICATION III*** *(O-26 to O-29)*
Grand Ballroom **OPENING CEREMONY:** **16:45** Guests to be seated in the Ballroom **17:00** Arrival of His Majesty King Maha Vajiralongkorn Phra Vajiraklaochaoyuhua and Her Majesty Queen Suthida Bajrasudhabimalalakshana **17:05** Report by Professor Dr. HRH Princess Chulabhorn **17:10** Opening Address by His Majesty King Maha Vajiralongkorn Video Presentation in honor of the 6th cycle (72 years) birthday of King Maha Vajiralongkorn Phra ajiraklaochaoyuhua Video Presentation in honor of His Late Majesty King Bhumibol Adulyadej the Great	**PLENARY LECTURES:** **PL1:** One Health Innovations: Predicting and Preempting Spillover Infections ***Julie L. Gerberding*** *(U.S.A.)* **PL2:** AMR in 2024: We Still Have an Awareness Problem ***Helen W. Boucher*** *(U.S.A.)*		
	L U N C H		
	POSTER PRESENTATION	*POSTER PRESENTATION*	*Grand Ballroom*
	13:00 SYMPOSIA: **S1:** Antimicrobial Resistance (AMR) and One Health: Critical Issues (S-1 to S-3) **S2:** One Health: Climate Change and Communicable diseases (S-4 to S-7) **S3:** Role of Chemical and Biological Sciences for Discovery of Modern Drugs I (S-8 to S-13) **S4:** Genetic Diseases: From Detection to Therapy (S-14 to S-19)	**13:00 SYMPOSIA:** **S5:** Early Detection and Prevention of Cancer (S-20 to S-22) **S6:** Antimicrobial Resistance: One Health Perspectives (S-23 to S-26) **S7:** Zoonotic Diseases (S-27 to S-30) **S8:** One Health and Therapeutics (S-31 to S-33) **S9:** Environmental Risk Factors Affecting Health (S-34 to S-38) ***SPECIAL PLATFORM SESSION ON ONE HEALTH*** *(sO-1 to sO-4)* ***FREE COMMUNICATION I*** *(O-1 to O-11)* ***FREE COMMUNICATION II*** *(O-12 to O-25)*	**13:00 – 15:30** • **ROUNDTABLE DISCUSSION:** Regulatory Innovation • **ROUNDTABLE DISCUSSION:** AMR Policy Impacting South and Southeast Asia **16:00 CLOSING CEREMONY:** • Presentation of the Princess Chulabhorn Gold Medal Award of Appreciation • Closing Remarks by Professor Dr. HRH Princess Chulabhorn
	CONGRESS DINNER at Royal Thai Navy Convention Hall		

SCIENTIFIC PROGRAM

SUMMARY

Monday, December 16, 2024

Time	***Grand Ballroom***
09:00	**Keynote Lecture:** From Environmental Exposures to Disease Manifestation: The Lasting Impacts of Arsenic *Professor Dr. H.R.H. Princess Chulabhorn Mahidol (Chulabhorn Research Institute, Thailand)*
09:45	**Special Remarks:** *Tedros Adhanom Ghebreyesus (WHO, Switzerland)*
	Chairpersons: Ram Sasisekharan (U.S.A.) and *M.R.* Jisnuson Svasti (Thailand)
10:00	**Plenary Lecture 1:** One Health: Predicting and Preempting Spillover and Infection Dissemination **PL-1** *Julie L. Gerberding (U.S.A.)*
10:45	**Plenary Lecture 2:** AMR in 2024: We Still Have an Awareness Problem **PL-2** *Helen W. Boucher (U.S.A.)*
11:30	*L U N C H*

POSTER PRESENTATION: **PA-01 to PB-30** (Discussion 08:30-09:00 and 12:00-13:00)

Time	***Ballroom I***	***Ballroom II***	***Ballroom III***
13:00	***SYMPOSIUM I:*** ***Antimicrobial Resistance (AMR) and One Health: Critical Issues*** ***Chairperson:*** Helen W. Boucher (U.S.A.) ***Co-chairperson:*** Mayuree Fuangthong (Thailand) ***Abstract No.*** AMR Governance: Critical Beyond the Science **S-1** *Adam Kamradt-Scott (U.S.A.)* Is This the End of the Antibiotic Era? **S-2** *David Paterson (Singapore)* Ensuring Sustainable Access to Effective Antibiotics: Key Messages from the Lancet Series **S-3** *Ramanan Laxminarayan (U.S.A.)* ***SYMPOSIUM II:*** ***One Health: Climate Change and Communicable diseases*** ***Chairperson:*** Paul R. Hunter (U.K.) ***Co-chairperson:*** Mayuree Fuangthong (Thailand) World Health Organization Calls for Urgent Action to Mitigate Climate-Related Health Risks and Promote Resilience through A One Health Perspective **S-4** *Maria Neira (WHO, Switzerland)* Climate Change and Emerging Infectious Diseases **S-5** *Paul R. Hunter (U.K.)* Rational Design of Vaccines against Zika and Dengue Viruses **S-6** *Lianpan Dai (P.R. China)* Elimination of Hepatitis in Thailand by the Year 2030 **S-7** *Yong Poovorawan (Thailand)*	***SYMPOSIUM III:*** ***Role of Chemical and Biological Sciences for Discovery of Modern Drugs I*** ***Chairperson:*** Geoffrey A. Cordell (U.S.A.) ***Co-chairperson:*** Charnsak Thongsornkleeb (Thailand) ***Abstract No.*** Phytochemicals of Liverworts: Structures, Biological Activity, and Their Application to Foods and Medicinal Drugs **S-8** *Yoshinori Asakawa (Japan)* Phenotypic Assays and Analog Synthesis: Effective Tools for the Discovery and Development of Marine Natural Product Drug Leads **S-9** *Raymond Andersen (Canada)* Discovery of Antiviral Natural Products Based on Native Mass Spectrometry and Molecular Networking **S-10** *Yang Ye (P.R. China)* Natural Product Biosynthesis and Associated Molecular Innovation **S-11** *Wen Liu (P.R. China)* Efficient Synthesis of Bioactive Steroid and Terpenoid Natural Products **S-12** *Jinghan Gui (P.R. China)* One Health - An Aspect of the Food, Medicines, and Environment Conundrum for 2040 and Beyond **S-13** *Geoffrey A. Cordell (U.S.A.)*	***SYMPOSIUM IV:*** ***Genetic Diseases: From Detection to Therapy*** ***Chairperson:*** Pornswan Wasant (Thailand) ***Co-chairperson:*** Suthat Fucharoen (Thailand) ***Abstract No.*** Thalassemia: A Model of Genetic Disease **S-14** *Suthat Fucharoen (Thailand)* Over 3 Decades of Organizing Services for Inherited Metabolic Disorders in Thailand **S-15** *Pornswan Wasant (Thailand)* Gaucher Disease as A Model for Rare Disorders **S-16** *Ari Zimran (Israel)* Rare Inborn Metabolic Disorders: From Mystery to Diagnosis and Management **S-17** *Duangrurdee Wattanasirichaigoon (Thailand)* Newborn Screening by Tandem Mass Spectrometry: From Early Detection of Rare Diseases to Therapy **S-18** *Nithiwat Vatanavicharn (Thailand)* Lysosomal Storage Diseases: Progress in Diagnosis and Therapy in Thailand **S-19** *Thipwimol Tim-Aroon (Thailand)*

PROGRAM SUMMARY

Tuesday, December 17, 2024

Time	Grand Ballroom	Abstract No.
	PLENARY LECTURES: **Chairpersons:** John M. Essigmann (U.S.A.) and Skorn Mongkolsuk (Thailand)	
09:00	**Plenary Lecture 3:** Drug Development and Regulatory Sciences: Turning Crises into Opportunities — A Valuable Perspective *Ram Sasisekharan (U.S.A.)*	**PL-3**
09:45	**Plenary Lecture 4:** The Two Sides of Precision Medicine: Proteomic Enablement of Biomarkers and Therapeutics *Jennifer van Eyk (U.S.A.)*	**PL-4**
10:30	**Plenary Lecture 5:** Programmable Therapeutics for Genetic Diseases *John M. Essigmann (U.S.A.)*	**PL-5**
11:15	**Plenary Lecture 6:** The Lessons Learned from COVID-19 *George F. Gao (P.R. China)*	**PL-6**
12:00	*L U N C H*	

POSTER PRESENTATION: **PC-01 to PF-07** (Discussion 12:30-13:00 and 16:30-17:00)

Time	Ballroom I	Ballroom II	Ballroom III
13:00	***SYMPOSIUM VII:*** ***Zoonotic Diseases*** ***Chairperson:*** Khongsak Thiangtum (Thailand) ***Co-chairperson:*** Waraphon Phimpraphai (Thailand) ***Abstract No.*** Protecting Animals and Human Lives from Rabies Project: One Health Approach for Rabies Control and Elimination in Thailand **S-27** *Professor Dr. H.R.H. Princess Chulabhorn Mahidol (Thailand)* One Health in Actions on Zoonoses and Agrifood Systems: Perspectives for Global Health and Food Security **S-28** *Thanawat Tiensin (FAO, Italy)* Successful One Health Approach to Reduce Zoonotic Disease from Pigs **S-29** *Cate Dewey (Canada)* Complexity, Context, and Community Engagement: Centering Anthropology Within the One Health Paradigm **S-30** *Elizabeth Finnis (Canada)* ***SYMPOSIUM VIII:*** ***One Health and Therapeutics*** ***Chairpersons:*** Helen W. Boucher (U.S.A.) ***Co-chairperson:*** Ram Sasisekharan (U.S.A.) Vaccine Manufacturing Considerations for Pandemic Preparedness **S-31** *Rahul Singhvi (U.S.A.)* Design and Patterns of Optimized Drug Combinations for TB **S-32** *Bree Aldridge (U.S.A.)* Development of Multi-Epitope Subunit Vaccines Against *Acinetobacter baumannii* **S-33** *Bernard Arulanandam (U.S.A.)*	***SYMPOSIUM V:*** ***Early Detection and Prevention of Cancer*** ***Chairperson:*** Xin Wei Wang (U.S.A.) ***Co-chairperson:*** Jittiporn Chaisaingmongkol (Thailand) ***Abstract No.*** Adductomic Strategies for Emerging Risk Factors in Liver Cancer **S-20** *John D. Groopman (U.S.A.)* Liver Cancer Prevention **S-21** *Hashem B. El-Serag (U.S.A.)* Emerging Strategies of Early Detection of Liver Cancer **S-22** *Xin Wei Wang (U.S.A.)* ***SYMPOSIUM VI:*** ***Antimicrobial Resistance: One Health Perspectives*** ***Chairperson:*** Peter Dedon (U.S.A.) ***Co-chairperson:*** Nisanart Charoenlap (Thailand) One Health Selection and Transmission of Antibiotic Resistance in Thailand, Argentina and the United Kingdom **S-23** *Matthew B. Avison (U.K.)* Lessons from Wastewater Surveillance: Impact of COVID-19 on AMR **S-24** *Motoyuki Sugai (Japan)* Mining the Epitranscriptome for First-In-Class Antimicrobials **S-25** *Peter Dedon (U.S.A.)* AMASS: AutoMated Tool for Antimicrobial Resistance Surveillance System – How Thailand Utilizes Timely Data from 127 Public Hospitals for Actions at the Facility and National Levels **S-26** *Direk Limmathurotsak*	***SYMPOSIUM IX:*** ***Environmental Risk Factors Affecting Health*** ***Chairperson:*** Martin van den Berg (The Netherlands) ***Co-chairperson:*** Piyajit Watcharasit (Thailand) ***Abstract No.*** Towards One Health:Toxicokinetics at the Intersection of Human and Ecological Health **S-34** *Michelle Embry (U.S.A.)* Food Safety from a One Health Perspective **S-35** *Maged Younes (U.K.)* Per- and Polyfluoroalkyl Substances (PFAS): The Complexity in Toxicity and Risk Assessment **S-36** *Martin van den Berg (The Netherlands)* The Air We Breathe – Chemical Challenges to Health, A UK-Thailand Perspective **S-37** *Dudley E. Shallcross (U.K.)* $PM_{2.5}$ Toxicity and Adverse Health Effects **S-38** *Herman Autrup (Denmark)* ***SPECIAL PLATFORM SESSION ON ONE HEALTH*** *(sO-1 to sO-4)*

FREE COMMUNICATION I *(O-1 to O-11) at* ***The Study***

FREE COMMUNICATION II *(O-12 to O-25) at* ***The Garden Gallery***

PROGRAM SUMMARY

Wednesday, December 18, 2024

Time	Ballroom I	Ballroom II	Ballroom III
9:00	***SYMPOSIUM X:*** ***Protein Changes in Disease*** ***Chairperson:*** John R. Yates III (U.S.A.) ***Co-chairperson:*** Sittiruk Roytrakul (Thailand) ***Abstrcat No.*** How a Single Mutation in CFTR Causes the Systemic Disease Cystic Fibrosis: Interactions, PTMs, and Structure **S-39** *John R. Yates III (U.S.A.)* Ultrasensitive Proteomics for Precision Oncology **S-40** *Yu-Ju Chen (Chinese Taipei)* Navigating Drug-Targetome-Phenotype Interaction and Its Translational Implications **S-41** *Ho Jeong Kwon (Republic of Korea)* Advancement in Peptidoproteomics for Cancer Research **S-42** *Sittiruk Roytrakul (Thailand)* Exploring Microproteins and Interferon Pathways in Colorectal Cancer: Implications for Disease Progression and FOLFOX Chemoresistance **S-43** *Teck Yew Low (Malaysia)*	***SYMPOSIUM XI:*** ***Relationships between Genetic Mutation and the Environment in Cancer and Infectious Diseases*** ***Chairperson:*** Tariq Enver (U.K.) ***Co-chairperson:*** Panida Navasumrit (Thailand) ***Abstract No.*** Genetic and Epigenetic Cues Shape Initiation, Promotion and Therapy Resistance in Childhood Leukaemia **S-44** *Tariq Enver (U.K.)* Towards an Understanding of Air Pollution Driven Lung Cancer Promotion **S-45** *Charles Swanton (U.K.)* Axonal Injury Initiates Glioblastoma **S-46** *Simona Parrinello (U.K.)* What Does Our Knowledge of Innate Immune Regulation during Infection Tell Us about Innate Immune Contribution to Cancer Outcome? **S-47** *Greg Towers (U.K.)* ***SPECIAL PLATFORM SESSION ON CANCERS*** *(sO-5 to sO-8)*	***SYMPOSIUM XII:*** ***Role of Chemical and Biological Sciences for Discovery of Modern Drugs II*** ***Chairperson:*** Roderick Bates (Singapore) ***Co-chairperson:*** Jutatip Boonsombat (Thailand) ***Abstract No.*** The Infinite Loop: Machine Learning for Discovery, Delivery, and Rapid Manufacturing of Potential Medicines **S-48** *Bradley L. Pentelute (U.S.A.)* Antibody-Drug Conjugates (ADCs) – A Perfect Synergy? **S-49** *John A. Hartley (U.K.)* Developments of Molecular Probes for Detection of Biological Targets **S-50** *Sun-Joon Min (Republic of Korea)* From Structural Biology to Organic Synthesis: A Collaborative Search for New Anti-Mycobacterial Drugs **S-51** *Roderick Bates (Singapore)* ***FREE COMMUNICATION III*** *(O-26 to O-29)*
12:00	*L U N C H*		
Time			***Grand Ballroom***
13.00	CLOSING SESSION: ***REGULATORY INNOVATION*** ***Moderators:*** Ram Sasisekharan (U.S.A.) and Surachoke Tangwiwat (Thailand) • U.S. FDA's Efforts to Advance Gene Therapy Development [Virtual Presentation] RD *Peter Marks (U.S.A.)* • Perspectives from the Thai FDA *Worasuda Yoongthong (Thailand)* ***Roundtable Discussion:*** ***Panelists:*** • Helen W. Boucher (U.S.A.) • Rahul Singvi (U.S.A.) • Worasuda Yoongthong (Thailand) • Ram Sasisekharan (U.S.A.) ***Roundtable Discussion - AMR Policy Impacting South and Southeast Asia*** ***Moderators:*** Helen W. Boucher (U.S.A.) and Julie L. Gerberding (U.S.A.) ***Panelists:*** • Helen W. Boucher (U.S.A.) • George F. Gao (P.R. China) • Julie L. Gerberding (U.S.A.) • Ramanan Laxminarayan (U.S.A.) • David Paterson (Singapore)		***Abstract No.***
16:00	CLOSING CEREMONY: • **Presentation of the Princess Chulabhorn Gold Medal Award of Appreciation** • **Closing Remarks by Professor Dr. H.R.H. Princess Chulabhorn**		

DAILY PROGRAM

Monday, December 16, 2024

Grand Ballroom

09:00 ***KEYNOTE LECTURE:***

From Environmental Exposures to Disease Manifestation:
The Lasting Impact of Arsenic

Professor Dr. H.R.H. Princess Chulabhorn Mahidol

(Chulabhorn Research Institute, Thailand)

09:45 ***SPECIAL REMARKS:*** *[VDO Presentation]*

Tedros Adhanom Ghebreyesus *(World Health Organization,Switzerland)*

PLENARY LECTURES:

Chairpersons: **Ram Sasisekharan** (U.S.A.)

M.R. **Jisnuson Svasti** (Thailand)

Abstract No.

10:00 **Plenary Lecture 1** **PL-1**

One Health: Predicting and Preempting Spillover and Infection Dissemination

Julie L. Gerberding *(Foundation for the National Institutes of Health, U.S.A.)*

10:45 **Plenary Lecture 2** **PL-2**

AMR in 2024: We Still Have an Awareness Problem

Helen W. Boucher *(Tufts University, U.S.A.)*

11:30 LUNCH

Ballroom I

***SYMPOSIUM I:* Antimicrobial Resistance (AMR) and One Health: Critical Issues**

Chairperson: Helen W. Boucher (U.S.A.)

Co-chairperson: Mayuree Fuangthong (Thailand)

Abstract No.

13:00 AMR Governance: Critical Challenges Beyond the Science **S-1**
Adam Kamradt-Scott *(Tufts University, U.S.A.)*

13:30 Is This the End of the Antibiotic Era? **S-2**
David Paterson *(National University of Singapore, Singapore)*

14:00 Ensuring Sustainable Access to Effective Antibiotics: Key Messages from the Lancet Series **S-3**
Ramanan Laxminarayan *(One Health Trust, U.S.A.)*

***SYMPOSIUM II:* One Health: Climate Change and Communicable Diseases**

Chairperson: Paul R. Hunter *(*U.K.)

Co-chairperson: Mayuree Fuangthong (Thailand)

14:30 World Health Organization Calls for Urgent Action to Mitigate Climate-Related Health Risks and Promote Resilience through A One Health Perspective [VDO presentaion] **S-4**
Maria Neira *(World Health Organization, Switzerland)*

14:45 Climate Change and Emerging Infectious Diseases **S-5**
Paul R. Hunter *(University of East Anglia, U.K.)*

15:15 Rational Design of Vaccines against Zika and Dengue Viruses **S-6**
Lianpan Dai *(Chinese Academy of Sciences, P.R. China)*

15:45 Elimination of Hepatitis in Thailand by the Year 2030 **S-7**
Yong Poovorawan *(Chulalongkorn University, Thailand)*

Ballroom II

***SYMPOSIUM III:* Role of Chemical and Biological Sciences for Discovery of Modern Drugs I**

Chairperson: Geoffrey A. Cordell (U.S.A.)

Co-chairperson: Charnsak Thongsornkleeb (Thailand)

		Abstract No.
13:00	Phytochemicals of Liverworts: Structures, Biological Activity, and Their Application to Foods and Medicinal Drugs ***Yoshinori Asakawa*** *(Tokushima Bunri University, Japan)*	**S-8**
13:30	Phenotypic Assays and Analog Synthesis: Effective Tools for the Discovery and Development of Marine Natural Product Drug Leads ***Raymond Andersen*** *(University of British Columbia, Canada)*	**S-9**
14:00	Discovery of Antiviral Natural Products Based on Native Mass Spectrometry and Molecular Networking ***Yang Ye*** *(Chinese Academy of Sciences, P.R. China)*	**S-10**
14:30	Natural Product Biosynthesis and Associated Molecular Innovation ***Wen Liu*** *(Chinese Academy of Sciences, P.R. China)*	**S-11**
15:00	Efficient Synthesis of Bioactive Steroid and Terpenoid Natural Products ***Jinghan Gui*** *(Chinese Academy of Sciences, P.R. China)*	**S-12**
15:30	One Health - An Aspect of the Food, Medicines, and Environment Conundrum for 2040 and Beyond ***Geoffrey A. Cordell*** *(Natural Products Inc., U.S.A.)*	**S-13**

Ballroom III

SYMPOSIUM IV: *Genetic Diseases: From Detection to Therapy*

Chairperson: Pornswan Wasant (Thailand)

Co-chairperson: Suthat Fucharoen (Thailand)

		Abstract No.
13:00	Thalassemia: A Model of Genetic Disease ***Suthat Fucharoen*** *(Mahidol University, Thailand)*	**S-14**
13:30	Over 3 Decades of Organizing Services for Inherited Metabolic Disorders in Thailand ***Pornswan Wasant*** *(Mahidol University, Thailand)*	**S-15**
14:00	Gaucher Disease as a Model for Rare Disorders ***Ari Zimran*** *(Hebrew University of Jerusalem, Israel)*	**S-16**
14:30	Rare Inborn Metabolic Disorders: From Mystery to Diagnosis and Management ***Duangrurdee Wattanasirichaigoon*** *(Mahidol University, Thailand)*	**S-17**
15:00	Newborn Screening by Tandem Mass Spectrometry: From Early Detection of Rare Diseases to Therapy ***Nithiwat Vatanavicharn*** *(Mahidol University, Thailand)*	**S-18**
15:20	Lysosomal Storage Diseases: Progress in Diagnosis and Therapy in Thailand ***Thipwimol Tim-Aroon*** *(Mahidol University, Thailand)*	**S-19**

Grand Ballroom

PLENARY LECTURES:

Chairpersons: **John M. Essigmann** (U.S.A.)
Skorn Mongkolsuk (Thailand)

Abstract No.

09:00 **Plenary Lecture 3** **PL-3**
Drug Development and Regulatory Sciences: Turning Crises into Opportunities — A Valuable Perspective
Ram Sasisekharan *(Massachusetts Institute of Technology, U.S.A.)*

09:45 **Plenary Lecture 4** **PL-4**
The Two Sides of Precision Medicine: Proteomic Enablement of Biomarkers and Therapeutics
Jennifer van Eyk *(Cedars-Sinai Medical Center, U.S.A.)*

10:30 **Plenary Lecture 5** **PL-5**
Programmable Therapeutics for Genetic Diseases
John M. Essigmann *(Massachusetts Institute of Technology, U.S.A.)*

11:15 **Plenary Lecture 6** **PL-6**
The Lessons Learned from COVID-19
George F. Gao *(Chinese Academy of Sciences, P.R. China)*

12:00 LUNCH

Ballroom I

SYMPOSIUM VII: Zoonotic Diseases

Chairperson: Khongsak Thiangtum (Thailand)

Co-chairperson: Waraphon Phimpraphai (Thailand)

		Abstract No.
13:00	Protecting Animal and Human Lives from Rabies Project: One Health Approach for Rabies Control and Elimination in Thailand ***Professor Dr. H.R.H. Princess Chulabhorn Mahidol*** *(Chulabhorn Research Institute, Thailand)*	**S-27**
13:30	One Health in Actions on Zoonoses and Agrifood Systems: Perspectives for Global Health and Food Security ***Thanawat Tiensin*** *(Food and Agriculture Organization of the United Nations (FAO), Italy)*	**S-28**
14:00	Successful One Health Approach to Reduce Zoonotic Disease from Pigs ***Cate Dewey*** *(University of Guelph, Canada)*	**S-29**
14:30	Complexity, Context, and Community Engagement: Centering Anthropology within the One Health Paradigm ***Elizabeth Finnis*** *(University of Guelph, Canada)*	**S-30**

SYMPOSIUM VIII: One Health and Therapeutics

Chairperson: **Helen W. Boucher** (U.S.A.)

Co-chairperson: **Ram Sasisekharan** (U.S.A.)

15:00	Vaccine Manufacturing Considerations for Pandemic Preparedness ***Rahul Singhvi*** *(National Resilience, Inc., U.S.A.)*	**S-31**
15:30	Design and Patterns of Optimized Drug Combinations for TB ***Bree Aldridge*** *(Tufts University, U.S.A.)*	**S-32**
16:00	Development of Multi-Epitope Subunit Vaccines Against *Acinetobacter baumannii* ***Bernard Arulanandam*** *(Tufts University School of Medicine, U.S.A.)*	**S-33**

Ballroom II

***SYMPOSIUM V:* Early Detection and Prevention of Cancer**

Chairperson: Xin Wei Wang (U.S.A.)

Co-chairperson: Jittiporn Chaisaingmongkol (Thailand)

		Abstract No.
13:00	Adductomic Strategies for Emerging Risk Factors in Liver Cancer ***John D. Groopman*** *(Johns Hopkins School of Medicine, U.S.A.)*	**S-20**
13:30	Liver Cancer Prevention ***Hashem B. El-Serag*** *(Baylor College of Medicine, U.S.A.)*	**S-21**
14:00	Emerging Strategies of Early Detection of Liver Cancer ***Xin Wei Wang*** *(National Cancer Institute, U.S.A.)*	**S-22**

***SYMPOSIUM VI:* Antimicrobial Resistance: One Health Perspectives**

Chairperson: Peter Dedon (U.S.A.)

Co-chairperson: Nisanart Charoenlap (Thailand)

		Abstract No.
14:30	One Health Selection and Transmission of Antibiotic Resistance in Thailand, Argentina and the United Kingdom ***Matthew B. Avison*** *(University of Bristol, U.K.)*	**S-23**
15:00	Lessons from Wastewater Surveillance: Impact of COVID-19 on AMR ***Motoyuki Sugai*** *(National Institute of Infectious Diseases, Japan)*	**S-24**
15:30	Mining the Epitranscriptome for First-In-Class Antimicrobials ***Peter Dedon*** *(Massachusetts Institute of Technology, U.S.A.)*	**S-25**
16:00	AMASS: AutoMated Tool for Antimicrobial Resistance Surveillance System – How Thailand Utilizes Timely Data from 127 Public Hospitals for Actions at the Facility and National Levels ***Direk Limmathurotsakul*** *(Mahidol University, Thailand)*	**S-26**

Ballroom III

SYMPOSIUM IX: Environmental Risk Factors Affecting Health

Chairperson: **Martin van den Berg** (The Netherlands)

Co-chairperson: **Piyajit Watcharasit** (Thailand)

		Abstract No.
13:00	Towards One Health: Toxicokinetics at the Intersection of Human and Ecological Health ***Michelle Embry*** *(Health and Environmental Sciences Institute, U.S.A.)*	**S-34**
13:30	Food Safety from a One Health Perspective ***Maged Younes*** *(Robert Gordon University, U.K.)*	**S-35**
14:00	Per- and Polyfluoroalkyl Substances (PFAS): The Complexity in Toxicity and Risk Assessment ***Martin van den Berg*** *(Utrecht University, The Netherlands)*	**S-36**
14:30	The Air We Breathe – Chemical Challenges to Health, A UK-Thailand Perspective ***Dudley E. Shallcross*** *(University of Bristol, U.K.)*	**S-37**
15:00	$PM_{2.5}$ Toxicity and Adverse Health Effects ***Herman Autrup*** *(University of Aarhus, Denmark)*	**S-38**

Special Platform Session on One Health

Chairperson: **Bette Meek** (Canada)

Co-chairperson: **Kwanrawee Sirikanchana** (Thailand)

15:30	World Health Organization Calls for Enhanced Multisectoral Action to Improve Chemical Safety and Protect Public Health, Including Through A One Health Approach ***Virunya Bhat*** *(World Health Organization, Switzerland)*	**sO-1**
15:45	Development of Guidance on Problem Formulation for Implications for One Health Approaches ***Bette Meek*** *(University of Ottawa, Canada)*	**sO-2**
16:00	Harnessing Nano- and Bio-Technology Platforms for Human and Plant Health ***Archana Bhaw-Luximon*** *(University of Mauritius, Mauritius)*	**sO-3**
16:15	Tracking Communicable Diseases Using Wastewater in an Environmental One Health Framework ***Kwanrawee Sirikanchana*** *(Chulabhorn Research Institute, Thailand)*	**sO-4**

The Study

Free Communication I:

Chairperson: James Dubbs (Thailand)

Co-chairperson: Rojana Sukchawalit (Thailand)

		Abstract No.
13:00	The Impact of Glyphosate on Emergence of Antimicrobial Resistance ***Nisanart Charoenlap*** *(Chulabhorn Research Institute, Thailand)*	**O-1**
13:15	Biocide-associated Resistance Mechanisms in *Pseudomonas Aeruginosa:* Impacts on Antibiotics and Host Immunity ***Adisak Romsang*** *(Mahidol University, Thailand)*	**O-2**
13:30	Nano Boost: Enhancing Antibacterial Efficacy of Ceftriaxone with Magnesium Oxide Nanoparticles to Tackle Antimicrobial Resistance ***Qaiser Akram*** *(University of Veterinary and Animal Sciences, Pakistan)*	**O-3**
13:45	Evaluating AMR Surveillance Methodologies in Cholistani and Sahiwal Cattle: A Comparative Study of Sampling Strategies and Testing Techniques in Pakistan ***Muhammad Amjad Ali*** *(Bahauddin Zakariya University, Pakistan)*	**O-4**
14:00	Emergence of Multi-Drug Resistant Bacteria Due to Irrational Antibiotic Use in Livestock ***Dhanushka Darshana Silva Nammuni*** *(University of Ruhuna, Sri Lanka)*	**O-5**
14:15	The Gut Microbiota in Children from an E-waste Recycling Area with 6PPDQ and Other Pollutant Exposure ***Xia Huo*** *(Jinan University, P.R. China)*	**O-6**
14:30	Heavy Metal (Loid) Exposure Increases the Risk of Childhood Anemia: Evidence from a Typical E-waste Recycling Area in China ***Bo Xie*** *(Jinan University, P.R. China)*	**O-7**
14:45	Influence of E-waste Exposure on DNA Damage and DNA Methylation in People Living Near Recycling Sites ***Jinhan Wang*** *(Chinese Academy of Medical Sciences, P.R. China)*	**O-8**
15:00	The Health Status Assessment of Personnel Handling Electronic Waste in Developing Country: A Situation Analysis ***Ravichandran Beerappa*** *(ICMR Regional Occupational Health Centre, India)*	**O-9**
15:15	Blood Heavy Metals, DNA Damage, and Intelligence Quotient Among Children from an Informal E-waste Processing Village in Vietnam ***Hien Thi Thu Ngo*** *(Phenikaa University, Vietnam)*	**O-10**
15:30	Environmental Health Risks of Post-Ban Paraquat Residues in Phitsanulok's Nan River and Vicinity Areas ***Pantip Hinhumpatch*** *(Naresuan University, Thailand)*	**O-11**

Free Communication II

The Garden Gallery

Chairperson: Daam Settachan (Thailand)

Co-chairperson: Chotirat Rattanasinchai (Thailand)

		Abstract No.
13:00	Enhancing Anti-glioma Activity with Combined Epigenetic Agents and MAGE-D4 Peptide-specific T Cells ***Xiaoxun Xie*** *(Guangxi Medical University, P.R. China)*	**O-12**
13:15	Adipose Tissue Levels of PBDEs in Relation to Chemotherapy Toxicity, Chemoresistance and Prognosis in Breast Cancer Patients ***Lin Peng*** *(Shantou University Medical College, P.R. China)*	**O-13**
13:30	Genetic Underpinnings of Hypoglycemia-Induced Epilepsy: Insights from GYS2 Gene Mutations ***Muhammad Ilyas*** *(Riphah International University, Pakistan)*	**O-14**
13:45	*Candida albicans* DNA-protein crosslink repair and its roles in stress response ***Oranart Matangkasombut*** *(Chulabhorn Research Institute, Thailand)*	**O-18**
14:00	Differential Requirement for IL-2 And IL-23 in the Differentiation and Effector Functions of T Cell Against Cancer ***Cai Ping Koh*** *(Quest International University, Malaysia)*	**O-16**
14:15	Clinical Feasibility of MALDETEC-CTC For Detection of Circulating Tumor Cells in Osteosarcoma Using Whole-Cell MALDI-TOF Mass Fingerprint ***Santhasiri Orrapin*** *(Chiang Mai University, Thailand)*	**O-17**
14:30	Aminoacyl-tRNA Synthetase Deficiencies: Clinical Presentations, Biochemical Analyses, and Functional Studies ***Parith Wongkittichote*** *(Mahidol University, Thailand)*	**O-15**
14:45	Metabolic Dysfunction in Mice with Adipocyte Specific Ablation of the Adenosine A2A Receptor ***Narendra Verma*** *(Centre of Biomedical Research Lucknow, India)*	**O-19**
15:00	PI3K/AKT Mediate Collagen Type 1- Induced Osteogenesis of Dental Pulp Stem Cells Via Focal Adhesion Mechanism ***Norhayati Yusop*** *(Universiti Sains Malaysia, Malaysia)*	**O-20**

(To be continued)

The Garden Gallery

Free Communication II (continued)

		Abstract No.
15:15	Synergistic Effects of AMPK Activation and TBK1 Inhibition to Improve Metabolic Health and Insulin Sensitivity in Obese Mice ***Churaibhon Wisessaowapak*** *(University of California San Diego, U.S.A.)*	**O-21**
15:30	Unraveling the Intracellular Action of Antimicrobial Peptide A11 through Proteomic Analysis in *Acinetobacter baumannii* ***Ratchaneewan Aunpad*** *(Thammasat University, Thailand)*	**O-22**
15:45	Glycolipid Biosurfactant and Its Potential Applications in Biomedicines ***Nitnipa Soontorngun*** *(King Mongkut's University of Technology Thonburi, Thailand)*	**O-23**
16:00	Genome Persistence & Survival of *Clostridium perfringens* from Ichthyofauna of Dal Lake Himalaya-A Possible Future Public Health Hazard ***Irfan Ahmad*** *(University of Agricultural Sciences & Technology of Kashmir, India)*	**O-24**
16:15	Toxicological Impacts of Clofibric Acid, Diclofenac and Ibuprofen on Fish: An Environmental Health Issue ***Manoharan Saravanan*** *(Bharathiar University, India)*	**O-25**

Ballroom I

SYMPOSIUM X: ***Protein Changes in Disease***

Chairperson: John R. Yates III (U.S.A.)

Co-chairperson: Sittiruk Roytrakul (Thailand)

		Abstract No.
09:00	How a Single Mutation in CFTR Causes the Systemic Disease Cystic Fibrosis: Interactions, PTMs, and Structure ***John R. Yates III*** *(The Scripps Research Institute, U.S.A.)*	**S-39**
09:30	Ultrasensitive Proteomics for Precision Oncology ***Yu-Ju Chen*** *(Academia Sinica, Chinese Taipei)*	**S-40**
10:00	Navigating Drug-Targetome-Phenotype Interaction and Its Translational Implications ***Ho Jeong Kwon*** *(Yonsei University, Republic of Korea)*	**S-41**
10:30	Advancement in Peptidoproteomics for Cancer Research ***Sittiruk Roytrakul*** *(National Center for Genetic Engineering and Biotechnology, Thailand)*	**S-42**
11:00	Exploring Microproteins and Interferon Pathways in Colorectal Cancer: Implications for Disease Progression and FOLFOX Chemoresistance ***Teck Yew Low*** *(Universiti Kebangsaan Malaysia, Malaysia)*	**S-43**
11:30	LUNCH	

Ballroom II

***SYMPOSIUM XI:* Relationships between Genetic Mutation and the Environment in Cancer and Infectious Diseases**

Chairperson: **Tariq Enver** (U.K.)

Co-chairperson: **Panida Navasumrit** (Thailand)

		Abstract No.
09:00	Genetic and Epigenetic Cues Shape Initiation, Promotion and Therapy Resistance in Childhood Leukaemia ***Tariq Enver*** *(University College London, U.K.)*	**S-44**
09:30	Towards an Understanding of Air Pollution Driven Lung Cancer Promotion ***Charles Swanton*** *(Francis Crick Institute, U.K.)*	**S-45**
10:00	Axonal Injury Initiates Glioblastoma ***Simona Parrinello*** *(University College London, U.K.)*	**S-46**
10:30	What does Our Knowledge of Innate Immune Regulation during Infection Tell Us About Innate Immune Contribution to Cancer Outcome? ***Greg Towers*** *(University College London, U.K.)*	**S-47**

Special Platform Session on Cancers

Chairperson: **Christopher Loffredo** (U.S.A.)

Co-chairperson: **Benjarath Javed** (Thailand)

11:00	The Landscape of Etiological Patterns of Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma in Thailand ***Benjarath Javed*** *(Chulabhorn Research Institute, Thailand)*	**sO-5**
11:15	Integrated Genomic Applications Discern Molecular Subgroups and Treatment Response in Liver Cancer ***Anuradha Budhu*** *(National Cancer Institute, U.S.A.)*	**sO-6**
11:30	Ionizing Radiation-associated Liver Cancers in the Mayak Worker Cohort ***Christopher Loffredo*** *(Georgetown University, U.S.A.)*	**sO-7**
11:45	Urinary Biomarkers for Early Lung Cancer Detection in Non-smokers: A Step Toward Personalized Screening ***Daxeshkumar P. Patel*** *(National Cancer Institute, U.S.A.)*	**sO-8**
12:00	LUNCH	

Ballroom III

***SYMPOSIUM XII:* Role of Chemical and Biological Sciences for Discovery of Modern Drugs II**

Chairperson: **Roderick Bates** (Singapore)

Co-chairperson: **Jutatip Boonsombut** (Thailand)

		Abstract No.
09:00	The Infinite Loop: Machine Learning for Discovery, Delivery, and Rapid Manufacturing of Potential Medicines ***Bradley L. Pentelute*** *(Massachusetts Institute of Technology, U.S.A.)*	**S-48**
09:30	Antibody-Drug Conjugates (ADCs) – A Perfect Synergy? ***John A. Hartley*** *(University College London, U.K.)*	**S-49**
10:00	Developments of Molecular Probes for Detection of Biological Targets ***Sun-Joon Min*** *(Hanyang University (ERICA), Republic of Korea)*	**S-50**
10:30	From Structural Biology to Organic Synthesis: A Collaborative Search for New Anti-Mycobacterial Drugs ***Roderick Bates*** *(Nanyang Technological University, Singapore)*	**S-51**

Free Communication III:

Chairperson: **Poonsakdi Ploypradith** (Thailand)

Co-chairperson: **Rapeepat Sangsuwan** (Thailand)

11:00	Exploring and Characterizing the Biosynthetic Machineries for Synthesizing Fungal Natural Products ***Hsiao-Ching Lin*** *(Academia Sinica, Chinese Taipei)*	**O-26**
11:15	*In Vitro* Screening Anti-Viral and Virucidal Effects Against HIV-1 RT and SARS-COV-2 By Phenylamino-Phenoxy-Quinoline ***Arthit Makarasen*** *(Chulabhorn Research Institute, Thailand)*	**O-27**
11:30	Design and Application of Ultrasensitive & Selective Sensor for the Detection of An Anticancer Drug Using Nanoparticles ***Amber Solangi*** *(University of Sindh, Pakistan)*	**O-28**
11:45	Unraveling Heparan Sulfate–SARS-COV-2 Spike Protein Interaction: Strategy to Develop Novel Viral Inhibitors ***Michela Parafioriti*** *(Istituto di Ricerche Chimiche e Biochimiche "G. Ronzoni", Italy)*	**O-29**
12:00	LUNCH	

CLOSING SESSION:

Grand Ballroom

13:00-15:30

REGULATORY INNOVATION

Moderators: **Ram Sasisekharan** (U.S.A.) and **Surachoke Tangwiwat** (Thailand)

Abstract No.

- U.S. FDA's Efforts to Advance Gene Therapy Development RD
  Peter Marks *(Food and Drug Administration, U.S.A.)* [Virtual presentation]
- Perspectives from the Thai FDA
  Worasuda Yoongthong *(Food and Drug Administration, Thailand)*

Roundtable Discussion

Panelists:
- **Helen W. Boucher** (U.S.A.)
- **Rahul Singhvi** (U.S.A.)
- **Worasuda Yoongthong** (Thailand)
- **Ram Sasisekharan** (U.S.A.)

Roundtable Discussion – AMR Policy Impacting South and Southeast Asia

Moderators: **Helen W. Boucher** (U.S.A.) and **Julie L. Gerberding** (U.S.A.)

Panelists:
- **Helen W. Boucher** (U.S.A.)
- **George F. Gao** (P.R. China)
- **Julie L. Gerberding** (U.S.A.)
- **Ramanan Laxminarayan** (U.S.A.)
- **David Paterson** (Singapore)

CLOSING CEREMONY

16:00 - Presentation of the Princess Chulabhorn Gold Medal Award of Appreciation

Recipients: *(in alphabetical order)*
- Professor Martin van den Berg (The Netherlands)
- Dr. Norbert Frank (Germany)
- Dr. Curtis C. Harris (U.S.A.)
- Professor Leonard Ritter (Canada)
- Dr. Xin Wei Wang (U.S.A.)
- *Thanphuying* Putrie Viravaidya (Thailand)

- Closing Remarks by Professor Dr. HRH Princess Chulabhorn

INVITED SESSIONS

ABSTRACTS

NOBEL LAUREATE LECTURE

HARNESSING EVOLUTION TO MAKE NEW MEDICINES

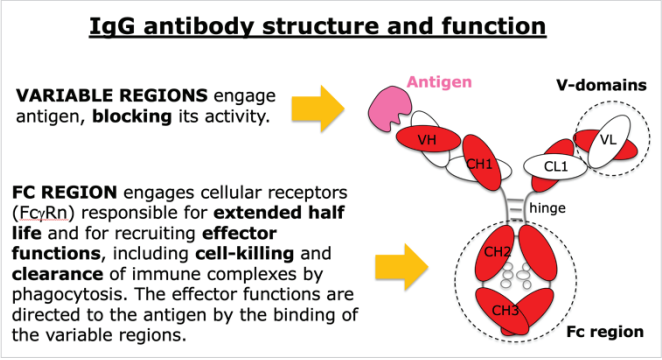
Sir Gregory P. Winter

Fellow, Trinity College, Cambridge, U.K.

E-mail: gpw22@cam.ac.uk

In recent years there has been a revolution in medicine due to the use of antibodies as pharmaceutical drugs. Currently many of the top-selling drugs are antibodies, and their main application has been for the treatment of cancer and inflammatory disease such as rheumatoid arthritis.

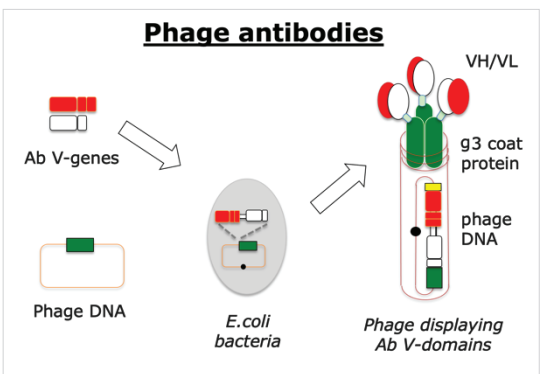
Antibodies are nature's own pharmaceuticals, created to order by the immune system against infectious agents and toxins. IgG Abs are large (150,000 Da) Y-shaped proteins with four chains, two heavy and two light. At the tips of the arms of the Y the heavy and light chains come together through their variable domains and create the antigen binding site. Typically both chains contribute to antigen binding.



Antibodies achieve their therapeutic effects by engaging with their target, blocking its activity, and/or by recruiting immune effector cells to attack and clear the target. Antibodies are able to sustain a long half-life in the serum due to their large size (and avoiding clearance in the kidneys) and rescue from the lysosomal destruction in the endothelial cells by binding to a recycling receptor. This means that antibodies can blockade a target in the serum for a month or more.

Although antibodies were evolved by nature to attack infectious agents and toxins, they can also be used to treat non-infectious disease, and it is in this embodiment that we see the revolution in medicine. However, the development of such antibodies required several technological advances. In one approach we developed, mice are immunized with the target antigen, and the antibody-producing cells fused with a myeloma cell line to create "hybridomas" - cells that secrete antibodies and can be grown in large scale culture. As mouse antibodies provoke an immune response in humans, the antibody genes are isolated from these hybridomas, and the antigen-binding regions incorporated into human antibodies. This creates "humanized" antibodies that are much less immunogenic and with similar binding activities to the mouse hybridomas.

In another approach we developed, human antibodies are selected directly from libraries of human antibody genes. The libraries are usually created by harvesting the antibody heavy and light chain genes from a population of human lymphocytes by PCR, and then shuffling them together. This readily creates an extremely large and diverse library of human antibody genes. The antibody genes are then fused to the p3 coat protein gene of filamentous phage, leading to display of the antibody on the surface of the phage. Phage with binding activities are selected by passing the phage library over solid phase antigen. The phage that stick to the antigen can then be eluted by high or low pH buffers. These phage are then used to infect bacteria, they



multiply, are harvested and subjected to a further round of selection. A selection factor of 1000 fold in one round becomes a million-fold in two rounds and a billion-fold in three rounds. It is this iterative selection that enables the selection of the rare phage antibodies with binding activities.

At the time Darwin proposed his theory of evolution in 1859 it was popularised by phrases such “survival of the fittest”, and “nature, red in tooth and claw”. The theory required a diverse population and a selection process in which the individuals that survive go on to multiply, so that the population becomes enriched in the progeny of the survivors. Our approach is therefore a form of accelerated evolution using huge populations (billions of phage antibodies) and ferocious selection pressures (1000/1) applied to a very large (> 1010) and diverse population. Instead of taking thousands of years, or more, as is typical for the evolution of organisms in nature, our process takes days.

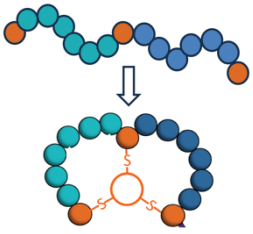
Furthermore, by mutating the selected phage antibody genes (for example, randomly by growth in bacterial mutator strains or at defined places on the antibody DNA by PCR mutagenesis), we can generate mutants with stronger binding activity. These mutants can be isolated from wild-type binders by increasing the stringency of selection, for example by extended wash cycles.

With this technology, the same antibody library, the same tube of bacteriophage, could be used to isolate antibodies against one target after another. It can be regarded as a bottled immune system, at least for the purposes of generating antibodies. It was this technology that led to Humira, the first human therapeutic antibody to be approved by the US FDA and for many years the world’s top selling pharmaceutical drug.

The beauty of the phage technology is that it can be used to evolve any protein that can displayed on the phage. We have used it to evolve antibody mimics that may overcome some of the disadvantages of antibodies as medicines. One disadvantage of antibodies is their size. Although this helps sustain the long half-life of antibodies in the serum, it also limits their penetration deep into tissues. To overcome this, we have tried to create small antibody mimics using the phage technology.

We considered using a peptide as a small antibody mimic. However unstructured peptides tend to have weak binding activities due to their high conformational entropy, and they are also readily cleaved by proteases in the serum. We therefore constrained the peptide by attachment to a chemically reactive scaffold molecule (for example tris bromomethylbenzene) at three cysteine residues. This creates a bicyclic peptide, or “bicycle”, essentially a scaffold with two short peptide loops attached.

To obtain bicycles against a target antigen, we created a large phage display library of linear peptides each with three invariant cysteines. A typical peptide library is encoded by synthetic DNA comprising three cysteine codons, separated by two 18 base segments of random nucleotides encoding two six residue peptide loops. The phage peptide libraries are then linked to the scaffold via the three cysteines, and the phage are selected by binding to antigen, as already described for phage antibodies. The sequence of the phage bicycles with binding activities can be deduced by sequencing the phage DNA; the soluble free peptide is then chemically synthesized at scale using routine methods.



Bicycle: Two Ag-binding peptide loops (randomized) on a chemical scaffold attached through invariant cysteine. MW = 1,500 (15 aa)

With large enough libraries (>10exp10), we can obtain phage bicycles with potent binding affinities to a wide range of targets. The bicycles are relatively resistant to proteolysis and can be made even more resistant if non-natural amino-acids are incorporated into the free bicycles by chemical synthesis. The bicycles do not seem to be immunogenic, and are tolerant of other entities chemically linked to their C-termini - this reflects their selection while anchored to the phage. It

is therefore possible to append other entities such as chemical toxins; or to assemble bicycles together to create multimers with increased avidity of binding or to recruit effector functions.

For example, bicycles have been created that are able to block viral (SARS-Cov2) infection, deliver toxins to cancer cells, recruit NK cells or activate cytotoxic T-cells to kill tumour cells. Several bicycles are in pre-clinical and clinical development. Currently the lead clinical programme in Bicycle Therapeutics is a bicycle toxin conjugate (zelenectide pevedotin) that targets nectin-4. This is showing acceptable toxicity and clinical responses in patients with metastatic urothelial cancer.

In conclusion, by harnessing evolution in the laboratory we can make valuable antibody drugs, and we believe that we can do likewise with peptide drugs.

The work described is from a large number of workers, including those from the MRC Laboratory of Molecular Biology, Cambridge; the former Centre for Protein Engineering, Cambridge; the former company Cambridge Antibody Technology and the company Bicycle Therapeutics and its collaborators.

Please see the following links:

- https://www.nobelprize.org/prizes/chemistry/2018/winter/biographical
- https://www.bicycletherapeutics.com/media/science-publications

PLENARY LECTURES

ONE HEALTH: PREDICTING AND PREEMPTING SPILLOVER AND INFECTION DISSEMINATION

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One Health Background

One Health is an integrated approach that recognizes the interconnection between human health, animal health, and environmental health. According to the U.S. Centers for Disease Control and Prevention (CDC), One Health is a "collaborative, multisectoral, and transdisciplinary approach, working at the local, regional, national, and global levels, with the goal of achieving optimal health outcomes for people, animals, and the global ecosystem." In recent years, this framework has become increasingly relevant, so much so that globally, the World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Organisation for Animal Health (OIE) have made this framework a central theme of their work and many other governmental and civil society organizations are now highly engaged.

The One Health framework is relevant to many contemporary health challenges, but infectious diseases are an especially prominent area of concern. Approximately 60% of emerging infectious pathogens among humans are zoonotic, meaning they originate in animals before spilling over to humans. Important examples include HIV, Ebola virus, Zika virus, and SARS-CoV-2. Antimicrobial resistance is a related One Health threat. Widespread use (and all-too-often misuse) of antimicrobials in humans and animals has created bacteria so resistant to available antimicrobials that they are essentially impossible to treat. These pathogens can move from one species to another, a problem made worse by contamination of water supplies and food products.

Emerging and Re-Emerging Zoonotic Infectious Disease Threats: A Perfect Storm

Emerging and re-emerging infectious disease threats are among the most important challenges facing the global community, and several convergent factors are highly likely to increase the threat. Factors especially relevant to zoonotic infections include:

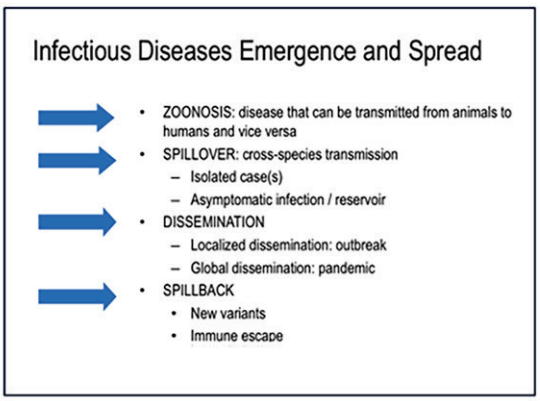
1. Population demographics: Increasing urbanization, urban poverty, and the incursion of people and communities into previously isolated areas increase the opportunities for human exposure to animal and vector sources of infectious pathogens and rapid dissemination once these pathogens are acquired.
2. Global travel: Increases in global transportation – in the air, on water, and across land - increase the potential for admixing of people and the pathogens they carry. According to the World Air Traffic Forecast, 4 billion passengers will travel internationally and 5.7 billion will travel domestically in 2024. Animals are also traveling and despite efforts to reduce illegal trade, many are smuggled into new regions without health inspection. Animal trade is a well-documented source of infection outbreaks, as seen in the 2003 U.S. monkeypox (now known as mpox) outbreak when an infected rodent from Western Africa caused a large chain of animal and human pox infections.
3. Climate change: Climate change is likely to be a powerful driver of infection emergence. Human, animal, plant, and vector habitats are changing, which affects the entire ecosystem of infectious disease transmission and vulnerability. Chaotic weather conditions, including extreme drought, flooding, fires, and other weather-related calamities add to the risk in myriad ways.
4. Social disruptions and displacement: The United Nations estimated that in 2023, there were

more than 117 million forcibly displaced people in the world. Infectious diseases threats loom large for those who are unstably housed and lack access to food, water, hygienic services, and health services. Given the current state of global conflict and expanding geographies of chaotic weather, it is likely this number will only increase.

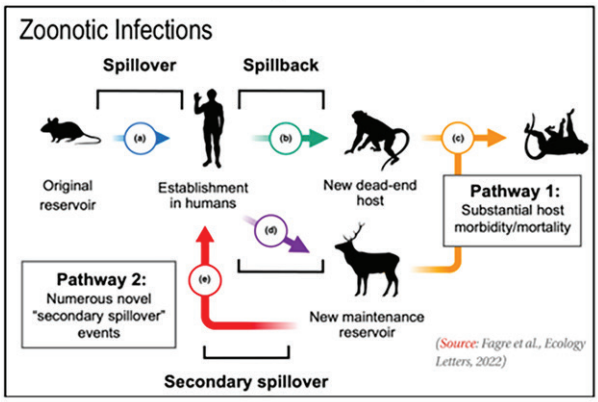
These and many other evolving conditions create a perfect storm for the emergence, re-emergence, and dissemination of pathogens that afflict humans, animals, and even plants. In this context, it is not surprising that we recently experienced a devastating pandemic with a new zoonotic pathogen, and all indicators are that this is unlikely to be an isolated episode.

Genesis of Emergence: Spillover and Spillback

Box 1: Definitions



Box 2: Emergence and Spread



SARS-CoV-2 emergence is an exemplar of zoonotic infection emergence and dissemination (Box 1 and Box 2). The original reservoir is unproven but is probably a bat species. Spillover from the reservoir to an intermediate host, or potentially directly to a human, started what proved to be an extraordinarily rapid cascade of dissemination, reaching global proportions as travelers quickly moved the virus from one community to another. While not surprising in retrospect, SARS-CoV-2 also spills back to other animal species, including whitetail deer, minks, several feline species, and many others. The impact of non-human infections is variable, ranging from inapparent or very mild infections to fatal outcomes.

Even more worrisome is the phenomenon known as secondary spillover – when the virus – or its new variants – are transferred back to humans. These recurring cycles of transmission create a potential incubator for the emergence of new variants that evade current diagnostic test detection and are resistant to current-generation antiviral therapies.

Beyond Surveillance and Response

Traditional approaches to emerging infectious diseases rely on standard epidemiological practices: case detection, diagnostic testing, outbreak investigation, clinical and public health interventions to treat disease and prevent spread, and broader social containment measures when necessary. Not surprisingly, preparedness efforts have largely focused on surveillance strategies, laboratory capacity development, countermeasure development, and emergency response training. These are certainly necessary but not sufficient in today's world of potential threats. Prevention of spillover and earlier preemption are increasingly feasible strategies, aided by geosurveillance tools, enhanced ecological, animal and human testing, advanced modeling, and other predictive tools.

The application of artificial intelligence (AI) is an especially promising frontier for predicting spillover and detecting events before they disseminate:

1. Predictive Modeling: Large data models that include climate data, weather patterns, human demographics, food production, animal population changes, land use, water, and air monitoring, etc., are being used to predict “hotspots” for human-animal connection and sites for enhanced surveillance.
2. Early Warning: Real-time monitoring of human healthcare engagement, health records, social media, and other patterned behaviors can be analyzed with machine learning tools to identify patterns suggestive of infection spillover and dissemination. Though currently less developed, similar algorithms can be applied to food production animal status, wildlife health, and ecological monitoring data.
3. Early outbreak intervention: Global Positioning System (GPS) tools and other means to assess and monitor human-animal and human-human interactions can aid in the analysis of transmission modes and high-risk settings, allowing earlier opportunities for containment and other interventions.
4. Predictive Research: AI approaches enhance the elucidation of disease transmission dynamics, risk factors for spillover and spread, and intervention strategies. For example, AI can process genomic data from pathogens to identify characteristics associated with virulence or resistance. AI can examine human and animal behavior patterns and connectivity to better understand and predict spillover. Likewise, environmental data climate models and other environmental sensors can contribute to better understanding and prediction of transmission dynamics.

Summary

New tools, technologies, and countermeasures will improve prediction, early recognition, and preemption of emerging zoonotic diseases. However, the most effective strategies for preventing spillover are even further upstream: protection of biodiversity, reduction in deforestation, sustainable land use, safer farming practices, improved human and animal health monitoring, and regulation of wildlife trade. These are all part of a comprehensive ONE HEALTH approach. Success requires more research support, international disease monitoring, policy coordination, and global cooperation.

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AMR IN 2024: WE STILL HAVE AN AWARENESS PROBLEM

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In 2013, the United States Center for Disease Control and World Health Organization declared antimicrobial resistance (AMR) a public health crisis. Combating antimicrobial resistance (AMR) and strengthening the antibiotic pipeline are top priorities for the United Nations (UN), World Health Organization (WHO) and many Nongovernmental Organizations (NGOs), all of whom recognize that this is a One Health problem impacting humans, animals and the environment globally.

Patients with infections that are extremely difficult or in some cases impossible to treat due to resistance are seen more frequently by physicians around the globe. They are further challenged by the dwindling arsenal of safe and effective antibiotics. Despite over a decade of effort, the problem continues to increase, and progress has been unacceptably slow. Importantly, the burden of AMR is disproportionately felt in developing countries, where access to antibiotics remains the major challenge.

Recent years have seen increased recognition of the antibiotic resistance crisis and the need for action, but this has not yet been enough to impact lasting change. While some progress has been made in infection prevention and control, antibiotic stewardship, surveillance, developing the infectious diseases workforce, access to antibiotics and research and development of new antibiotics, the costs of AMR, human and economic, continue to grow.

The US National Action Plan for Combatting Antibiotic Resistant Bacteria has 5 goals:

1. Slow emergence and prevent spread of AMR
2. Strengthen national One Health surveillance
3. Advance development and use of rapid and innovative diagnostic tests
4. Accelerate research and development of new antibiotics, therapeutics and vaccines
5. Improve international collaboration and capacities for prevention, surveillance, controlantibiotic research & development and establish a Federal champion for AMR.

Murray and colleagues recently published a comprehensive estimate of deaths associated with AMR and forecasted to 2050; they showed an estimated 4.71 million deaths associated with AMR and 1.14 million deaths attributable to AMR in 2021, with a forecast of 1.91 million deaths attributable to AMR and 8.22 million deaths with AMR by 2050, the highest being in south Asia, Latin America and the Caribbean and among individuals 70 years old or older. Importantly, the authors modeled a “better care scenario” in which 92 million deaths could be averted between 2025 and 2050 through improved care and greater access to antibiotics; in addition 11.1 million AMR deaths could be prevented through the development of a gram-negative drug pipeline (ref & figures).

On 26 September 2024, the UN held the 2nd High Level Meeting on AMR and adopted a political declaration which established global goals, commitments and targets for combating AMR, including a goal to reduce global deaths associated with bacterial resistance by 10% by 2030. The declaration articulates specific prevention interventions including:

- Immunization (reduces infections and antimicrobial use)
- Early and accurate detection of pathogens
- Infection prevention and control
- Water, sanitation and hygiene (WASH)

This issue was the focus of the 2019 Aspen Health Strategy, co-chaired by two former Secretaries

of Health and Human Services, Kathleen Sebelius (2009-2014) and Tommy Thompson (2001-2005). Expert bodies, including the World Health Organization and the President's Council of Advisors on Science and Technology, have also called for action to combat antibiotic resistance and foster the research and development of urgently needed new antibiotics. These challenges are a key focus of numerous professional society meetings, ECCMID, IDWeek—IDSA's annual scientific meeting—which convenes experts aimed at advancing scientific approaches to resistance, antibiotic development and antibiotic stewardship, and most recently the September 2024 UN High Level Meeting. Patient advocacy groups have started (https://www.who.int/groups/task-force-of-amr-survivors) and there has been modest interest from the lay press (Race Against Resistance: The Life And Death Struggle To Save Antibiotics | BBC StoryWorks), but as yet we have no single voice to raise awareness for AMR.

A 22 September 2024 panel with CDC and One Health Trust (https://onehealthtrust.org/), acknowledged the limited consistent global data on knowledge, attitudes, and beliefs about antimicrobial resistance. To address this data gap, CDC is partnering with CDC Foundation and Gallup to develop the Global AMR Monitor which will use the Gallup World Poll to gather representative data on antimicrobial resistance knowledge, attitudes, and behaviors from countries across the world. CDC and Global Partners Commit to Collective Action to Combat Antimicrobial Resistance | Blogs | CDC The second phase of the Global AMR Monitor project, which is contingent on funding becoming available, will leverage the information gained in the first phase to monitor and track antimicrobial resistance knowledge, attitudes, and behaviors over time and in more than 140 countries. This sounds promising but is yet unfunded.

This presentation will address the current state of response to AMR with a focus on awareness and advocacy. The path forward should focus on global One Health solutions with emphasis on measures for both resource replete and low and middle income countries.

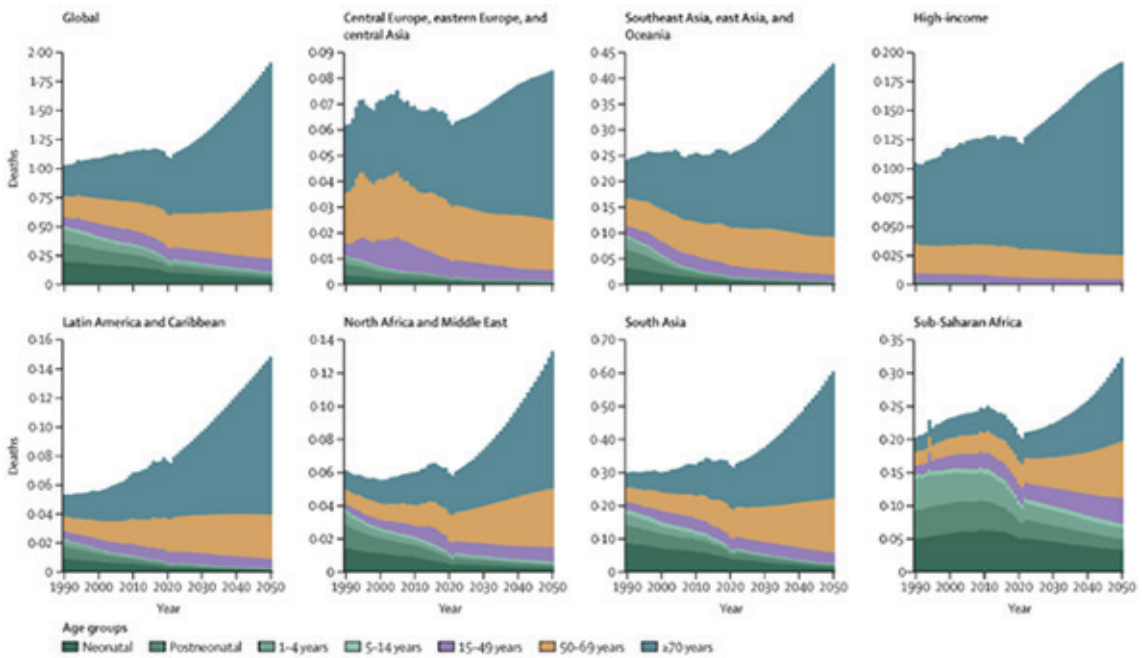

Figure: Deaths attributable to AMR by age group and location in the reference scenario, 2022–2050 Units are in millions.

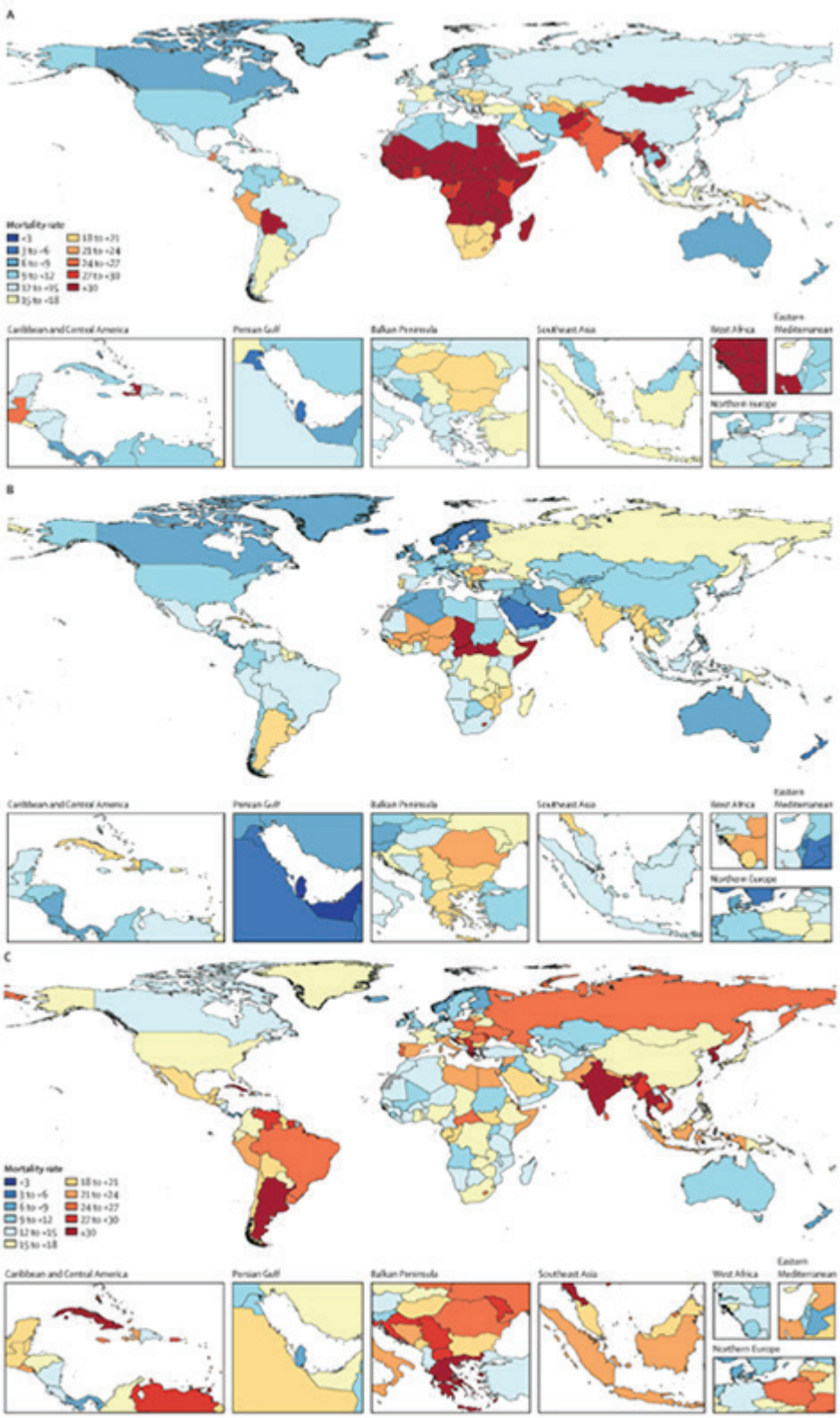

Figure: Death rates per 100 000 attributable to AMR, all ages, 1990, 2021, 2050

(A) Death rate attributable to AMR, all ages, 1990. (B) Death rate attributable to AMR, all ages, 2021. (C) Death rate attributable to AMR, all ages, 2050. AMR=antimicrobial resistance.

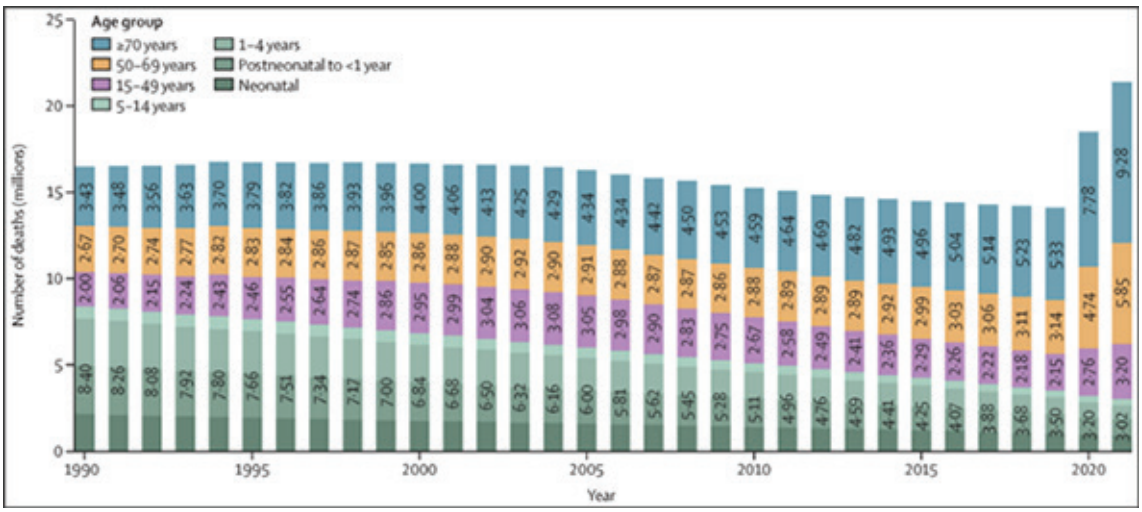

Figure: Global time trend of sepsis, by age, 1990–2021

Bar labels represent the number of sepsis deaths in a given year for people aged 0–14 years, 15–49 years, 50–69 years, and ≥70 years. Values for the age group of 0–14 years represent the sum of sepsis deaths among neonates, postneonates to <1 year, 1–4 years, and 5–14 years.

DRUG DEVELOPMENT AND REGULATORY SCIENCES: TURNING CRISES INTO OPPORTUNITIES – A VALUABLE PERSPECTIVE

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The drug development process involves several key steps. It begins with drug discovery, then testing the drugs in laboratory settings and suitable animal models. These preliminary stages are collectively known as preclinical work. Once this initial testing is complete, a regulatory submission known as an Investigational New Drug (IND) application is filed to obtain permission for testing the drug in humans. Next, a series of studies on humans is conducted. First, drug safety is established through a Phase I study. Then, effective dosage through Phase II study is established. Finally, in Phase III trials, the drug efficacy is demonstrated through statistical significance in treating the intended condition. In parallel, an essential step is the drug's production, known as Chemistry, Manufacturing, and Controls (CMC). This process follows Good Manufacturing Practices (GMP). GMP is vital for clinical testing in humans and the commercial production necessary to market the drug. The results from the clinical (phases I, II, and III) studies, taken together with a robust manufacturing data package, are needed for a New Drug Application (NDA), which regulatory bodies carefully evaluate to approve the drug for human use and the commercialization of the drug.

This development process is primarily designed to demonstrate that drugs are safe and establish their effectiveness to the standard of care for which the drug is developed. This process serves as the regulatory framework for determining whether a drug is approved for commercial use by humans.

While the above is a general framework for the drug development and regulation process, challenges over time have created significant opportunities, starting from its creation as a framework and the necessary innovation to support its evolution to help address new drug modalities (from small molecules to gene therapy).

In this plenary lecture, I will provide a historical context for drug regulation and discuss how current scientific approaches have helped address regulatory challenges and the opportunities they presented in the recent past. There are two examples that I will use to illustrate essential concepts. First, the approaches that were instrumental in solving the world's heparin contamination crisis, saving hundreds of lives, and demonstrating how regulatory frameworks can use science to address the risks of regulatory unknowns. Second, navigating regulatory frameworks to speed up the development of therapeutics, in response to Influenza, Zika, Dengue, Yellow Fever, and the COVID-19 pandemic, making these efforts a real game-changer in the field.

The convergence of life sciences, physical sciences, and engineering represents an exciting development at the forefront of research and development. Scientists and engineers are increasingly moving beyond traditional disciplinary boundaries, recognizing the vast opportunities that emerge when molecular-level life science research is combined with technological advancements in engineering. This transformation is further enhanced by the advent of artificial intelligence and machine learning. The potential impact of these changes on drug development and regulation raises fascinating questions for all of us.

THE TWO SIDES OF PRECISION MEDICINE: PROTEOMIC ENABLEMENT OF BIOMARKERS AND THERAPEUTICS

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Underlying precision medicine is the concept that an individual's Omic signature (including the proteome) will provide a physician with clinically actionable diagnosis and a subsequent mechanistic therapeutic route. This requires i) having an array of mechanistic therapies for each person's disease state and ii) a means to diagnosis (identify) which therapy (or combination) will be appropriate for a particular person. Proteomics has played a role in discovery and defining potential mechanistic routes. Now it is time to move into implementation. We will discuss the progress in cancer and heart disease focusing on how technology is now allowing scalable robust protein measurements that enables translation.

Precision medicine requires having an appropriate clinically useful diagnostic. Here, we use the example of our Molecular Twin program where multi-omic data was used to determine an individual's molecular phenotype in context to cancer. By comparing a patient specific multi-omic molecular profile to other we can identify their "molecular twin", determine their mechanism/outcome and response commonalities providing clinical insight into the appropriate therapeutic intervention. Using the molecular twin approach for from pancreatic cancer, we carried out genetics, transcriptomics, proteomics, and lipidomic of matched tissue and plasma samples found that plasma proteomics was predictive, which was then validated in two independent cohort. We have moved on to determine whether plasma proteome profile alone or in combination with pancreatic imaging can classify the individuals who benefit from Whipple surgery from those who should move directly to other interventions (immunotherapy, chemotherapy, etc.). The Molecular Twin approach is now being used in other cancers.

As the plasma proteomics was so informative, it suggests that new blood collection approaches that allows for remote sampling, where an individual can take their own blood sample, anytime and anywhere, can be used to promote precision medicine. Remote blood sampling methods allow for deep proteomic profiling as well as targeted protein assays. Remote blood sampling devices are stable at room temperature and can be easily shipped by mail reducing the barrier for collection and can be used in epidemiological studies, in the study of natural disease progression and longitudinal intervention studies and in clinical trials. We will illustrate how our health surveillance targeted multiplex protein panel representing >20 FDA approved and other clinical laboratory tests compare between traditional plasma and remote blood sampling devices. This is the first step in moving remote sampling into a physician's routine

Precision medicine also requires having an appropriate clinical intervention. Here, we will provide example of proteomics usefulness to uncover mechanistic underpinnings of new drug class (exemplified by PR-364). With a single dose of PR-364 immediately following a heart attack is sufficient to stop heart remodeling and death. Using a combination of proteomics, metabolomics and functional assay we uncovered the molecular mechanisms behind the drug effect in mouse and cardiac-like cells. PR-364 activates Parkin, an enzyme that regulates the mitophagy and the clearance of injured and dying mitochondria. Surprisingly PR-364 does even more. Using proteomics to screen potential drug candidates in a semi-high throughput method (100 samples/day) will allow for earlier molecular phenotyping of a drugs affect. This is true for PR-364 especially as it does not work to the same degree on all cardiac cells. Also a surprise and has ramification for how a drug effect within a person and across populations.

In summary, precision health requires clinically actionable diagnosis and a subsequent mechanistic therapeutic route and molecular profiling including proteomics is essential.

PROGRAMMABLE THERAPEUTICS FOR GENETIC DISEASES

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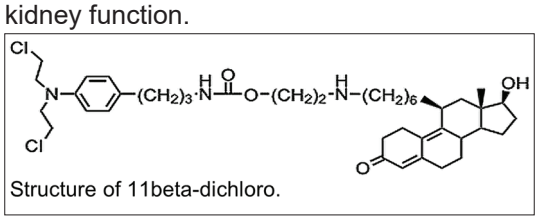
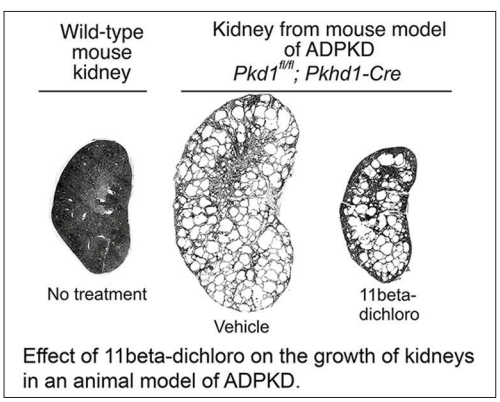
Oxidative stress is a two-edged sword. On the one hand, it damages nucleic acids and proteins, leading to aging, cancer, and neurologic diseases. On the other hand, some diseased cells are especially sensitive to oxidative stress, leading to the use of reactive oxygen generators as therapeutics. This presentation will chart the lineage of a suite of compounds, starting with the widely used metal-based antitumor drug, cisplatin, leading to bifunctional organic anticancer agents, and culminating in the development of a set of molecules that trigger oxidative stress that is selectively fatal to cyst-forming cells responsible for a major human kidney disease.

Autosomal dominant polycystic kidney disease (ADPKD) is a genetic disease affecting over 500,000 people in the United States and over 12 million people worldwide. While the cysts in ADPKD are not cancerous, they share several cancer-like properties, including metabolic network rewiring that leads to unnatural progressive growth. Histologically, large fluid-filled cysts form within the kidney and can double the size of the organ. ADPKD is not curable and, if untreated, it leads pain, high blood pressure and eventually kidney failure in about half of the patients by their 60s. There is one FDA approved drug for the disease, tolvaptan, which slows growth of the cysts, but it has significant side effects and can lead to liver damage. There is a need for novel agents that have fewer side effects and improved efficacy for reducing the size of the cysts in ADPKD, and thus preserving kidney function.



Structure of 11beta-dichloro.

Cystic cells in ADPKD, as with many cancer cells, up-regulate aerobic glycolysis for energy production to support enhanced growth, bypassing the more normal use of mitochondrial respiration for that purpose. Because of metabolic imbalances, cystic cells naturally experience enhanced levels of oxidative stress, and it was reasoned that additional oxidative stress provided by an external agent, such as a drug, might push the cells over a threshold for apoptosis. In our studies on agents developed to treat urogenital malignancies, we designed a multi-functional agent named 11beta-dichloro. This compound is surprisingly well tolerated by normal cells and even animals, and one of its operative mechanisms includes the induction of mild oxidative stress. Accordingly, 11b-dichloro was evaluated in cellular and mouse models of ADPKD.



Effect of 11beta-dichloro on the growth of kidneys in an animal model of ADPKD.

In the present work we demonstrated that 11beta-dichloro is effective in delaying cyst growth and its associated inflammatory and fibrotic events, thus preserving kidney function in perinatal and adult mouse models of ADPKD. In both models, the cyst cells with homozygous inactivation of a gene for ADPKD, Pkd1, show enhanced oxidative stress following treatment with 11beta-dichloro and undergo apoptosis. Co-administration of the antioxidant vitamin E negated the therapeutic benefit of 11beta-dichloro *in vivo*, supporting the conclusion that oxidative stress is a key component of the mechanism of action. One obstacle to the eventual use of 11beta-dichloro in the clinic is its previously shown ability to cause direct damage to DNA. Consequently, the structure of the compound was reprogrammed to eliminate its direct DNA damaging functionality, while preserving its ability to induce oxidative stress. This derivative shows favorable pre-clinical activity, maintains excellent anti-cystic properties *in vivo*, and emerges as the lead candidate for development.

THE LESSONS LEARNED FROM COVID-19

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During the recent COVID-19 pandemic, Gao Fu's team sequenced and isolated the novel coronavirus within a week, quickly alerting the global community. His group was the first to identify ACE2 as the receptor for the COVID-19 virus, the same receptor used by SARS-CoV, and further elucidated the structural basis of the receptor-binding interaction. In vaccine development, Gao's team created the world's first approved recombinant protein subunit vaccine for COVID-19 (ZF2001). They also conducted extensive evaluations of viral variants, discovering that the vaccine exhibited strong cross-neutralizing ability against the Omicron variants. Gao's group also demonstrated exceptional leadership in the development of therapeutic antibodies. In collaboration with international pharmaceutical companies, they developed Etesevimab, the first COVID-19 antibody in China to enter clinical trials. The antibody received emergency use authorization in several countries and nearly 1 million doses were sold globally. These achievements marked a significant milestone in China's pharmaceutical research and development. Additionally, Gao's team established a virus-cell immune monitoring platform, systematically analyzing T-cell epitopes of newly emerging and recurrent viruses. This work provided critical guidance for the optimization of vaccine strategies and the development of diagnostic reagents.

SYMPOSIA

AMR GOVERNANCE: CRITICAL CHALLENGES BEYOND THE SCIENCE

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The continued emergence and spread of microbially resistant pathogens present the global public health and scientific communities with one of the most significant public policy challenges of the 21st century. Sir Alexander Fleming, the scientist who discovered penicillin in 1928, raised the likelihood of antibiotic resistance becoming widespread in his acceptance speech of the Nobel Prize for his discovery in 1945; but resistance to other antibiotic compounds had already been documented some 20 years prior. Since then, the initial optimism of penicillin's discovery prompting a 'golden age' of medicine has been progressively, comprehensively undermined by increasing levels of resistant bacteria, parasites, fungi, and viruses. Diseases once presumed conquered have resurged even as the successful development and commercialization of new microbial treatments has slowed.

Despite the formation of the World Health Organization (WHO) coinciding with the first documented case of AMR, the international community of member states have been slow to recognise the phenomenon's catastrophic potential let alone develop and then implement plans to contain it. Indeed, while the WHO released a strategy for the containment of AMR in 2001, the WHO's first global action plan to tackle the threat was not adopted until 2015, and only then reportedly because of the United Kingdom's advocacy – much of which has been attributed to Dame Sally Davies, the UK's then-Chief Medical Officer. Since 2015 there has been increased attention given to the AMR 'problem', including two United Nations High-Level Meetings on Antimicrobial Resistance (2016, 2024), a memorandum of understanding between three (later four) international agencies to work collaboratively in addressing AMR (otherwise known as the 'Quadripartite'), the formation of an Interagency Coordination Group to better facilitate cooperation between governments and relevant intergovernmental agencies, and even ministerial conferences. Notably, however, while the COVID-19 pandemic understandably diverted government attention away from many important public health matters, by late 2024 only half of the world's countries had implemented some type of surveillance system to detect AMR. Even more concerning is the fact there has been no evidence of an increase in global testing over several years while many of the national action plans to address AMR that are intended to drive action at the national level have either already expired or are set to expire in the next 12 months. COVID-19 aside, these results suggest the current governance structures and policy settings are not achieving their objective in successfully mitigating AMR.

This paper examines the challenges surrounding the existing governance structures for coordinating international action on AMR. The paper briefly explores the complex nature of the phenomenon prior to evaluating the existing approach to addressing the AMR threat being promoted by the Quadripartite agencies (World Health Organization, World Organization for Animal Health, Food and Agriculture Organization, and the United Nations Environment Programme) via national action plans. The paper then considers alternative governance structures and the various existing political impediments to their creation and/or execution.

More specifically, the paper argues that while microbial resistance is an evolutionary biological process that has been underway for millennia, the problem humanity now confronts has largely arisen and continues to be driven by human interaction with the microbial world. In fact, the upsurge in AMR across multiple organisms extending from bacteria, viruses, fungi, and parasites has emerged precisely because of humanity's proclivity to utilise antimicrobial substances to eliminate various forms of disease. As a result, the Anthropocene has witnessed a change in how microbes are responding to this latest threat of elimination by evolving resistant strains –

strains that are progressively, incrementally, spreading globally to the extent that many medical experts now openly anticipate a 'post-antibiotic' era.

Like human-induced climate change, AMR is a 'slow burn' problem that has been emerging over decades. It is widely accepted that AMR cannot be wholly eliminated; it can only be controlled or mitigated either via enhanced stewardship of existing treatments, or the development of new antimicrobial agents. Herein lies one of the most insidious problems though, in that virtually as soon as a new treatment becomes available, resistance to that treatment habitually emerges – on average, five years after an agent becomes commercially available. Accordingly, this has led to a much greater emphasis on antimicrobial stewardship as a long-term strategy for prevention, control and mitigation, to preserve the longevity of existing and future medical countermeasures.

In this broader context, responsibility for coordinating the international response has been delegated to now-four intergovernmental agencies that are described as the Quadripartite. This cross-institutional initiative was instigated in 2015 when the WHO's principle decision-making body, the World Health Assembly, adopted resolution *WHA68.7 Global action plan on antimicrobial resistance* that had been jointly developed by the WHO, FAO and the World Organization for Animal Health (now WOAH, formerly OIE). The resolution directed the intergovernmental human health agency to strengthen collaboration with the FAO and WOAH to combat AMR as part of a One Health approach to global health security. The following year further impetus in tackling AMR was then provided by the *United Nations High-Level Meeting on Antimicrobial Resistance* that adopted a political declaration which emphasised again the need for these three specialised agencies to collaborate in tackling AMR while also calling on the UN Secretary-General to establish an ad hoc inter-agency coordination group (IACG). In response, on 30 May 2018 the three international agencies – the WHO, FAO, and WOAH – signed a memorandum of understanding to cooperate on One Health issues. This initial tripartite agreement was then expanded to a quadripartite arrangement on 17 March 2022 when the United Nations Environment Programme (UNEP) was invited to join. In both versions of the MoU agreements, AMR is listed as the top priority area for multilateral cooperation, with specific actions and responsibilities for each of the organisations to be outlined in a detailed work plan.

In 2022, the Quadripartite released its detailed joint action plan that outlined six work packages that all four organisations would pursue. Curtailing the "silent pandemic of AMR" was listed as the fifth "action track", with prioritisation being given to i) raising awareness and capacity for tackling AMR via policy, legislation, and programs, ii) strengthening global and regional AMR initiatives, and iii) enhancing global governance arrangements. To give effect to these priorities, the Quadripartite also released a strategic framework that describes the "comparative advantage and catalytic role" the four organisations can engender, as well as outlining "a theory of change" that governments are encouraged to utilise (with the four organizations' assistance) in building domestic capacity to address AMR.

Indeed, central to all current existing global strategies and frameworks for countering the spread of AMR is the development and implementation by governments of their own national action plans (NAPs). Since 2015 and the adoption of the AMR global strategy, the tripartite organisations have released a range of guidelines that urge all their respective member states to create a national, comprehensive, multisectoral strategy to address AMR in their individual jurisdictions. Even so, and arguably predictably given the functionalist origins of the intergovernmental agencies involved, each of these organisations have continued to approach their assistance to member states from their respective technical mandate, releasing guidance on how to develop NAPs within their discreet areas of expertise (see Table 1.1). While this has resulted in the publication of some 141 NAPs to date, only 30 jurisdictions have developed NAPs that address AMR in the plant and animal sectors, and only eight countries have published a One Health AMR NAP. Added to this, it is often unclear whether the actions outlined in the NAPs reflect measures that have already been taken or if they yet to be enacted.

Table 1. AMR National Action Plan Implementation (as of October 31, 2024)

AMR NAP Guidelines	***Countries***
Territories that have published a WHO AMR NAPs[1] * indicates countries have also developed a FAO-PMP-AMR plan ± indicates countries that have published a second (and/or third), updated AMR NAP § indicates countries that have published a One Health AMR NAP	Afghanistan, Albania, Algeria, Argentina, Armenia, Australia, Austria±, Bahrain, Bangladesh, Barbados, Belgium*±§, Benin, Bhutan, Brazil, Brunei Darussalam, Burkina Faso, Burundi, Cabo Verde, Cambodia*, Cameroon*±, Canada±, Cayman Islands, Chad, Chile, China (People's Republic of)±, Colombia*, Comoros, Cook Islands, Costa Rica, Cote d'Ivoire*, Congo (Democratic Republic of the)*, Croatia, Cyprus, Czech Republic±, Denmark, Ecuador, Egypt, El Salvador, Eritrea, Eswatini, Ethiopia±§, Fiji±§, Finland, France±, Gabon, Germany±, Ghana*, Greece, Guinea, Guinea-Bissau, Iceland, India, Indonesia±, Iran, Iraq, Ireland±§, Italy*±, Japan±, Jordan, Kenya*, Korea (Democratic People's Republic of), Korea (Republic of)±, Kosovo, Kuwait, Kyrgyzstan*, Lao (People's Democratic Republic of)*, Latvia§, Lebanon, Liberia*, Libya, Lithuania, Luxemburg, Macedonia, Madagascar*, Malawi, Malaysia±, Maldives, Mali*, Malta, Marshall Islands, Mauritius*, Micronesia (Federated States of), Moldova, Mongolia*±, Montenegro, Morocco*, Mozambique, Myanmar, Namibia, Nauru, Nepal±, Netherlands, New Zealand, Nigeria*, Norway, Oman, Pakistan*, Palestine, Papua New Guinea, Paraguay, Peru, Philippines§, Poland, Portugal±, Russian Federation, Rwanda, Samoa, Saudi Arabia (Kingdom of)±, Senegal*, Serbia, Sierra Leone*, Singapore, Slovakia, Slovenia§, Solomon Islands, Somalia, South Africa, Spain±, Sri Lanka±, Sudan, Sweden, Switzerland, Syrian Arab Republic, Tajikistan*, Tanzania (United Republic of)±, Thailand, Timor-Leste±, Togo, Tonga, Tunisia*, Turkmenistan, Tuvalu, Uganda*, United Arab Emirates, United Kingdom±, United States±, Uruguay, Vietnam, Yemen, Zambia*, Zimbabwe*§
Territories that have published an FAO-PMP-AMR but not a WHO AMR NAP[2]	Bolivia, Niger, Saint Kitts and Nevis, Seychelles
WHO Member States that have yet to publish an AMR NAP	Andorra, Angola, Antigua and Barbuda, Azerbaijan, Bahamas, Belarus, Belize, Bolivia, Bosnia and Herzegovina, Botswana, Bulgaria, Central African Republic, Congo, Cuba, Djibouti, Dominica, Dominican Republic, Equatorial Guinea, Estonia, Gambia, Georgia, Grenada, Guatemala, Guyana, Haiti, Honduras, Hungary, Israel, Jamaica, Kazakhstan, Kiribati, Lesotho, Mauritania, Mexico, Monaco, Nicaragua, Niger, Niue, North Macedonia, Palau, Panama, Qatar, Republic of Korea, Republic of Moldova, Romania, Saint Kitts and Nevis, Saint Lucia, Saint Vincent and the Grenadines, , San Marino, Sao Tome and Principe, Seychelles, South Sudan, Suriname, Tokelau, Trinidad and Tobago, Türkiye, Ukraine, Uzbekistan, Vanuatu, Venezuela (Bolivarian Republic of).

[1]WHO, "Library of AMR national action plans" (Webpage, last accessed 9 May 2023).

[2]FAO, "FAO Progressive Management Pathway for Antimicrobial Resistance (FAO-PMP-AMR)" (Webpage, last accessed 9 May 2023).

Given that it is widely acknowledged containing AMR will require comprehensive, multisectoral collaboration at the national level, and cooperation and coordination at the global level, the evidence to date for how the international community is responding to this challenge is far from encouraging. If it is accepted the current approach being pursued by the Quadripartite is potentially contributing to decoupled AMR policies, programs, and governance arrangements in domestic contexts, it warrants questioning whether an alternative, more inclusive approach is necessary. Importantly, however, this is not the first time the public health community has navigated a problem that required a more integrated method, but in that instance, rather than a Quadripartite arrangement involving four separate intergovernmental entities, it prompted the creation of an entirely new intergovernmental organization: the Joint United Nations Programme for HIV/AIDS, or UNAIDS. This paper explores what a United Nations agency on AMR, or UNAMR, might offer for the international community seeking to contain the AMR threat, as well as alternative global health partnership arrangements similar to the Global Fund for HIV/AIDS, TB, and Malaria. The paper then concludes with evaluating the benefits and drawbacks of the subsequent alternative governance arrangements, including what such arrangements might mean for national governments struggling to address AMR as a public health menace.

IS THIS THE END OF THE ANTIBIOTIC ERA?

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In 2022, a Lancet report showed that 4.95 million deaths were associated with bacterial antimicrobial resistance (AMR) globally in 2019, including 1.27 million deaths directly attributable to it (Antimicrobial Resistance Collaborators, Lancet 2022). The majority of these deaths were when AMR occurred in the context of bacteria causing lower respiratory tract infections (such as ventilator-associated pneumonia – VAP) and bloodstream infection (BSI). The top-ranking pathogens as causes of death are Gram negative bacilli (Lancet 2022). Approximately 50% of all global deaths due to AMR occur in Asia (Lancet 2022). This underscores the need to address this problem.

E. coli and *Klebsiella* are members of the Enterobacterales. They are common causes of bloodstream infection and may produce extended-spectrum beta-lactamases (ESBLs). ESBL producers are typically resistant to third-generation cephalosporins such as ceftriaxone, but susceptible to carbapenem antibiotics. A landmark clinical trial comparing piperacillin-tazobactam to meropenem in the treatment of bloodstream infection due to ESBL-producing *E. coli* and *Klebsiella* spp (Harris et al JAMA 2018), showed that piperacillin-tazobactam was associated with higher mortality than meropenem. However, the widespread use of carbapenems (such as meropenem) may cause selection pressure leading to carbapenem-resistant organisms. This is a significant issue as carbapenem-resistant organisms are treated with last-line antibiotics such as colistin or need to be treated with newer antibiotics that are expensive or limited in their availability in most low-income or middle-income countries.

The market for expensive newer antibiotics is relatively limited in high-income countries. This has led to bankruptcy of companies developing new antibiotics and created difficulties in fundraising for the large, clinically relevant trials that can direct their appropriate use. Despite the great need for new antibiotic options, especially in Asia, economic forces are leading us closer and closer to the beginning of the post-antibiotic era.

ENSURING SUSTAINABLE ACCESS TO EFFECTIVE ANTIBIOTICS: KEY MESSAGES FROM THE LANCET SERIES

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Each year, an estimated 7.7 million deaths are caused by bacterial infections, and 4·95 million of these deaths are associated with bacterial pathogens resistant to the antibiotics available to treat them. Antibiotics, if used as indicated, can avert many deaths from bacterial infections, and access to second-line antibiotics can even prevent deaths from some drug-resistant infections. I will present the key results from the Lancet Series on Sustainable Access to Effective Antibiotics aims to frame the issue of antimicrobial resistance (AMR) so actions to prevent infections (through vaccination; water, sanitation and hygiene; and infection prevention and control), ensure that patients everywhere have access to affordable, effective antibiotics. Investment in innovation of new affordable treatments, vaccines, and diagnostics must be geared towards patients, supporting access and serving as cornerstones for ambitious global action.

WORLD HEALTH ORGANIZATION CALLS FOR URGENT ACTION TO MITIGATE CLIMATE-RELATED HEALTH RISKS AND PROMOTE RESILIENCE THROUGH A ONE HEALTH PERSPECTIVE

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The World Health Organization (WHO) highlights the urgent need for action to mitigate climate-related health risks and promote resilience through a One Health perspective. Climate change poses significant threats to human health, animal health, and environmental integrity, necessitating an integrated response that embodies the One Health framework.[1,2] This approach recognizes the interconnectedness of human, animal, and envir82onmental health, promoting collaborative efforts across various sectors to address complex health challenges.

The Quadripartite collaboration on One Health consisting of the WHO, the Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), and the World Organisation for Animal Health (WOAH), have united to tackle these challenges.[3] This vision is based on the common understanding that One Health, as defined by the One Health High-Level Expert Panel, is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals, plants, and ecosystems. It acknowledges the interconnections between human, animal, and environmental health and the necessity of collaborative, cross-sectoral efforts to address these interdependencies.

Climate change contributes to a myriad of health issues, including increased prevalence of vector-borne diseases, respiratory ailments from air pollution, and heat-related illnesses.[1,4] Extreme weather events, such as floods and heatwaves, disproportionately affect vulnerable populations, exacerbating health inequities. Changes in ecosystems due to climate shifts also disrupt food security and water availability, further endangering health outcomes. Furthermore, climate change is undermining many of the social determinants for good health, such as livelihoods, equality and access to health care and social support structures. These climate-sensitive health risks are disproportionately felt by the most vulnerable and disadvantaged, including women, children, ethnic minorities, poor communities, migrants or displaced persons, older populations, and those with underlying health conditions. The WHO underscores the necessity of addressing these health risks through coordinated efforts that align public health, veterinary services, and environmental policies. Figure 1 is an overview of climate-sensitive health risks, their exposure pathways and vulnerability factors.[4] Figure 1 also illustrates how climate change impacts health directly and indirectly, and is strongly mediated by environmental, social and public health determinants.

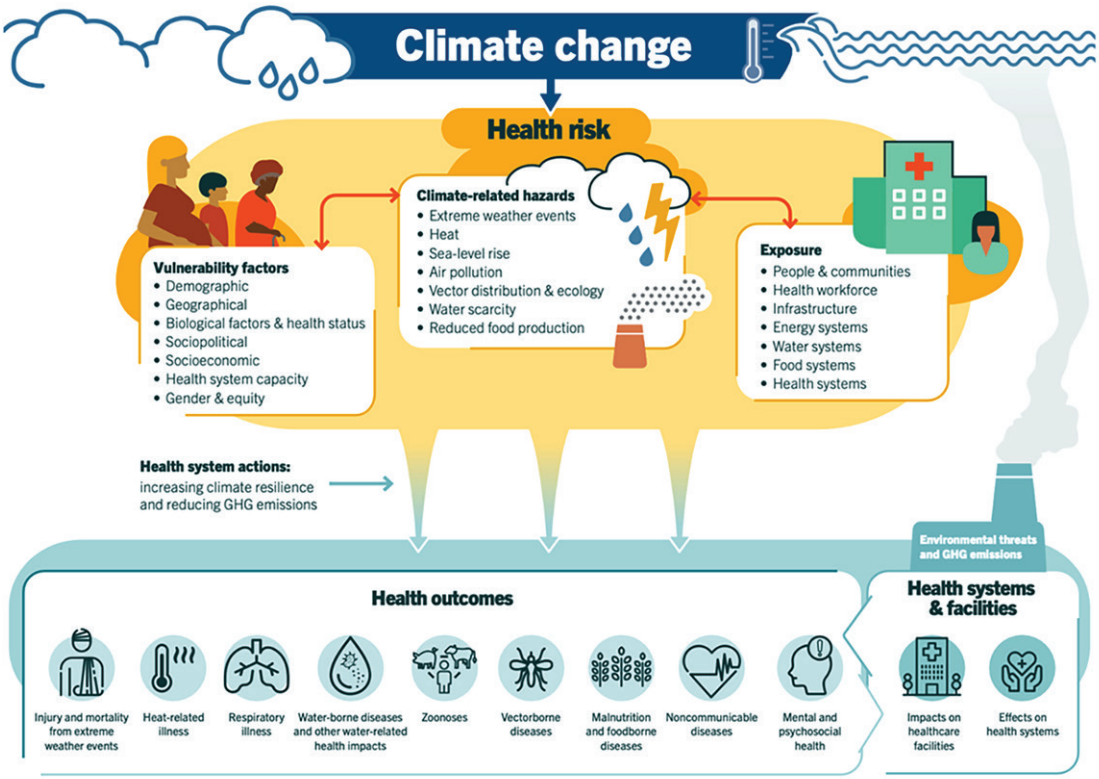
[1] A health perspective on the role of the environment in One Health. Copenhagen: WHO Regional Office for Europe; 2022. https://www.who.int/europe/publications/i/item/WHO-EURO-2022-5290-45054-64214

[2] COP28 Declaration on Climate and Health. February 2024. https://www.cop28.com/en/cop28-uae-declaration-on-climate-and-health

[3] One health joint plan of action (2022–2026): working together for the health of humans, animals, plants and the environment. https://www.who.int/publications/i/item/9789240059139

[4] World Health Organization. Fact Sheet. Climate Change and Health. October 2023. https://www.who.int/news-room/fact-sheets/detail/climate-change-and-health

Figure 1: An overview of climate-sensitive health risks, their exposure pathways and vulnerability factors.



WHO data indicate 2 billion people lack safe drinking water and 600 million suffer from foodborne illnesses annually, with children under 5 bearing 30% of foodborne fatalities.[4] Climate stressors heighten waterborne and foodborne disease risks. In 2020, 770 million faced hunger, predominantly in Africa and Asia. Climate change affects food availability, quality and diversity, exacerbating food and nutrition crises. In 2020, 98 million more experienced food insecurity compared to the 1981–2010 average.

Temperature and precipitation changes enhance the spread of vector-borne diseases. Without preventive actions, deaths from such diseases, currently over 700 000 annually, may rise.[4] Recent research attributes 37% of heat-related deaths to human-induced climate change. Heat-related deaths among those over 65 have risen by 70% in two decades.

The climate crisis threatens to undo the last 50 years of progress in development, global health and poverty reduction, and to further widen existing health inequalities between and within populations.[4] It severely jeopardizes the realization of universal health care, including by compounding the existing burden of disease and by exacerbating existing barriers to accessing health services, often at the times when they are most needed. Over 930 million people – around 12% of the world's population – spend at least 10% of their household budget to pay for health care. With the poorest people largely uninsured, health shocks and stresses already currently push around 100 million people into poverty every year, with the impacts of climate change worsening this trend.

The One Health approach offers a holistic strategy to tackle the multifaceted challenges posed by climate change.[3] To effectively implement the One Health approach in the context of climate change, the WHO recommends several strategies which include: (1) Strengthening Surveillance Systems: Enhanced monitoring of health indicators related to climate change

will facilitate early detection of outbreaks and the emergence of new diseases, (2) Promoting Sustainable Practices: Encouraging sustainable agricultural and land-use practices can mitigate environmental degradation and reduce the impacts of climate change on health, (3) Fostering Intersectoral Collaboration: Building partnerships between health, agriculture, and environmental sectors is essential for developing comprehensive policies that address the root causes of health issues linked to climate change, and (4) Enhancing Community Engagement: Empowering communities through education and resources can foster resilience and adaptive capacity, allowing populations to better cope with the health impacts of climate change.[1]

The Quadripartite developed the One Health Joint Plan of Action to drive a coordinated and constructive One Health agenda globally.[3] Recognizing the needs from countries to strengthen the implementation of One Health approaches at national level, the Quadripartite also developed an implementation guide, which provides a step-by-step approach and a general logical framework that countries can readily adapt at the national level. The 4Cs of a One Health approach are coordination, communication, collaboration, and capacity building.

In addition, the integration of One Health into climate action plans can enhance adaptive capacity and improve health outcomes and provides an opportunity for countries to embed nature-based solutions in their One Health strategies and existing mechanisms, maximizing co-benefits for people, animals, plants and ecosystems in the context of a changing climate.

The first-ever Health Day was hosted at the 28th United Nations Climate Conference of the Parties (COP28) in Dubai in 2023, and at COP29 in November 2024 in Baku, the Republic of Azerbaijan, WHO in collaboration with the Wellcome Trust and the health community will host the Health Pavilion to convene the global health community and key multisectoral stakeholders to ensure health and equity are at the centre of climate negotiations.[5] In May 2024, the World Health Assembly approved WHO's 14th General Programme of Work 2025–2028 (GPW14), which prioritizes climate change and health as the first of its six strategic objectives.

In summary, the interlinked nature of climate change and health necessitates a unified approach that embraces the principles of One Health. By prioritizing collaboration and integrating efforts across sectors, it is possible to develop effective strategies that not only mitigate the impacts of climate change on health but also promote a sustainable future for all. The WHO calls for urgent action to embrace the One Health framework, ensuring that health systems are prepared to face the challenges posed by a changing climate while safeguarding human, animal, and environmental health.

[5] https://www.who.int/teams/environment-climate-change-and-health/climate-change-and-health/advocacy-partnerships/talks/health-at-cop29/

CLIMATE CHANGE AND EMERGING INFECTIOUS DISEASES

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Emerging infectious diseases are those infections that have recently appeared and have become or are likely to become more common. The obvious example for this is covid-19. Re-emerging infectious diseases are those that were common in the past then became uncommon and are now returning. A good example of a re-emerging infectious disease is cholera which has seen a global serge in infections over the past few years.

There are several reasons why an infectious disease becomes an emerging or re-emerging infection such as:

1. The infection may have been with us for generations but only recently identified.as a cause of human disease. Examples of these infections include cryptosporidiosis and Helicobacter pylori.
2. New diagnostics have allowed us to diagnose infections that previously were undiagnosable such as hepatitis C.
3. Infections such as legionnaires' disease that are able to take advantage of changes in the human environment.
4. Breakdowns in human societies with failures in basic public health systems such as cholera and polio.
5. Existing human or animal infections that have evolved to become more transmissible or virulent in humans. We see this in influenza pandemics and was the cause of the global spread of Shiga Toxin producing E. coli. Some would include the appearance and spread of antimicrobial resistant organisms in this category.
6. Changes in the distribution of vectors such as dengue fever.
7. The infection may have jumped from an animal into humans and then subsequently spread through human populations. There are multiple examples of this including HIV and most recently covid-19.

The major threats to global human society probably comes from zoonotic infections that make the jump from wildlife species into humans and then subsequently spread.[1]

Climate change may impact on the emergence or re-emergence of infectious diseases in multiple ways. Of the possible mechanisms of emergence listed above, I suspect the main concerns for climate change are changes of the distribution of vectors, increased potential for infections to jump to humans from animal hosts and breakdown of human societies. I will discuss these in more detail.

Those vector-borne diseases associated with the Aedes mosquitos have been a particular interest of mine. Most of our work has been on dengue fever. Predicting the future impact of climate change on vector-borne diseases such as dengue is not easy. There have been very many models published in the literature that have attempted to do just that.[2] We developed our own models based on a Generalized Additive Model (GAM) as this permitted non-linear relationships between outcome and predictor variables. Our initial work was based on surveillance data from Mexico.[3] In one of our first papers we showed that the relationship between climate variables and dengue risk is far from linear. The risk with maximum daily temperature peaked at around 32^{o}C and average daily rainfall of between 550-650 mm. We were then able to draw up estimates of future dengue fever incidence under different climate scenarios

Aedes albopictus one of the vectors of dengue fever is now distributed through much of Southern Europe https://www.ecdc.europa.eu/en/publications-data/aedes-albopictus-current-known-distribution-may-2024 and is spreading northwards. But that does not mean that dengue risk is present just because the mosquito is present. We used our GAM models in an effort to predict future dengue risks in Europe.[4] We found the risks must more narrowly localised than the entomological data alone would suggest. In the ten years since publication of our paper there have been multiple outbreaks of autochthonous dengue all limited to areas where our models highlighted as primary risk.

Another Aedes related infection is the Zikavirus which was associated with a dreadful epidemic of microcephaly in newborn babies in Brazil. We used GAM models to predict future epidemiology of the virus in Southern and Latin America.[5] In our models under climate change the main focus of infection will remain Brazil and Latin America. Since publication countries highlighted in our model continue to report infections but not at the same level as was the case in 2016. Reports of microcephaly have declined substantially as well. However, our main concern was areas not at high risk of infection but could expect to see occasional epidemics. It is not known how long immunity to Zikavirus will last. However, argue that in countries with high transmission risk most women would have their first infection prior to getting pregnant. In such women a re-infection during pregnancy is likely to be very mild and not put the foetus at risk. Women in borderline areas may not have that protection and so the risk of microcephaly could be significantly higher.

I will only touch briefly on increased risk of animal to human transmission associated with climate change. But this is certainly a major concern. In areas where climate change leads to habitat destruction or extreme weather displaces populations and animals, people and wild animals are forced into closer contact. There are numerous examples of where this may have played a major role. Such happenings likely contributed to recent epidemics of Nipah, Ebola, Lassa and mpox viruses.

Finally, I will discuss the issue of Cholera. Cholera is a climate sensitive disease being affected by the El Niño-Southern Oscillation.[6] Cholera is well known to follow major flooding and droughts.[7] Following the 1998 major flooding in Dhaka, Bangladesh, we documented some of the risk factors for cholera both during and after the main floods.[8] Climate change can have significant impacts on cholera risk associated with water, sanitation and hygiene. But this is only part of the story. Throughout history Cholera has been linked with conflict, political instability and mass displacement. Climate change itself is predicted to increase political instability and conflict in future years.[9] Conflict can lead to the destruction of civil and public health infrastructure, disrupts social care networks and displaces people from their homes. This is illustrated in the conceptual model in figure 1, showing the pathways between one type of extreme weather event and cholera incidence and diseases severity.

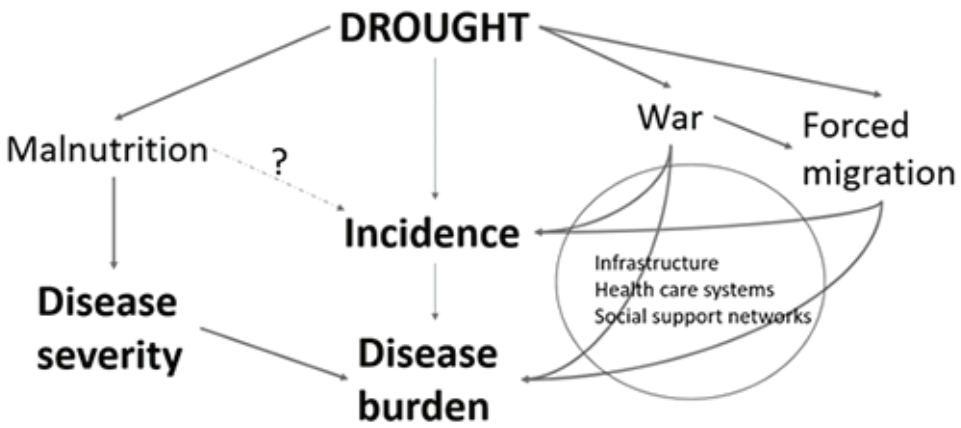

Figure 1. A conceptual model of the impact of drought on cholera

It is the impact of the vicious circle of climate change, drought, famine, malnutrition, conflict, mass migration and societal collapse on cholera and many other infectious diseases that, to my mind, pose one of the biggest threats to humanity over the coming century. This concern reflects not just about cholera, but many other infectious diseases could be affected in similar ways.

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RATIONAL DESIGN OF VACCINES AGAINST ZIKA AND DENGUE VIRUSES

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Antibody-dependent enhancement (ADE) is an important safety concern for vaccine development against dengue virus and its antigenically related Zika virus because vaccine may prime deleterious antibodies to enhance natural infections. In this talk, I would present both published (Nature Immunology, 2021) and unpublished data from our lab regarding to re-design Zika immunogen with elimination of dengue ADE. We adopted a structure-guided antibody-to-antigen strategy to engineered the key epitope targeted by ADE-prone antibodies in fusion loop (FL), but maintained neutralizing epitopes. The new immunogen induced sterilizing immunity and abrogate maternal-fetal transmission of Zika virus in mice, without ADE for all serotypes of dengue viruses. Unlike the wild-type-based vaccine inducing predominately cross-reactive ADE-prone antibodies, B cell profiling revealed that the engineered vaccines switched immunodominance to dispersed patterns without DENV enhancement. The crystal structure of the engineered immunogen showed the dimeric conformation of the envelope protein with disruption of FL epitope. From the unpublished data, we would describe our exciting findings in preclinical study of this FL-engineered Zika vaccine in non-human primate (NHP) model. An undefined pattern of B-cell response is found in NHPs who had received FL-engineered Zika vaccine, which account for the vaccine protection and ADE elimination. This study paved the way for a safe and effective Zika vaccine, and provided immunological insights to flavivirus immunity. I will also introduce our latest progress in the dengue vaccine development.

ELIMINATION OF HEPATITIS IN THAILAND BY THE YEAR 2030

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Hepatitis, particularly Hepatitis B and C, is a global health challenge, leading to chronic liver disease, cirrhosis, liver failure, and liver cancer. The World Health Organization (WHO) has set a goal to eliminate hepatitis as a public health threat by 2030. This initiative aims to reduce new hepatitis infections by 90%, ensure that at least 90% of the population knows their hepatitis status, and provide treatment to at least 80% of infected individuals. Additionally, by 2030, deaths from liver diseases caused by hepatitis should be reduced by at least 65%.

Thailand has adopted this WHO initiative, starting with the elimination of Hepatitis B. The program began in 1988 with the introduction of the universal hepatitis B vaccine for newborns in Chonburi and Chiang Mai as pilot provinces, expanding to 10 more provinces in 1990, and covering the entire country by 1992. The vaccination schedule involved the first dose given within 12 hours after birth, followed by doses at 2 and 6 months. Later, the vaccine was integrated into combination vaccines that also covered diphtheria, pertussis, and tetanus. In 2009, Thailand introduced a separate Hepatitis B vaccine at 1 month for infants born to Hepatitis B carrier mothers, followed by a combined vaccine at 2, 4, and 6 months. Currently, a 5-in-1 combination vaccine is used, covering diphtheria, pertussis, tetanus, hepatitis B, and Haemophilus Influenzae, with coverage rates exceeding 95% in the first year of life.

Additionally, screening for Hepatitis B in pregnant women and providing hepatitis B immunoglobulin (HBIG) to newborns has been implemented. For mothers with high viral loads (HBeAg-positive or viral load exceeding 200,000 IU/ml), antiviral treatment with Tenofovir is provided in the last trimester to reduce viral transmission. These efforts aim to achieve zero mother-to-child transmission of Hepatitis B.

The National Blood Center also implemented blood screening nationwide since 1992, adopting nucleic acid testing (NAT) in 2006. Measures to reduce injection drug use and prevention efforts similar to HIV have further contributed to lowering Hepatitis B infection rates. Recent studies conducted every 10 years (2004, 2014, and 2024) showed a significant decline in Hepatitis B infections, with current rates in children under 10 years old below 0.1% and an overall national infection rate of 1.6%.

For Hepatitis C, Thailand had an anti-HCV prevalence of nearly 2% in the past. However, through measures such as universal blood screening and prevention efforts for people who inject drugs (PWID), the incidence has significantly decreased. Nevertheless, people over 30 years old with chronic Hepatitis C still face risks of cirrhosis and liver cancer. Today, antiviral therapies can effectively cure Hepatitis C.

Phetchabun Province has the highest liver cancer rate in Thailand. While its Hepatitis B prevalence is similar to other regions, the prevalence of Hepatitis C is notably high, particularly among those over 30 years old, with infection rates between 5-10%. In northern Phetchabun, the rate is around 8%. To address this, the Center of Excellence in Clinical Virology, in collaboration with the provincial health office, developed the “Phetchabun Model” for Hepatitis C elimination, initiated in 2017. The program involves screening all residents over 30 years old using a simple strip test (rapid diagnostic test) for anti-HCV, followed by sensitive qualitative HCV RNA confirmation via real-time RT-PCR for positive anti-HCV cases. Infected individuals are then treated. To date, approximately 300,000 people have been screened, and over 5,000 have received treatment.

With these comprehensive measures, Thailand is on track to achieve the WHO and the Ministry of Public Health’s goal of eliminating hepatitis as a public health threat by the year 2030.

PHYTOCHEMICALS OF LIVERWORTS: STRUCTURES, BIOLOGICAL ACTIVITY, AND THEIR APPLICATION TO FOODS AND MEDICINAL DRUGS

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The present paper concerns with the characteristic chemical structures of liverworts, their biological activity and application to foods and medicinal drugs (1-8). Over several hundred new terpenoids, polyketides and aromatic compounds have been isolated from the bryophytes (mosses, liverworts and hornworts) and their stereostructures elucidated. Especially, most of liverworts which contain oil bodies in their cells (*Fig. 1*) elaborate characteristic such as odiferous, pungent and bitter compounds many of which show antimicrobial, antifungal, antiviral, anti-HIV, NO production inhibitory, anti-obesity, anticancer, muscle relaxing, antitrypanosomal, superoxide anion radial release inhibitory, plant growth regulatory, insecticidal, neurotrophic, piscicidal and allergenic contact dermatitis inducing activity. The most significant chemical feature of liverworts is that most of mono-, sesqui- and diterpenoids isolated from liverworts are enantiomers of those found in higher plants.

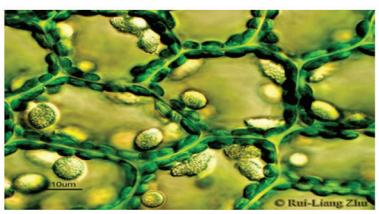
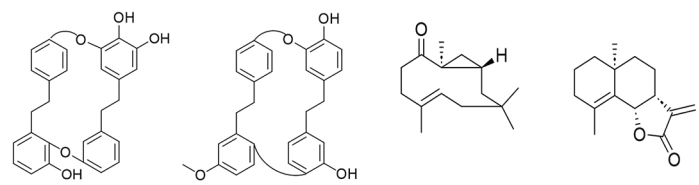


Fig. 1. Oil bodies of *Jungermannia* sp.

Fig. 2. Marchantia polymorpha

It is also noteworthy that different species of the same genera, such as the *Frullania species* produce the same sesquiterpenoid enantiomers. The liverworts, *Blasia, Dumortiera, Marchantia* (*Fig.2*), *Plagiochasma, Plagiochila, Radula, Riccardia,* and *Reboulia* species produce naturally very rare bis-bibenzyls (**1, 2**) which show cytotoxic, anti-micro-, antifungal and antiinfluenzal as well as antimalarial and muscle relaxing activity.



1 (marchantin A) **2** (riccardin A) **3** (bicyclohumulenone) **4** (tamariscol)

The three liverworts, the Greek *Fossombronia angulosa* and Tahitian *Chandonanthus hirtellus* (*Fig. 3*) and the Chilean *Anastrophyllopsis involutifolia* biosynthesize the exactly same sex pheromones as those found in some brown algae. The latter species is chemically very characteristic since it produces the cembrane, fusicoccane and verticillane diterpenoids. The liverworts, *Plagiochila sciophila* and *Frullania tamarisci* subsp. *tamarisci* contain strong mossy and carnation odorous sesquiterpenoids, bicyclohumulenone (**3**) and tamariscol (**4**), respectively. Surprisingly, the *Radula* species, such as the Japanese *R. perrottetii* (*Fig. 4*) and the New Zealand *R. emarginata* produce both psycho and antiinflammatory active perrottetinene (**5**) the structure of which is very similar to that of tetrahydrocannabinoid (**6**) obtained from the higher plant, *Cannabis sativa*.

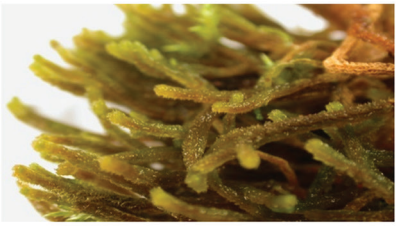


Fig. 3. Chandonanthus hirtellus

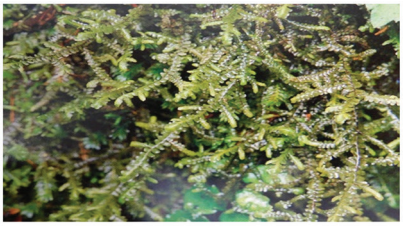
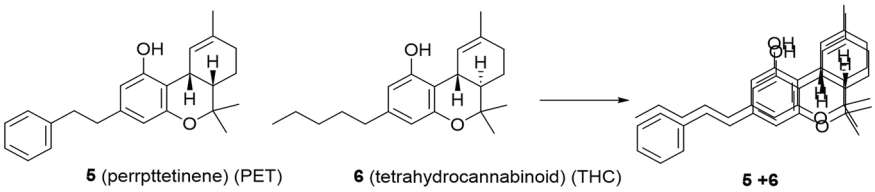


Fig. 4. Radula perrottetii



5 (perrpttetinene) (PET) **6** (tetrahydrocannabinoid) (THC) **5 +6**

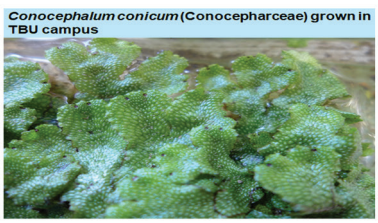


Fig.5. Conocephalum conicum

Fig. 6. Marchantia paleacea subsp. *diptera*

The highly efficient production of mushroomy odorous components from *Conocephalum conicum* (*Fig. 5*) and (S)-(-)-perillaldehyde from *Marchantia paleacea* subsp diptera (*Fig. 6*) will be discussed.

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PHENOTYPIC ASSAYS AND ANALOG SYNTHESIS: EFFECTIVE TOOLS FOR THE DISCOVERY AND DEVELOPMENT OF MARINE NATURAL PRODUCT DRUG LEADS

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Natural products continue to be an important source of chemical inspiration for the development of drugs to treat human diseases. Historically, these inspirational natural products have been isolated from terrestrial plants and microorganisms. Examples include the antibiotics penicillin and vancomycin, the anticancer agents pacitaxel and doxyrubicin, the immunosuppressive drug rapamycin, the antimalarial drug artemisinin, and the cholesterol lowering drug lovastatin. Recently, a small number of drugs inspired by 'marine natural products' have also been approved for clinical use. These include the analgesic Prialt (ziconotide) and the anticancer drugs Yondelis (trabectedin), Halaven (eribulin mesylate), and Adcetris (brentuximab vedotin). There are many more marine natural product inspired drug candidates in various stages of preclinical development and clinical trials.

The world's oceans contain vast biodiversity that remains a relatively unexplored source of natural product chemical inspiration for drug lead discovery and development. Our research group at UBC has focused its efforts on exploring the natural products chemistry of marine sponges and microorganisms as sources of inspiration for drug development in response to important unmet medical needs.[1]

The lecture will describe our discovery of potent broad spectrum 'host directed' antiviral agents against COVID4 and other respiratory infections of serious medical concern and the synthesis and SAR of analogs.[5] It will also describe our discovery of a potent antimitotic sponge natural product hemiasterlin[2,3] that served as inspiration in our laboratory for synthesis of an analog HTI-286[6,7,8,9] currently used as a cytotoxic warhead in an antibody drug conjugate approved for orphan drug status by the USA FDA for treatment of pediatric Acute Myeloid Leukemia-RAM phenotype.[10]

Development of a natural product discovery into a viable clinical trials candidate involves overcoming many hurdles that are common to all drug development projects whether or not the inspirational starting point is a natural product or a completely synthetic combinatorial chemistry compound. These include: 1) addressing an important unmet medical need, 2) hitting a new or well validated cellular target, 3) demonstrated ability to bind and modulate the molecular target in a cellular environment with a suitable effectiveness to toxicity ratio, 4) a commercially viable supply of the compound, 5) strong intellectual property, 6) good chemical/metabolic stability and oral bioavailability, and 7) effectiveness in an animal model of the disease. Our marine natural products research at UBC is designed to address these requirements as much as possible at the early discovery stages to give our inspirational compounds the best possible chance to generate high quality clinical trials candidates. We address most of these development issues by using a combination of phenotypic cell-based assays for bioactivity screening and hit compound analog synthesis.[1]

The lecture will illustrate the advantages of using phenotypic assays[1,2,3,4] and analog synthesis[5,6,7,8] to generate promising natural product drug leads,[9,10] and approved drugs.

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DISCOVERY OF ANTIVIRAL NATURAL PRODUCTS BASED ON NATIVE MASS SPECTROMETRY AND MOLECULAR NETWORKING

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Mass spectrometry (MS), known for its high-resolution and high sensitivity, is a powerful tool for identifying and analyzing natural products. MS analysis enables researchers to determine the composition and structure of compounds in complex mixtures, facilitating the rapid screening and validation of target components with potential medicinal value. Natural products play a crucial role as sources for the development of antiviral drugs. This study focuses on the application of high-resolution mass spectrometry in the discovery of antiviral natural products. Employing two techniques, native mass spectrometry (native MS) and molecular networking (MN), the study explores efficient methods for the discovery of target compounds.

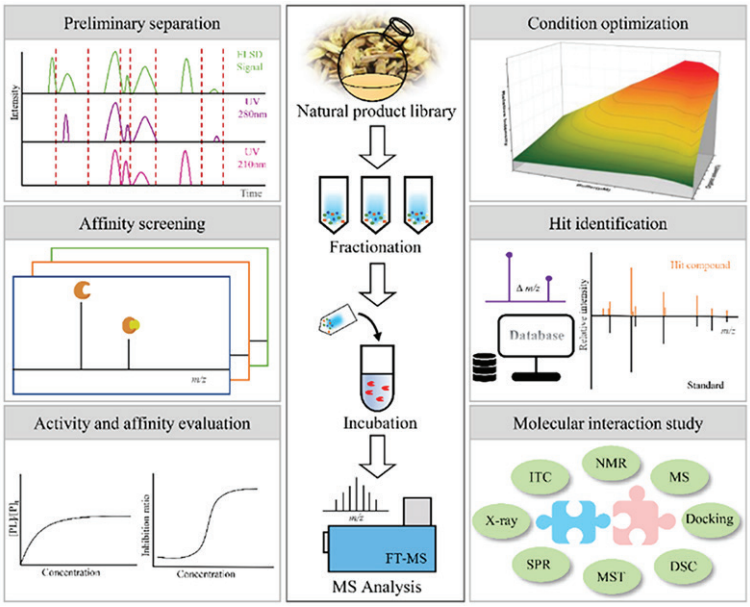

Figure 1. Application flow of native MS in natural product research

First, a native MS affinity screening method for SARS-CoV-2 3CLpro is established. Through screening six herbal extracts, 16 compounds capable of binding to 3CLpro are quickly identified. Subsequent MS analysis further explores the relationship between the activity and affinity of these compounds, testing their biological activity. Results show that baicalein, scutellarein, and ganhuangenin exhibit significant inhibitory effects on SARS-CoV-2 3CLpro, with IC_{50} values of 1.14, 3.04, and 0.84 μM, and K_d values of 1.43, 3.85, and 1.09 μM, respectively. Native MS, utilizing soft ionization techniques, can transfer non-covalent weakly bound biomolecular complexes intact to the gas phase under conditions close to physiological pH and mild desolvation. This facilitates a deeper understanding of interactions between biomolecules in complex mixtures. The method enables the rapid screening of compounds capable of binding to target proteins from complex mixtures. Moreover, the quantification of complexes and the strength of compound-protein binding can be directly observed in the mass spectrum. This offers a new approach to studying the interaction between small-molecule drugs and large biomolecules, providing important clues for discovering active components in natural products and drug development.

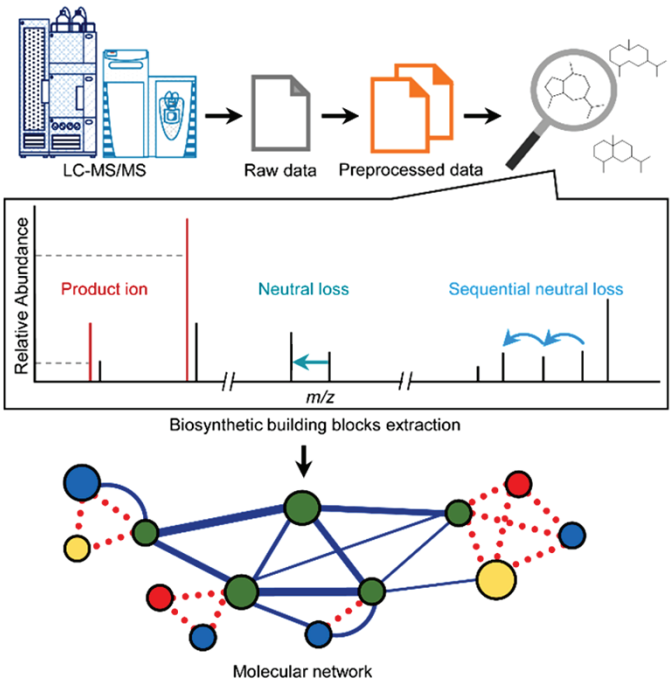

Figure 2. Schematic workflow of the application of BBE

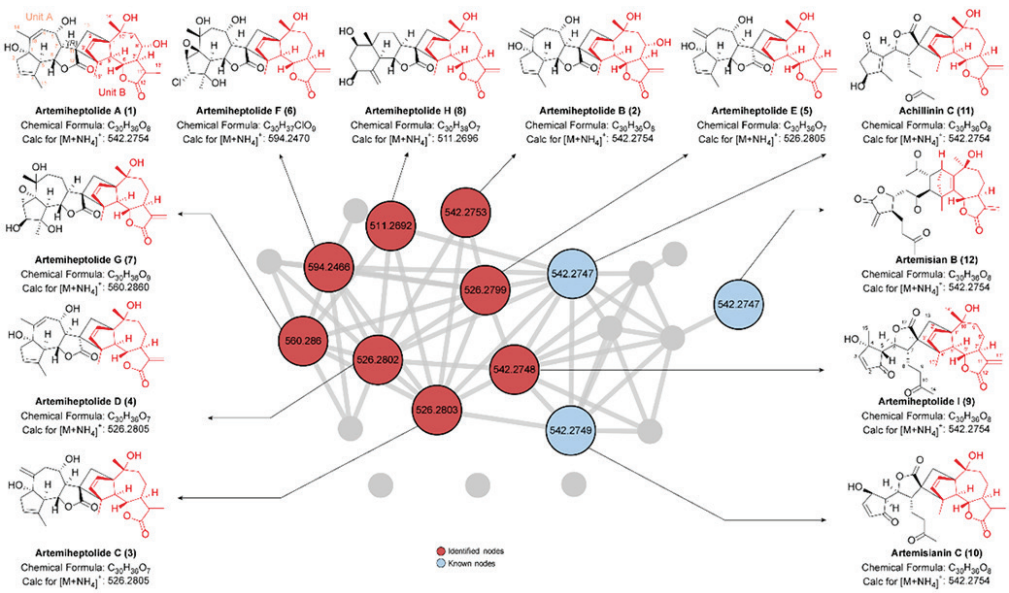

Figure 3. Building blocks-based molecular network of the spectra with the extracted features

Second, a program named “Building Block Extractor (BBE)” is developed, accompanied by a user-friendly web interface. This tool not only categorizes product ions and neutral losses as building blocks but also innovatively integrates the abundance of product ions and sequential neutral losses as features within building blocks. With this strategy, compounds with MS fragmentation patterns can be efficiently screened from MS/MS data, enabling the rapid discovery of target natural products. This method is applied in the chemoinformatic analysis of Artemisia heptapotamica, a plant from Kazakhstan. Visualizations through molecular networking (MN) are employed. By targeting features of dimeric sesquiterpene lactones, 12 dimeric

sesquiterpenes, including 9 novel compounds, are successfully separated and identified. The dimeric sesquiterpene artemiheptolide I exhibits an IC_{50} of 8.01 ± 6.19 μM against Influenza A/Hongkong/8/68 (H3N2). Additionally, two known monomeric sesquiterpenes 3α-chloro-4β,10α-dihydroxy-1β,2β-epoxy-5α,7αH-guai-11(13)-en-12,6α-olide and 3β-chloro-4α,10α-dihydroxy-1α,2α-epoxy-5α,7αH-guaia-11(13)-en-12,6α-olide, exhibit significant antiviral activity against Influenza A/Puerto Rico/8/1934 (H1N1), H3N2, and Influenza B/Lee/40, with IC_{50} values ranging from 3.46 to 11.77 μM, comparable to the positive control oseltamivir. This method expands the annotation capabilities of LC-MS analysis and is significant for the comprehensive discovery and tracking of natural product analogs with exceptional activity, as well as the detection of derivatives with specific fragments.

Natural products serve as a treasure trove for drug discovery, playing a crucial role in addressing the growing global challenges posed by antiviral diseases. As the threats from viral diseases such as influenza, Ebola, and COVID-19 continue to escalate, efficient screening and identification methods based on mass spectrometry have become key to accelerating new drug development. These technologies not only significantly enhance the speed of discovering natural products with antiviral activity but also effectively reduce development costs, providing strong support in the fight against viral diseases.

NATURAL PRODUCT BIOSYNTHESIS AND ASSOCIATED MOLECULAR INNOVATION

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Nature utilizes simple substrates, e.g., short carboxylic acids, amino acids and sugars, to prepare various building blocks; and enzymatic polymerization, combination and permutation of these monomers, in many cases followed by various post-modifications, eventually lead to the generation of diverse natural products (NPs) such as polyketides, peptides, terpenoids, alkaloids, and their hybrids in living organisms (**Fig. 1**). These chemical entities exhibit an extremely wide range of biological activities, which underlie the critical roles of NPs in both the history and context of medicinal chemistry and chemical biology as drugs, biological tools and synthetic targets. Of the chemotherapeutical agents that have been clinically approved, particularly those with anti-infection, antitumor and immunosuppressive activities, approximately 50% are NPs and their semisynthetic derivatives or are chemically synthesized but inspired by the pharmaceutically important moieties of NPs. Many of these small molecules are capable of specifically targeting bio-macromolecules in cellular networks, including proteins and nucleic acids, thereby enabling strategies using chemical probes to exquisitely control and examine life phenomena in biological systems. The generation of NP-like libraries is one of the major areas of current interest in diversity-oriented synthesis, which has emerged in aid of searching biologically active small molecules based on the expansion of both their chemical and functional spaces. However, advances in chemical synthesis may not always keep pace with the high-throughput screening (HTS) technique, largely due to the tremendous challenge posed by the structural complexity of NPs.

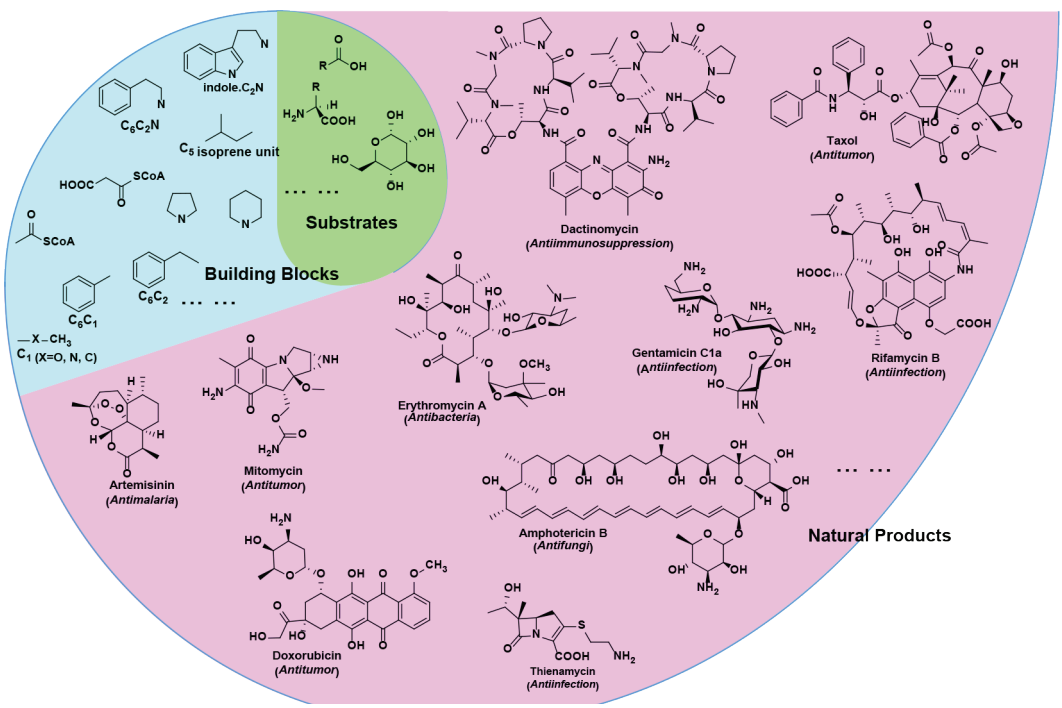

Fig. 1. Substrates, building blocks and representative NPs
(from: Chen, M.; Liu, J.; Duan, P.; Li, M.; Liu, W. Nat. Sci. Rev. **2017**, *4*, 553).

What we should always keep in mind is that NPs do not exist initially for the purposeful use of human beings. In fact, the living organisms produce them, usually as secondary metabolites, to modulate or participate in numerous internal biochemical processes and to fight against external environmental concerns, such as signal transduction, competition and adaptation. These biological processes are often dynamic, and could feed back to the related biosynthetic machineries for the diversification, optimization and, ultimately, selection of suitable active small-molecules to combat the associated biochemical or environmental changes. Consequently, interactions between the metabolites of living organisms and the macromolecular targets that mediate their biological functions are considered an intrinsic engine that drives the co-evolution of these metabolites and their targets in nature. It now seems that NP evolution occurs over a continuous spectrum spanning millennia, and the imaginable permutations or mutations of prototypes (e.g., biosynthetic pathways, biochemical reactions, catalytic enzymes and encoding genes) are manifested to some extent to create NPs that possess a multitude of biological functions and display an unimaginable diversity of molecular architectures. This evolution has a constant theme, with the generality that typically accounts for the relevance of NPs in biosynthesis and structure and with the specificity for unique members that can be individually recognized in nature.

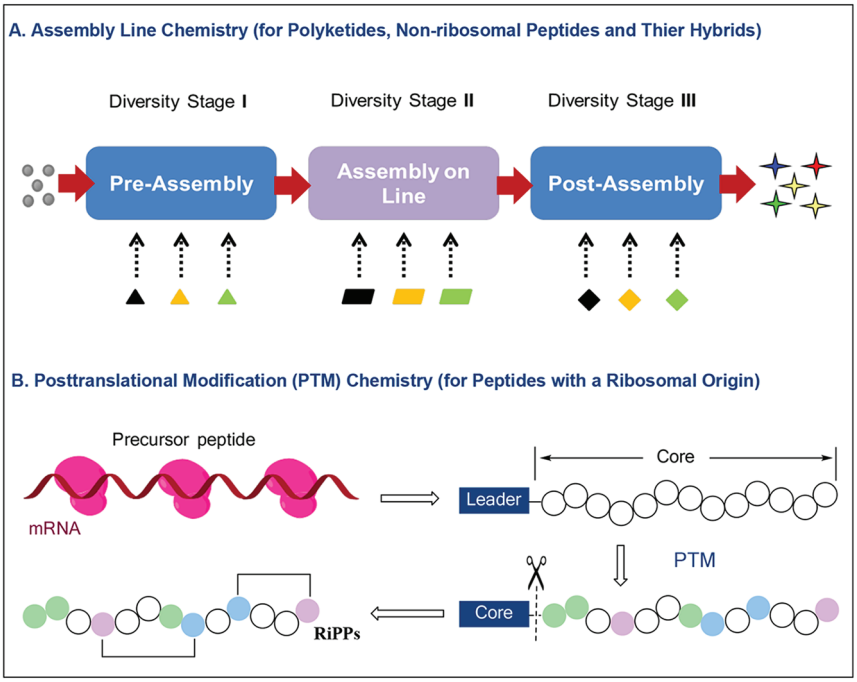

Fig. 2. Biosynthesis of natural products with a templated logic

With a focus on templated bacterial NPs, this talk examines the recent progress of biosynthesis by exemplifying the conceptual and technological leaps. In contrast to oligosaccharides, isoprenoids and other NPs that are produced by non-templated pathways, the target products described here typically include polyketides and peptides (either ribosomally synthesized or non-ribosomally synthesized), both of which share a “template”-biosynthetic logic that proceeds through the entire process for molecular assembly and modifications (**Fig. 2**). It should be noted that we do not intend to provide a comprehensive review of the biosynthesis of these NPs, as the incredible and rapidly increasing volume of research that has been completed precludes such an effort. Instead, we report on the examples in which we are involved or with which we are most familiar, to highlight the practices of what we have learned from nature, how we recognize the generality and specificity in NP biosynthesis, and following the natural theme in the development of biologically active small molecules, whether we are able to accelerate the NP-diversification process to advance the expansion of their molecular utility.

EFFICIENT SYNTHESIS OF BIOACTIVE STEROID AND TERPENOID NATURAL PRODUCTS

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Steroids have a wide variety of biological activities, including anti-inflammatory, antishock, immunosuppressive, stressresponse-enhancing, and antifertility activities, and steroid research has made great contributions to drug discovery and development. According to a chart compiled by the Njardarson group at the University of Arizona, 15 of the top 200 small-molecule drugs (by retail sales in 2023) are steroid-related compounds (Figure 1).[1] Therefore, synthetic and medicinal chemists have long pursued the chemical synthesis of steroid natural products (SNPs) with diverse architectures, and vital progress has been achieved.

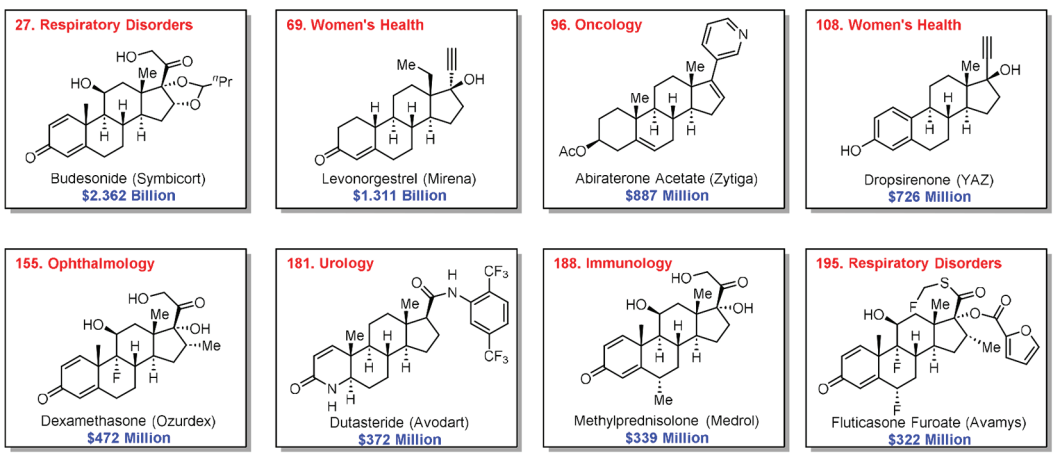

Figure 1. Selected examples of steroid drugs in the top 200 small-molecule drugs in 2023.

Despite the incredible advances of steroid chemistry in the past century, the efficient and scalable preparation of bioactive SNPs still presents a formidable challenge to the scientific community due to the high structural complexity. Our group has a long-standing interest in the efficient synthesis of bioactive steroid and related terpenoid natural products,[2] and we aim to develop creative, concise, and scalable routes to these compounds to facilitate an in-depth investigation of their biological activities. To increase the synthetic efficiency, we draw inspiration from proposed biogenetic pathways and seek to use inexpensive, readily available starting materials. We find that bioinspired skeletal reorganization via cationic rearrangements or radical relay cyclization is an efficient strategy for synthesizing challenging SNPs.[2] Using this strategy, we have achieved concise syntheses of several different kinds of SNPs (cyclocitrinols,[3] propindilactone G,[4] bufospirostenin A,[5] pinnigorgiol B,[6] and aspersteroids[7]) with considerably rearranged skeletons from abundant, inexpensive and commercially available starting materials (Figure 2).

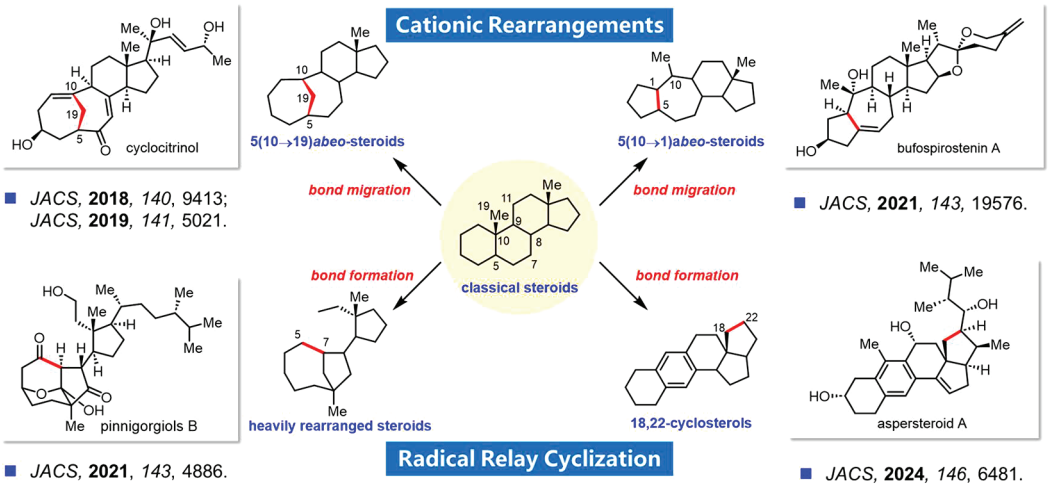

Figure 2. Bioinspired skeletal reorganization approach to steroid natural products.

Our synthetic endeavors provide facile access to a wide range of steroid natural products and congeners with diverse polycyclic scaffolds, which facilitate their biological evaluation. In collaboration with medicinal chemists, we found that these compounds exhibit some interesting anti-cancer, anti-infective, neurological activities, etc. Currently we are working on structure-activity relationship studies for lead compound identification.

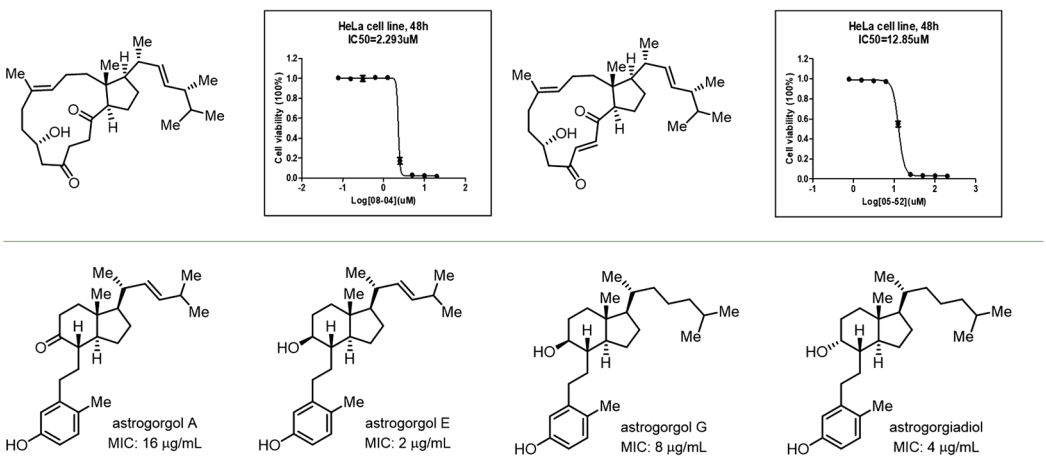

Figure 3. Biological evaluation of steroid natural products and congeners.

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ONE HEALTH - AN ASPECT OF THE FOOD, MEDICINES, AND ENVIRONMENT CONUNDRUM FOR 2040 AND BEYOND

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A Healthy People require a Healthy Earth. The inexorable interdependence of humankind on all of nature has been recognized since at least the 12th century. Natural resources remain a fundamental presence in the healers' armamentarium globally. However, dramatic population increases, degradation of biodiversity, environmental change, globalization of food production, and a variety of economic factors, threaten the accessibility to those medicinal and nutritive resources. In addition, as new and known acute and chronic diseases impact lifestyles due to dynamic environmental modulation, with no treatments available, creating healthier populations, resolving existing healthcare gaps, and re-establishing our holistic interconnectedness with all things natural, plant, animal, and microbial are within the integrated human-animal-environmental approach which "One Health" begins to embrace.

Since "One Health" emerged as a pathway in 2004, the world has transformed very rapidly in terms of climate and environmental issues, biodiversity losses, the depletion of fossil-based resources, and the enhanced levels of atmospheric carbon dioxide and methane. A single global disease, SARS-Covid-2, has killed over 7 million people, and over 700 million are currently diagnosed. Stunningly, it shut down meaningful lifestyles for extended time periods and devastated local economies to a greater or lesser extent. It highlighted how rapidly new, unforeseen, untreatable, lethal zoonotic diseases can be spread through globalization, and the vulnerability of our lives.

In May 2021, the Food and Agriculture Organization (FAO), the World Organization for Animal Health (OIE), the United Nations Environment Programme (UNEP), and the World Health Organization established a "One Health High-Level Expert Panel" (OHHLEP) of 26 global representatives. In June 2024 OHHLEP provided a new definition of Open Health, with the intention to create "policies and concrete actions". The partial definition is that "One Health is an integrated, unifying approach that aims to sustainably balance and optimize the health of people, animals and ecosystems." It continues "The approach mobilizes multiple sectors …….to work together to foster well-being and tackle threats to ecosystems, while addressing the collective need for healthy food, water, energy, and air, taking action on climate change and contributing to sustainable development." The definition is intended to activate four principal areas, Communication, Coordination, Collaboration, and Capacity building. Initiatives are based on the principles of equity, inclusivity, equal access, parity, socioecological equilibrium, stewardship. and transdisciplinarity and aims to be comprehensive (*1*).

The author has written extensively about the sustainability of medicinal resources, natural and synthetic, since 1992 (*2*). Several new terms have been introduced in the intervening years, among them ecopharmacognosy, cyberecoethnopharmacolomics (a fully integrated approach to the study and practical development of natural products), Q.S.E.C.A., and medicines security, as well as L.O.V.E. and other action-based acronyms in caring for the Earth and its future, particularly in relation to the Fifth Industrial Revolution and defossilization post-COP28 (*3*). Here, emphasis will be placed on integrating these aspects of the natural product sciences, thereby adding to the concept of "One Health" for 2040 and beyond. It is our moral responsibility to raise these alarms, "not for us, but for our descendants", as the global population expands, and the impacts of fossil fuel use take Earth closer to numerous global tipping points.

The primary focus of this conference is examining the challenges faced by "One Health". Enhancing health necessitates, among many other factors, sustainable and secure access to safe, effective medicines. This presentation will bring focus to considerations for the origins of future "medicines", the agents for prevention and healing, for humans, for animals, and for Earth for the duration of humanity. What will be discussed is no longer about choice, those options have dissipated. This is about urgent actions. Fossil fuel use is increasing leading to unimaginable outcomes, especially for the world's poorest and for nature in all its surviving diversity.

A critical challenge to discussions and actions at the government level in some countries is the inability to accept that Earth's resources are finite, and that unlimited economic growth is "a perilous illusion" (*4*). A myth supports this ill-informed and stubborn resistance to the Anthropogenic Era and inhibits action plan development. It is the presumption of the perpetual chemical, biological, and environmental availability of Earth and its assets, and that utilizing those assets has not caused irreparable damage (climate denial). This dangerous non-acceptance of reality, even after hundreds of conferences, thousands of reports, and endless "warnings" from gaggles of front-line scientists, undermines and profoundly challenges the calls within "One Health" to balance the use of natural materials with a transformed environment.

Five overarching global considerations impinge on the human-plant-environmental intentions of "One Health" as 2040 and beyond looms ominously: Population Expansion, Sustainability, Environmental Change, Defossilization, and Time. Integrating responses, through creative, scientifically and technologically holistic and sustainable approaches, enhancing diverse societal natural product practices with respect to their safety, efficacy, and consistency are fundamental to maintaining and balancing the basic parameters of human and animal health.

Major, very challenging, urgent factors are yet to be integrated into the "One Health" concept. They include Curtailment, Continuation, Consistency, Commitment, Creativity, Cost, and Care. Following the COP28 meeting commitment to "transition" from fossil fuels, defossilization will be a major factor as the world transitions in the Fifth Industrial Revolution. Barriers to "One Health" have been described (*5*) and include communication, integration of ecology in medical education, emphasizing translational research, promoting integrated research alliances, enhancing biosecurity, and factoring the economics of prevention versus treating outcomes.

Communication. Poor communication between human and veterinary medicine physicians, environmental scientists, chemists, biologists, agricultural and evolutionary scientists, and economists is an example. Network development and cooperation between the respective "disciplines" needs to be enhanced based on i) national, international, corporate, and foundation funding priorities, ii) University administrations supporting complex collaborative research programs with other institutions, industry, international agencies, and governments, and iii) a focus on translational research, rapid communication of results, and response implementation. Eliminating the barriers between human and veterinary medicine and the persistent ecological and agronomic issues for medicines, natural and synthetic (one aspect of medicines security), and for food security is paramount. Artificial scientific boundaries of research and industrial animal husbandry practices are enhancing multidrug resistance in both animals and humans. Thus, initiating programs which can span increasing food safety, reduce harmful vector transmission, and develop renewable, biodegradable antibiotics and pesticides for global use are necessary to meet human, animal, and environmental needs. These are not short term or even long term necessities. In parallel with the concepts and actions of sustainable economies and regeneration, these are now the core needs for the actual survival of humanity.

Global information access is a major communications issue, especially regarding past and current research, patents, and public versus commercial database systems for various data sets. Many countries, universities, and individuals cannot afford to purchase extensive information access, and is a reason why countries are resisting exorbitant publication fees, and examining alternatives. For implementation, "One Health" should develop strategies in support of parity for scientific information access.

"One Health" requires a commitment to biosecurity, reducing the prevalence of communicable and non-communicable diseases, monitoring the control of vector-borne disease transmission, and establishing reliable communications concerning impending wider risks. Systems for accumulation of incidence emergence data, local health and sanitary conditions, related climate and environmental conditions, and associated risk factors need to be functional and consolidated. Balancing food production, local and national health, and ecological and biodiversity conditions is needed to support a significantly greater reliance on plant-based protein sources.

Trends of the global warming of the air and the seas, the incrases in carbon dioxide and methane emissions, and reliable information on the impacts of non-renewable fossil fuels are essential base-line data for national and international action plans. In 2023, Earth was at, or very near, 25 of 35 "planetary vital signs", leading to the conclusion that: "We are on the brink of an irreversible climate catastrophe" (*4*); a shocking, yet realistic conclusion. The surface temperatures of the oceans were the highest ever in 2024 and are increasing. Humanity is probably too late to act in many instances. Polar warming and the rising sea levels, permafrost and landfill gas emissions of methane, ecosystem changes, and ocean and atmospheric warming, for example, are beyond human reach to consider restoration for our descendants. There is no "reverse the warming" gear to mitigate these trends. Ethically, effective responses cannot be created based on concealed or misleading data. Economic investments demand unbiased, accurate, and timely information, and must occur before supplies "runout" and Earth is beyond the tipping points. Estimates are needed for when the costs to produce, or the costs to buy, a commodity will become out of reach, at any level in society. There are a bevy of inconvenient truths for the survival of humanity which require immediate responses and lifestyle modifications. A period of climate upheaval is unfolding which humanity has never been witnessed, ever (*4*).

Persistent challenges for the concept of "One Health" are manyfold and span ecological, environmental, and human and animal health care systems locally and globally. Soil health and its stability during environmental change (flood, drought, wildfires, sea water intrusion, atmospheric pollution, etc.) can have a profound impact on what can be grown and its growth (i.e., biomass) and metabolite profiles. Contemporary concerns include the climate-induced decreased production of coffee, grapes, olives, and cocoa, and the economic impact of lost or disrupted supply chains. Major rice and cereal crops are under constant threat from climate sensitivities. Additional pertinent and evolving challenges for "One Health" include Curtailment, Continuation, Consistency, Commitment, Creativity, Cost, and Care.

Curtailment. Eliminating the concept of "waste"; introducing the practices of degrowth, and reducing overconsumption through accepting "old", "used", and "restored", rather than consistently demanding "new" from non-renewable resources; reducing fossil fuel use for energy, plastics, chemicals, etc.; transitioning to plant-based protein sources; replacing synthetic pesticides, herbicides, and antibiotics with sustainable options.

Continuation. Lifestyles that continuously deplete the assets of the planet are fundamentally unsustainable. Promoting a more rapid transition to alternative fuel use, monitoring climate change impacts, environmental degradation, and biodiversity transformations, enhancing food and medicines security, and emphasizing safe water quality and equitable global access are vital. Areas growing global crops and medicinal plants require assessment to secure the requirements for the food and biological and medicinal agents for least 9.5 billion people in 2050. Major implications exist for synthetic and natural products as non-renewable resources dissipate, and the towering importance of a bio-based, circular economy evolves.

Consistency. Lifestyles will be changing, while basic human and animal needs for quality foods and safe and effective medicines will remain (indeed expand). Consistent supply chains and applications of blockchain technology to provide traceable quality control are imperative. For all medicines, only the elimination of fake, adulterated, and contaminated products can assure consistency for patients.

Plant "health" also pertains to the biosynthesis of desired metabolites in a food or medicinal plant and to maintaining those attributes under diverse environmental conditions. For all plants of commercial interest, bioresponsible fertilizers, pesticides, and herbicides are an essential, ethical component for the consistent agricultural production of foods, medicines, and for animal life.

Commitment. Respective government ministries, foundations, and global institutions must be dedicated, philosophically, ethically, and economically, to investing in achieving sustainable supplies of safe foods and effective medicines; to reducing "waste" in food production, and potentiating the transformation of "waste' resources towards new, biorelevant applications; for assuring that natural and synthetic medicines are sustainable beyond the present generations; and developing a third category of safe, effective, consistent, and affordable sustainable medicines that are globally accessible. These are exciting times for innovative natural product research!

Creativity. It is said that creativity occurs at the junction of organization and chaos. There is no higher calling at the present than creating and implementing a sustainable planetary future for humans, animals, and the environment. Many areas where new pathways must originate have been cited herein, including new replacement medicinal agents for human and animal health, the quality control and accessibility of medicines, regeneration and replenishment strategies, and enhanced global accessibility.

Cost. Comprehensive, yet inevitable, societal lifestyle change over the next 20-30 years and the evolution of relationships of humans with nature, establishing a peace and not waging war, will be extremely expensive. Investment now is the initial cost for the survival of our descendants. The medicines of 2040 and beyond require investment to create the replacement drugs and address neglected needs as fossil fuel use "transitions" and evolution into sustainable biological agents for human and veterinary use expands. Affordability for patients/consumers must be enhanced.

Care. Developing transnational multidisciplinary teams that respect cultures, comprise diverse scientific and other areas of expertise, and can function effectively requires the trusted, care-filled leadership of patience, sharing, sorting ideas, prioritizing, and acting. Failure to act with mindful care will defeat the "One Health" mission and beyond.

Defossilization. The transition from, and eventual elimination of, non-renewable fossil fuels is an irrefutable element for human, animal, and environmental survival. A visionary framework for that era is needed, even though there is no timetable. For chemists, access to routine solvents and chemicals, and numerous other bulk chemical supply chains, will diminish in the next 20 years. Creative strategies and actions to unearth, literally and figuratively, sustainable medicinal agents are needed; acknowledging also the timeframe (10-15 years) for biological agent approval.

Ethical Challenges. In silico modeling and applications of AI and ML enhances the selection of organisms and compounds for new product development and minimizes biodiversity encroachment. Reality in the translation of environmental impact data to decision makers is essential. Minimizing profits to promote a sustainable, regenerative, and economically responsible society for environmental health is evolving. Sensitivity to the customs and mores of the local communities and the animal and biodiverse environments, including the toxic aspects, requires consultation, community collaboration, and negotiation (CBD and Nagoya Protocol) before developing specific plans and initiatives locally and nationally.

Animals and humans rely totally on plants, directly or indirectly, as their sole food source. Monitoring the health of the plants and the animals in their local environment (water, salinity, microbial contamination, and metal contamination) also relates to proximity to toxic plants. Sharing this knowledge is important as a health and economic issue. Exo- and endophytic microorganisms associated with plants may also cause nosocomial diseases or be present as enteric organisms resulting in the contamination of human and animal food supplies.

In summary, and as discussed by the author in 2011, “Plants are the Key to Global Health” (6). Chief Seathl wisely observed the importance of the state of the Earth that we leave for our descendants. King Charles III refocused this aphorism indicating “In 2050 our grandchildren won’t be asking what we said, they will be living with the consequences of what we did or didn’t do.” Will they also be very angry at us for what they must now deal with? What will our legacy be? Dithering or action? It must result from urgent actions, no more talk. “One Health” is a step on a transformational journey requiring unprecedented investment and creative initiatives to prioritize humanity, the fauna and flora of Earth, and the preservation, replenishment, and regeneration of the environment. It will require L.O.V.E., Learning to Optimize the Valuables of Earth, for a mutually healthy and sustainable future to support the lives of those who will follow.

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THALASSEMIA: A MODEL OF GENETIC DISEASE

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Thalassemia and abnormal hemoglobin (Hb) are the most common genetic disorders worldwide. Thalassemia is an autosomal recessive genetic disorder. All thalassemia carriers are asymptomatic (without anemia, jaundice or splenomegaly). Clinically, thalassemia syndromes are classified into three categories: thalassemia minor, thalassemia intermedia (non-transfusion-dependent thalassemia, NTDT) and thalassemia major (transfusion-dependent thalassemia, TDT).

The most common abnormal genes in Asia are alpha-thalassemia, beta-thalassemia, Hb E, and Hb Constant Spring (CS). These genes, in various combinations, result in more than 60 different thalassemia syndromes, making Asia the region with the most complex thalassemia genotypes. The complex gene-gene interactions between alpha- and beta-thalassemia lead to various thalassemia disorders, such as homozygous beta-thalassemia, beta-thalassemia/Hb E (beta-thal/Hb E), Hb H disease and Hb Bart's hydrops fetalis. It is estimated that the incidence of conception for homozygous beta-thalassemia and beta-thal/Hb E in Asia is 0.24 and 0.25 per 1000 birth, respectively. The pathophysiology of beta-thalassemia is mainly due to accumulation of excess unmatched alpha-globin, which precipitates in erythroid progenitor cells, leading to cell death, ineffective erythropoiesis, and severe anemia.

Beta-thalassemia/Hb E is the common thalassemic disease in Southeast Asia. Clinically, patients exhibit remarkable heterogeneity, varying from nearly asymptomatic to severely transfusion-dependent. Most patients manifest symptoms within the the first 10 years of life and survive without transfusions or with only occasional transfusions. Patients may come to the hospital due to anemia, abdominal masses, jaundice, infection, hypoxemia, pulmonary hypertension, endocrine and trace metal disturbances. In a recent study, GI disturbances were the most common presenting symptoms (34.6 %) leading patients to seek medical attention. Respiratory tract infections were found in 21.8 % of cases, and cardiovascular complications, including CHF were present in 11.9 % of cases. An abnormal glucose tolerance test was observed in 48 % of beta-thal/Hb E cases, even when fasting blood sugar levels were normal. The pathogenesis of pulmonary hypertension in thalassemia is complex, involving hypercoagulaion, NO formation, and other cytokines (such as endothelin-1 and placental growth factor) that affect vascular tone. Other complications include bone pain, chronic leg ulcers, paraplegia, hypertension-convulsion and cerebral hemorrhage (HCC) syndrome following multiple blood transfusions. Splenectomy is performed in about 25 % of cases. Approximately 67 % of patients die between the ages of 20 and 40 yrs, primarily from heart failure and septicemia, with septicemia usually resulting from Gram-negative bacterial infections. The clinical features of beta-thal/Hb E result from chronic anemia and iron overload. Studying the natural history and clinical course of the disease is essential to provide optimal management for these patients.

Hemoglobin levels in beta-thal/Hb E range from 3 to 12 g/dl, with an average level of 7 g/dl. The severity of anemia in beta-thal/Hb E reflects the degree of alpha- to non-alpha-globin chain imbalance and the excess of unmatched alpha-globin chains. Thus, any factors that reduce the degree of globin chain imbalance or the size of the free alpha-globin chain pool could moderate the clinical features of patients with beta-thal/Hb E disease. The primary modifying factor is the nature of the β-thalassemia mutation. The presence of a beta^{+}-thalassemia allele, which allows for some beta-globin chain production, results in a milder disease. In such cases, hemoglobin levels in beta-thal/Hb E range between 9 and 11 g/dl, typically not requiring any blood transfusions. Additionally, there is evidence that the amount of alternative spliced

beta^{E}-globin mRNA may influence the variability in the severity of beta-thal/Hb E disease. Co-inheritance of α-thalassemia leads to fewer excess alpha-globin chains and tends to result in less severe symptoms. The degree of amelioration depends on the number of functional alpha-globin genes; a defect in a single alpha-globin gene is sufficient to improve the clinical phenotype of beta-thal/Hb E patients. Conversely, co-inheritance of triplicated alpha-globin genes (ααα) may cause increased globin chain imbalance and severe anemia. Moreover, the role of increased fetal hemoglobin (Hb F; alpha$_2$gamma$_2$) as an ameliorating factor of beta-thalassemia becomes more evident. Its effect is mediated by reducing the degree of imbalance in globin chain synthesis. The Hb F level in beta-thal/Hb E varies widely, ranging from 5 % to 76 %, and is associated with disease severity.

Genetic linkage analysis and genome-wide association studies (GWAS) have identified susceptibility loci for the persistence of Hb F into adulthood or *trans*-acting quantitative trait loci (QTLs) controlling Hb F levels, including (i) the β-like globin cluster (11p locus), (ii) the HBS1L, MYB and HBS1L-MYB intergenic region (6q locus), and (iii) BCL11A (2p locus). Each of these genetic determinants can exhibit considerable heterogeneity, with allele frequencies varying widely among different populations. Consequently, the various interactions of these modifiers may lead to considerable variability in Hb F levels and clinical diversity among beta-thal/Hb E patients. This requires further confirmation in beta-thal/Hb E E cohorts from different ethnic populations. A better understanding of how genetic factors contribute to heterogeneity of beta-thal/Hb E disease may lead to new therapeutic approaches for patients.

The mainstay of treatment for thalassemia major is regular blood transfusion to maintain adequate hemoglobin levels. Iron overload in beta-thalassemia patients occurs due to multiple blood transfusions, increased iron absorption, or a combination of both. Iron chelation therapy, using agents such as desferrioxamine, or the oral iron chelators deferiprone and deferaxirox, is indicated for patients with severe iron overload. Iron overload can be assessed by measuring serum ferritin levels and using MRI techniques. Without iron chelation, death from iron-induced heart failure occurs by the mid-teenage years. The only cure for thalassemia is stem cell transplantation, while gene therapy is available only in some countries.

Laboratory diagnosis of thalassemia is conducted after CBC using automatic hemoglobin analysis techniques, such as HPLC and capillary zone electrophoresis, which were introduced in 1991 and 2000, respectively. With a better understanding of the molecular defects and improvements in laboratory technology using automated machines, a new era of thalassemia prevention and control of thalassemia has emerged in many countries. This includes screening high-risk couples, providing genetic counseling, performing prenatal diagnoses, and selectively aborting severe cases.

Future development

With advances in molecular biology, it is now possible to cure thalassemia through gene therapy. Following gene insertion, genome editing techniques, such as the CRISPR/Cas system, may be used to precisely correct the point mutation. Additionally, molecular techniques may be developed to enhance gamma globin gene production. Novel medicines, such as Luspatercept and other agents involved in abnormal erythropoiesis, are currently under development and evaluation. Progress in stem cell biology also offers hope that, in the future, red blood cells could be developed by biotechnology companies, potentially serving as universal donors without the need for blood grouping or matching.

Laboratory diagnosis of thalassemia may shift from traditional methods like CBC and Hb typing to molecular screening for abnormal gene in the population using next-generation sequencing (NGS) technology. Additionally, this “non-hypothesis-driven” approach in the genomic era may uncover genes that have been overlooked due to assumptions based on current understanding of biology, potentially identifying disease susceptibility or protective alleles in patients with varying degrees of severity. Prenatal diagnosis (PND) with selective abortion is crucial for the

effective prevention and control of thalassemia. In the near future, PND may be carried out using fetal DNA obtained from maternal blood, a non-invasive technique and can be carried out at an early stage of pregnancy.

Lastly, as everyone knows, thalassemia is a significant problem all over the world and all Asian countries. The Asian Thalassemia Network has been established to address this issue. This collaboration involves many faculties and departments within the country and worldwide, all aiming to improve the quality of life for those affected by thalassemia. Ultimately, we hope to control and prevent the disease at a global level. Finally, I would like to acknowledge and thank all thalassemia patients and their families for their cooperation and understanding of our goal.

OVER 3 DECADES OF ORGANIZING SERVICES FOR INHERITED METABOLIC DISORDERS IN THAILAND

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The study of inherited metabolic diseases (IMD) in Thailand was in its infancy when compare with developed countries. Prior to 1987, majority of IMD were clinically diagnosed since there were only a handful of clinicians and scientists with expertise in IMD, lack of well-equipped laboratory facilities and government support. In developing countries, IMD, were not considered a priority due to prevalence of infectious diseases (HIV and congenital infections). A multicenter survey conducted from 1994 to 2001 revealed the existence of numerous cases of IMD from all over the country. Case reports and publications on IMD in Thai (and international) medical journals in past 30 years undoubtedly raised its awareness among Thai pediatricians and scientists.

Milestones of IMD in Thailand:

1. The First Decade (1987-1997): In May 1990, only one Thai delegate (the author) attended the 5th International Congress on IEM held in Asilomar, California, the United States. In June 1993, the First Asia – Pacific Regional Meeting (APRM) of the International Society on Neonatal Screen (ISNS) was held in Sapporo, Japan. Experiences from these 2 international meetings had made a great impact on the development of IMD in Thailand. In July 1994, the First Asia – Pacific Conference on Medical Genetics was held in Bangkok, Thailand supported by Mahidol University and International Center for Medical Research (ICMR), Kobe University School of Medicine, Japan.

From 1993 to 1997, a pilot project on Newborn Screening was started at Siriraj Hospital, Mahidol University in Bangkok. In November 1995, the 2nd APRM of ISNS was held in Hong Kong. The Department of Medical Sciences, Ministry of Public Health (MOPH), Thailand initiated a pilot project in newborn screening in Thailand in 1996.

2. The Second Decade (1997-2017): In 1998, the 3rd APRM of the ISNS was organized in Chiangmai, Thailand and was attended by more than 200 people. From 1998 to 2000, Gas - Liquid Chromatography/mass spectrometry (GC/MS) was first introduced in Thailand through collaboration with Japanese scientists which led to previously undiagnosed organic acid disorders. Collaboration with US scientists (1993 to 2002) for tandem mass spectrometry (TMS) also led to identification of newly diagnosed fatty acid oxidation disorders. In 2003, the first Thai textbook on IMD, a collective data on IMD as well as 15 years experience of IMD referrals to Siriraj Hospital was completed.

In 2003, the IX International Congress on IMD (ICIEM) was held in Brisbane, Australia; 2 papers on urea cycle disorders and one oral presentation titled “IMD in Thailand – Siriraj Experience” were presented. In 2005 – the first Genetic Metabolic Symposium was held in Bangkok to raise awareness of IMD among Thai Pediatricians.

The X ICIEM was held in Chiba, Japan in September 2006. The author was invited to be a local organizing committee and invited speaker in “IEM in Asia” Symposium. Twenty –five newly reported cases of IMD were presented. The 6th ISNS meeting was held in Awaji Island, Tokushima, Japan and a poster “PKU infant from newborn screening at Siriraj Hospital” was presented. The invited lecture titled “Newborn Screening in Thailand - Challenges and Opportunities” was also presented. From 2005 - 2008, numerous publications on molecular characterization of IMD in Thai patients were accomplished.

(a) Research collaborations with United States (1981-1997)

(b) Research collaborations with Japan (1997-2007)

- Establishment of Genetics Metabolic Center in Thailand

Research collaborations with Chulabhorn Research Institute in Bangkok since 1998 has strengthened us in the area of amino acid analyses, enzyme assays and mutation analysis from which more than 20 publications were pioneering work on IMD in Thailand.

In 2001, the Genetic Metabolic Center was established at Siriraj Hospital Faulty of Medicine, the first of its kind in Thailand, with assistance from JICA (Japanese Intergovernmental agency) providing Gas-Liquid Chromatography & Mass Spectrometry (GC/MS) and technology tranfer; together with funding from Chaofa Maha - Chakri Pediatric Building for High – Performance Liquid chromatography (HPLC). Numerous new IMD cases and publications were identified since.

3. The Third Decade (2017-2023): Newborn Screening program in Thailand

National newborn screening program has been implemented in public health infrastructure by Ministry of Public Health (MOPH) since 1996. Approximately 80-90 percent of all newborns are being screened for congenital hypothyroidism (CH) and phenylketonuria (PKU).

Siriraj Hospital initiated ‘pilot project on newborn screening’ in 2005 with small support for Mahidol University and Foundation for the Mentally Handicapped. From 2005-2014 Department Pediatrics Siriraj Hospital was successfully performed the “Expanded Newborn Screening” (40 metabolic disorders) with technical assistance from Japan and Australia. In 2023, the Ministry of public health has agreed to include 40 inherited metabolic disorders into national policy, as per request from Siriraj Hospital.

4. In Summary

There had been quite a remarkable progress of work and research in inherited metabolic disorders in Thailand in past decades. Numerous publications both at national and international levels were published. Organization of services for IMD has successfully improved quality of life for Thai patients in past 3 decades.

GAUCHER DISEASE AS A MODEL FOR RARE DISORDERS

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Gaucher disease (GD) has played a pivotal role in advancing our understanding and treatment of genetic disorders at large: It has been among the first to demonstrate genotype-phenotype relationship using PCR-based techniques; it was the first lysosomal storage disease effectively treated with enzyme replacement therapy (ERT). It was also among the first orphan drugs to be approved by the FDA, and combining a huge medical success (in both safety and efficacy) with a great financial success for the manufactures, it has opened the door for the development of new therapies for other rare diseases, and in fact raising the interest of the big pharmaceutical industries to invest in rare disorders. GD was also among the first to be treated with oral substrate reduction therapy (SRT), and currently, albeit still in clinical trials, also of additional innovative therapeutic modalities, such as pharmacological chaperones and gene therapy.

Like all rare disease, GD patients often suffer from lack of awareness and hence delayed diagnosis, with the consequences of delayed initiation of therapy with the risk of irreversible complications, and the birth of another sibling by the time the diagnosis is made, as prenatal testing has not been offered. Research of rare disease may lead to innovative discoveries or to drug development that may impact on more common disorders, and in GD it is the relationship between GD and Parkinson. (See below).

From the epidemiology aspects - GD is pan-ethnic, with a global prevalence of 1:40,000–1:100,000, and possibly 1:1,000,000 in certain communities. It is significantly more common among Ashkenazi Jews wherein 1 of 17 is a carrier and about 1:800 is affected, a number bigger than the definition of an orphan drug.

GD is an autosomal recessive, multi-system glycolipid storage disorder caused by deficient glucocerebrosidase activity. This deficiency, caused by mutation in the glucocerebrosidase (GBA1) gene, leads to the accumulation of glucocerebroside in lysosomes within macrophages, resulting in hepatosplenomegaly (typically the spleen enlargement is much more pronounced than the liver enlargement), thrombocytopenia causing bleeding tendency, anemia, and bone problems (from non-specific pains, via acute episodes of bone crisis, to osteoporosis, debilitating osteonecrosis of large joints and pathological fracture). Additional clinical manifestations include delayed menarche, short stature, fatigue, and higher prevalence of monoclonal gammoapathies (MGUS).Patients with GD have a few co-morbidities, such as certain hematological malignancies (multiple myeloma, lymphoma) and Parkinson. The disease is characterized by a great phenotypic heterogeneity, even within families, with at times siblings sharing the same genotype presenting a totally different clinical course. While the existence of more than 860 different variants at the DNA level is clearly the main factor contributing to the diversity of clinical features, there are additional genetic and non-genetic factors behind this plethora of disease manifestations.

The disease has been traditionally classified into 3 clinical forms, based on the absence or (type 1; also known as adult type or chronic non-neuronopathic GD) presence of neurological manifestations (type 2 - acute neuronopathic, infantile form; and type 3 – the sub-acute juvenile form). Neuronopathic GD (nGD) is rare in the Western hemisphere (maybe less than 5%, whereas it is far more common in Asian countries, particularly Japan, Korea and Taiwan).

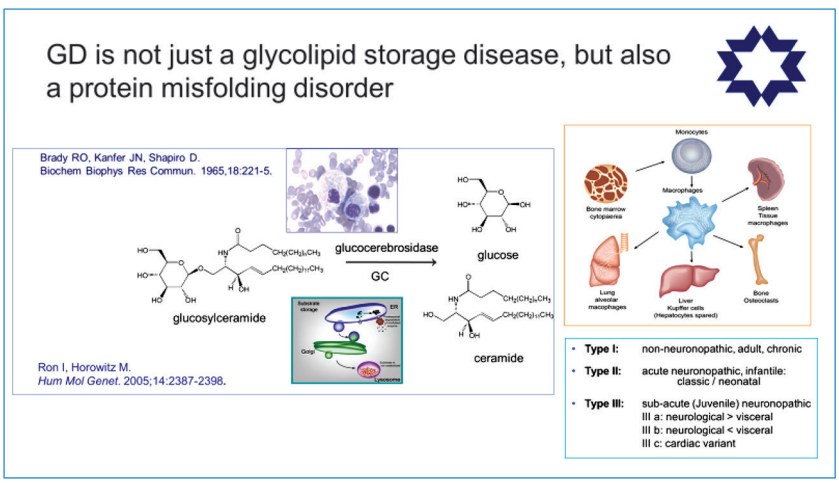
Once GD has been considered in the differential diagnosis (DD; and each of the disease manifestation or findings should lead to its inclusion in the DD) – then the actual diagnosis is easy, and can be performed on dried blood spot. Traditionally, the gold standard for the diagnosis was the demonstration of reduced enzymatic activity of the WBC glucocerebrosidase.

This is completed by the detection of mutations at the DNA level (bi-allelic variants), which may allow some prognostication, and to this measurement of the biomarker Lyso-Gb1 is added to provide both confirmation of the diagnosis as well as an effective follow-up tool whether the patient receives therapy (then the LysoGb1 should go down) or not (then it should be stable). Bone marrow aspiration or biopsy, although it is often performed due to lack of awareness and suspicion of hematological diseases like ITP or lymphoma, is unnecessary for GD diagnosis.

As mentioned above, despite being a rare disease, there are actually several treatment options, again adding the challenge of choosing the most suitable modality for the individual patient. Intravenous Enzyme Replacement Therapy (ERT) has revolutionized GD care with three FDA-approved enzymes and additional biosimilars available. For oral Substrate Reduction Therapy (SRT) there are currently two FDA-approved drugs. With a third drug in clinical trials for nGD. During the lecture, the similarities and differences between the different preparations will be discussed. Innovative emerging therapies, already in clinical trials, include oral pharmacological chaperones and gene therapy.

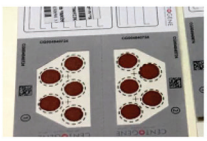
Remaining challenges include access to therapy in underdeveloped or poor countries, lack of registered treatment for nGD and the development of comorbidities, whether related or unrelated to the metabolic disorder. This group of diseases include malignancies (particularly lymphomas and multiple myeloma), cholelithiasis, certain autoimmune diseases, such as ITP, Hashimoto thyroiditis, and Celiac disease, and others, yet the most important association is between GD and even just a carrier status and the increased risk of developing a distinct variant of Parkinson disease (GBA1-related PD, or the Sidransky syndrome) where understanding of this unprecedent relationship between a rare genetic disease on the one hand and the second most common neurodegenerative disorder on the other hand, may lead to the development of disease modifying therapies and even the prevention of the devastating neurological illness.

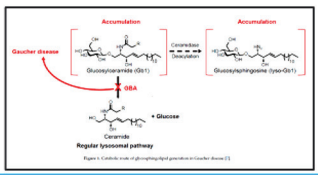
In conclusion, GD exemplifies how research into rare disorders can lead to groundbreaking advancements in medicine and improve outcomes for other diseases, rare and common alike.



Diagnosis

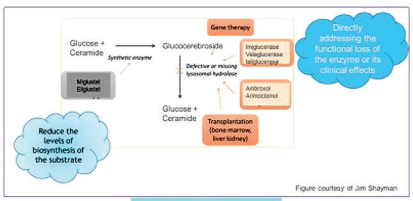
- Enzyme assay of glucocerebrosidase in WBC (DBS feasible)
- **Mutation analysis (whole gene sequencing)**
- **Biomarkers (LysoGb1; chitotriosidase and CCL18)**
- Bone marrow aspiration/biopsy not usually indicated





Gaucher disease: Treatment options

- Enzyme Replacement Therapy (ERT)
  - Imiglucerase
  - Velaglucerase alfa
  - Taliglucerase alfa
  - Imiglucerase biosimilars
- Substrate Reduction Therapy (SRT)
  - Miglustat
  - Eliglustat
- Investigational therapies
  - Pharmacological chaperones
  - Gene therapy



RARE INBORN METABOLIC DISORDERS: FROM MYSTERY TO DIAGNOSIS AND MANAGEMENT

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Inborn metabolic disorders are a diverse group of rare genetic conditions that disrupt metabolic pathways, usually leading to severe illness. These disorders pose significant diagnostic and therapeutic challenges due to their complex presentations and clinical and genetic heterogeneity. Clinical manifestations of these disorders may mimic symptoms of common disorders but with a lack of evidence confirming the diagnosis of the prevalent diseases.

Traditionally, diagnosing inborn metabolic disorders relied on clinical symptoms and biochemical tests such as urine organic analysis, plasma amino acid, and acylcarnitine profile (using tandem mass spectrometry). Biochemical and genetic testing can identify the underlying metabolic disorders before symptoms develop or in the early stages of the disease, often through newborn screening programs (NBS). For example, conditions like biotinidase deficiency, phenylketonuria (PKU), and methylmalonic acidemia can be diagnosed accurately, and quickly. Early identification enables prompt treatment, such as dietary changes, enzyme replacement, or vitamin supplementation, to prevent severe complications like developmental delays, organ damage, or neurological impairment. However, some inborn metabolic disorders are very rare, lack abnormalities of general and common biochemical analyses, and are not detectable by conventional NBS. Hence, the diagnosis can be delayed and occur after irreversible damage has begun.

Advanced genetic testing has significantly improved the early identification of rare inborn metabolic disorders, transforming patient outcomes through timely intervention. Technologies like next-generation sequencing (NGS), whole exome sequencing (WES), and targeted gene panels allow for the rapid and precise detection of genetic mutations associated with these disorders, even in newborns. Furthermore, advanced testing can uncover previously undiagnosed or atypical cases. Overall, advanced genetic testing and new therapeutic approaches have revolutionized the early detection and management of rare metabolic disorders, leading to improved health outcomes and quality of life for affected individuals.

In this presentation, a spectrum of rare inborn metabolic disorders, highlighting breakthroughs in diagnostic methods, current therapeutic approaches, and the critical role of precise diagnosis and treatment will be discussed, for example, pyruvate dehydrogenase complex deficiency, multiple carboxylase and biotinidase deficiency, transcobalamin defect, and vitamin-responsive metabolic disorders, aromatic L-amino acid decarboxylase (AADC) deficiency.

Pyruvate dehydrogenase complex deficiency (PDCD) is caused by mutations in the genes encoding the components of the pyruvate dehydrogenase complex (PDC), which is essential for linking glycolysis to the citric acid cycle. The deficiency leads to the accumulation of lactic acid and a reduction in cellular energy production, resulting in developmental delay, developmental delay, neurological impairment, poor muscle tone, seizures, and, in severe cases, early death. Management focuses on dietary modifications and supportive treatments to reduce lactic acid buildup and optimize energy metabolism. We present a 3-year-old boy, previously healthy, who came to the hospital due to a viral illness complicated with unexplained tachycardia, arrhythmia, severe metabolic acidosis, elevated lactate levels, and alteration of consciousness. Tandem mass spectrometry revealed no specific abnormalities of amino acids and acylcarnitine profiles. Rapid exome sequencing revealed a hemizygous *PDHA1* gene

mutation, confirming the diagnosis of PDCD. Treatment with the ketogenic diet, high doses of thiamine, and L-carnitine resulted in a clinical improvement. The diagnosis in the index case led to the diagnosis of two additional patients in the family.

Transcobalamin deficiency is a rare genetic disorder that affects the transport of vitamin B12 (cobalamin) within the body. This condition is caused by mutations in the *TCN2* gene, which leads to a shortage of functional transcobalamin, the protein responsible for carrying vitamin B12 to cells. As a result, the body cannot properly utilize vitamin B12, impairing crucial processes like DNA synthesis and red blood cell production. Symptoms typically appear in infancy and may include failure to thrive, developmental delays, anemia, neurological issues, and immune dysfunction. Early diagnosis and treatment with vitamin B12 injections is essential to prevent or reduce these complications and support normal development and health. We describe a 12-month-old infant who had a history of pancytopenia with acute hemolytic crisis, hepatosplenomegaly, and recurrent infection since the age of 3 months. Previous extensive hematologic and immunologic investigations failed to identify inherited bone marrow failure syndrome and hematologic malignancy. Whole exome sequencing identified a homozygous pathogenic *TCN2* mutation. All clinical abnormalities resolved, following hydroxocobalamin treatment.

Vitamin-responsive disorders are a group of metabolic conditions in which certain enzyme dysfunctions can be partially or fully corrected by the administration of specific vitamins. These disorders are typically caused by genetic mutations that impair enzyme activity or cofactor binding, which can be improved with high doses of vitamin supplementation. Examples include biotinidase deficiency, methylmalonic acidemia, and pyridoxine-dependent epilepsy. Early identification and appropriate vitamin therapy are crucial to prevent complications and support normal development.

Biotinidase deficiency is a disorder in which the recycling of biotin is impaired. Biotin is a vitamin essential for the metabolism of fats, carbohydrates, and proteins. Symptoms can vary widely in severity but may include developmental delay, chronic ataxia, seizures, skin rashes, hair loss, vision, hearing loss, and abnormal movement. If left untreated, the disorder can lead to irreversible serious neurological complications. Early diagnosis through newborn screening and biotin supplementation can effectively prevent symptoms and allow individuals to lead healthy lives. Unfortunately, NBS for biotinidase deficiency is available in a few countries. For those diagnosed through symptomatic presentation, timely diagnosis and administration of biotin can prevent complications and promote normal development. A 1-year-old child presented with acute illness, along with diffuse skin rashes and metabolic acidosis requiring respiratory and critical care support. Treatment with oral biotin led to rapid recovery. Whole exome sequencing confirmed a biallelic pathogenic mutation in the *BTD* gene.

Pyridoxine-dependent epilepsy is another vitamin-responsive disorder caused by mutations in the *ALDH7A1* gene, which encodes the enzyme antiquitin. Antiquitin is involved in the breakdown of the amino acid lysine, and its deficiency leads to the accumulation of toxic metabolites, including α-aminoadipic semialdehyde (AASA) and its cyclic form, piperideine-6-carboxylate (P6C). P6C reacts with and inactivates pyridoxal phosphate (PLP), the active form of vitamin B6, which is a crucial cofactor for many enzymes involved in neurotransmitter metabolism. This inactivation disrupts the synthesis of neurotransmitters such as gamma-aminobutyric acid (GABA) and dopamine, leading to seizures. Treatment with pyridoxine (vitamin B6) can restore adequate PLP levels, allowing for proper neurotransmitter function and seizure control. Early and continuous treatment with pyridoxine is essential for preventing neurological damage and improving developmental outcomes. An infant was presented with early onset seizure, in which general and specific biochemical testing revealed no abnormalities. Whole exome sequencing disclosed biallelic pathogenic mutations in the *ALDH7A1* gene. The patient's seizures were better controlled by high doses of vitamin B6.

Aromatic L-amino acid decarboxylase (AADC) deficiency is a rare autosomal recessive disorder that results in a synthetic defect of certain neurotransmitters, including dopamine and serotonin. The disorder is caused by mutations in the *DDC* gene, which provides instructions for producing the enzyme aromatic L-amino acid decarboxylase (AADC). This enzyme is essential for converting the amino acids L-DOPA and 5-hydroxytryptophan (5-HTP) into the neurotransmitters dopamine and serotonin, respectively. Patients with AAD deficiency may present with a range of neurological symptoms, including movement disorders, developmental delays, and autonomic dysfunction. The severity of the condition varies, but it often results in significant cognitive and motor impairments. Gene therapy for AADC deficiency has been recently approved, which is effective and safe, with improvement in motor and cognitive function. We detail a 4-month-old girl who developed oculogyric crises (involuntary upward-rolling movements of the eyes) and sustained dystonia. General and specific biochemical testing showed no abnormalities. Whole exome sequencing revealed biallelic mutations in the *DDC* gene, confirming the diagnosis of AADC deficiency. Neurotransmitter supplements with dopamine agonist, serotonin drug (5HT serotonin), and monoamine inhibitor (selegiline) for two years result in little clinical improvement. The patient received gene therapy at 2.5 years as part of the research, which led to a dramatic improvement.

NEWBORN SCREENING BY TANDEM MASS SPECTROMETRY: FROM EARLY DETECTION OF RARE DISEASES TO THERAPY

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Introduction

For over 60 years, newborn screening has been one of the most successful health policies for preventing death and disability in neonates and children in many countries. Expanded newborn screening (ENBS) for various rare inherited metabolic disorders (IMD) could be achieved through the development of tandem mass spectrometry (MS/MS) technology, which allows for the multiplex assay of targeted analytes. In the presentation, I will provide an overview of ENBS in Thailand and our research studies that shed light on the precise management of rare diseases.

Pilot expanded newborn screening program in Bangkok by Siriraj Hospital[1]

In Thailand, congenital hypothyroidism (CH) and phenylketonuria (PKU) were the only two conditions screened for nearly 30 years. In 2019, the National Health Security Office (NHSO) implemented universal coverage for the diagnosis and treatment of 24 small-molecule disorders. Seven rare disease centers, located in university hospitals with clinical geneticists, were established by the NHSO as referral centers for patients from across the country. However, the majority of patients still experienced delayed diagnoses due to nonspecific symptoms and a lack of awareness among physicians. With funding support from the NHSO-Bangkok, the Siriraj Center of Excellence for Inherited Metabolic Disorders and Newborn Screening implemented a pilot ENBS program in Bangkok, screening 180,070 newborn babies between May 2014 and December 2020.

The positive screening babies (0.2%) were recalled to repeat ENBS or receive diagnostic investigations. Diagnostic tests included plasma amino acids, urine organic acids, acylcarnitine assays, and molecular tests (Sanger sequencing or Next generation sequencing). We could detect newborns affected with a variety of small-molecule disorders with a total incidence of 1:7,829. Twenty-three newborns with IMD were identified including primary carnitine deficiency (PCD), glutaric aciduria type I (GA1), maple syrup urine disease, isovaleric acidemia, phenylketonuria, citrullinemia type 1, ornithine transcarbamylase deficiency, methylmalonic acidemia, propionic acidemia, methylcrotonyl-CoA carboxylase deficiency, carnitine palmitoyl transferase type I deficiency, short-chain acyl-CoA dehydrogenase deficiency, and very long-chain acyl-CoA dehydrogenase deficiency, giving an incidence of 1:7,829 live births. PCD is the most common disorder. In addition, we identified maternal disorders through ENBS including PCD, GA1, and beta-ketothiolase deficiency. Treatment was started promptly in urgent cases, and almost all confirmed cases were treated and followed at Siriraj Hospital. The median age of diagnoses for screened cases was 8 days of age compared with 106 days of age for non-screened cases.

Early diagnosis in screened babies resulted in significantly better neurodevelopmental outcomes. The success of the pilot ENBS and the findings from a feasibility study conducted by the Health Intervention and Technology Assessment Program led to the inclusion of ENBS by MS/MS in the Universal Coverage Scheme's benefit package in 2022, enabling the screening of all newborn babies in Thailand.

PKU: from a paradigm of newborn screening to precision medicine[2]

PKU is an autosomal recessive metabolic disorder of phenylalanine caused by mutations in the *PAH* gene, which encodes phenylalanine hydroxylase (PAH) with tetrahydrobiopterin (BH4) as its cofactor. The primary treatment for PKU is dietary restriction of phenylalanine. However, maintaining strict compliance becomes increasingly challenging for older patients. We conducted the study of the clinical phenotypes and genotypes of Thai PKU patients and evaluated PAH enzyme activity and expression of novel *PAH* variants. We also used the genotype results to assess the response to treatment with BH4.

The majority (66%) of Thai PKU patients were classified as mild phenotypes including mild PKU and mild hyperphenylalaninemia (HPA). This data is different from the global prevalence in which classical PKU is most prevalent and mild HPA is the smallest group. Eleven different *PAH* variants were identified: the most frequent variant was c.506G>T (p.Arg169Leu), accounting for 24% of all identified alleles. Two novel variants c.506G>T (p.Arg169Leu) and c.949T>A (p.Tyr317Asn) and previously reported variants at the same positions were expressed in COS-7 cells. These showed mildly impaired residual activity, and p.Arg169Leu mutant showed decreased protein expression of 55.7% compared to the wild-type enzyme. All patients with p.Arg169Leu variant identified in at least one pathogenic allele have a mild HPA metabolic phenotype. This explains the high prevalence of mild phenotypes in Thai PKU patients. The results of phenotypes and genotypes analyses led to the study of BH4 responsiveness and guided the use of BH4 as a precision treatment for Thai PKU patients.

Carnitine uptake defect: the most common IMD identified in ENBS with metabolic and molecular findings[3]

Carnitine Uptake Defect (CUD), also known as Primary Carnitine Deficiency (PCD), is typically detected by low blood levels of free carnitine and acylcarnitines, as measured by MS/MS. It is caused by *SLC22A5* mutations. As mentioned earlier, PCD was the most common IMD detected by the pilot ENBS. There were 14 cases with confirmed PCD: two clinically diagnosed cases, and 12 cases identified through NBS including five newborns, six mothers, and one older sibling. The incidence of PCD in neonates was 1:29,351. The high incidence of PCD in the Thai population is quite similar to that of Taiwan (1:30,000) and China (1:23,637–1:30,182).

All affected neonates and mothers were asymptomatic except one mother with dilated cardiomyopathy. One of the clinically diagnosed cases died from poor compliance with carnitine supplementation. This suggests that primary carnitine deficiency does not always follow a benign course. Molecular analysis identified 10 different *SLC22A5* variants, four of which were novel. The most prevalent were c.51C>G (p.Phe17Leu) and c.760C>T (p.Arg254Ter), which are also the most common variants found in PCD patients from China. For genotype-phenotype correlation, asymptomatic or mildly symptomatic women with PCD were neither homozygous nor compound heterozygous for null variants. In contrast, the only two clinically diagnosed patients were either homozygous or compound heterozygous for two null alleles of *SLC22A5* variants. In addition, cases with significant clinical features tended to have higher C0 clearance values. In conclusion, genotypes and C0 clearance can be used to diagnose and predict the development of clinical symptoms of PCD, aiding in the selection of cases that require carnitine treatment.

Current status of ENBS in Thailand and future direction

Currently, Thailand is one of three countries in Southeast Asia, out of a total of 10, along with Singapore and the Philippines, that have implemented national ENBS using MS/MS.[4] The Royal College of Pediatricians of Thailand, Birth Defects Association (Thailand), and Medical Genetics and Genomics Association developed the clinical practice guideline for ENBS and the initial management of small-molecule disorders for Thai pediatricians in 2024. Following the implementation of nationwide ENBS, the updated incidence of small-molecule disorders from all rare disease centers is 1 in 7,534 live births (data collected by Dr. Chulaluck Kuptanon),

which is similar to findings from the pilot ENBS in Bangkok. The highest prevalence is in the Northeastern region, with an incidence of 1 in 4,815 live births (data provided by Dr. Khunton Wichajarn). In 2024, more than 50 cases of IMD were detected through the national ENBS, with primary carnitine deficiency (PCD) remaining the most common disorder. Although ENBS now covers all newborns in Thailand, several obstacles hinder the full success of the policy. These include a shortage of pediatric geneticists in provincial areas, leading to limited access to diagnosis and treatment at rare disease centers, an insufficient supply of metabolic formulas or orphan drugs for rare disease treatment, and a lack of public awareness about the importance of newborn screening. Collaboration between the Ministry of Public Health and experts in this field is essential to overcome these challenges.

With Thailand's declining birth rate, preventing death and disability in children has become increasingly important. In the future, additional treatable rare diseases could be detected early through newborn screening. Ongoing pilot newborn screening projects for rare diseases, such as spinal muscular atrophy (SMA), are underway in Thailand. The genome data of the Thai population gained from the Genomics Thailand project, combined with next-generation sequencing technology, can be applied as a second-tier test, or even as a first-tier screening method for certain rare Mendelian disorders. However, the rapid advancement of screening and treatment technologies should be carefully adapted and considered alongside the ethical, legal, and socioeconomic aspects of Thai society.

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LYSOSOMAL STORAGE DISEASES: PROGRESS IN DIAGNOSIS AND THERAPY IN THAILAND

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Lysosomal Storage Diseases (LSDs) are a group of inherited inborn metabolic disorders resulting from enzyme deficiencies within lysosomes, leading to the accumulation of substrates and progressive cell damage or dysfunction in various organs. Lysosomes are cellular organelles responsible for breaking down waste products. Lysosomes contain about 40 types of hydrolytic enzymes, including proteases, nucleases, glycosidases, lipases, phospholipases, phosphatases, and sulfatases. Deficiencies in specific lysosomal enzymes can lead to LSDs, as shown in Table 1.

LSDs encompass over 70 distinct disorders, each linked to a specific enzyme deficiency. Previous reports indicated that the five most commonly observed LSDs globally include Gaucher disease (GD), Mucopolysaccharidosis (MPS) types I-VII, Pompe disease, Fabry disease, and Metachromatic leukodystrophy. In Thailand, however, the most frequently observed LSDs, based on survey data, are Gaucher disease, MPS type II, Pompe disease, MPS type VI, MPS type I, and MPS type IV. Each is characterized by progressive, multisystem involvement such as neurodegeneration, organomegaly, hematologic and skeletal abnormalities. Early diagnosis is crucial yet challenging due to the variability in symptom onset and presentations. Diagnostic approaches involve a combination of clinical evaluation, enzyme activity assays, genetic testing and biomarker evaluation, with advancements in newborn screening enabling early detection in some countries.

Treatment options for LSDs have advanced considerably, though they vary by disease. **Enzyme Replacement Therapy (ERT)** is a primary lifelong treatment for several LSDs, involving the intravenous administration of a synthetic version of missing enzyme to reduce accumulated substances in cells. For instance, ERT is available for Gaucher, Pompe, Fabry, and some MPS types, significantly improving patients' quality of life. However, ERT does not cross the blood-brain barrier, limiting its effectiveness for LSDs affecting the central nervous system. **Substrate Reduction Therapy (SRT)** is another approach that reduces the production of substances accumulating in cells by inhibiting specific pathways. SRT has been used in Gaucher and Niemann-Pick disease type C. **Hematopoietic Stem Cell Transplantation (HSCT)** offers an alternative for some neuronopathic LSDs, while **Gene Therapy** showed promise as a potentially curative approach. Additionally, symptomatic and supportive care remains essential for enhancing patient quality of life.

Table 1 Examples of lysosomal storage diseases (LSDs), including enzyme deficiencies and resulting accumulations

Disease	Enzymes	Accumulation
Gaucher disease	Beta-glucosidase	Sphingolipids
Pompe disease	Alpha-glucosidase	Glycogen
MPS type I (Hurler/Hurler-Scheie/Scheie)	Iduronidase	Dermatan/heparan sulfate
MPS type II (Hunter)	Iduronate sulfatase	Dermatan/heparan sulfate
MPS type III (A/B/C/D) (Sanfilippo)	A: N-fulfoglucosamine sulfohydrolase B: Alpha-N-acetylglucosaminidase C: Heparan-alpha-glucosaminide N-acetyltransferase D: Glucosamine (N-acetyl)-6-sulfatase	Heparin sulfate
MPS type IV (A/B) (Morquio)	A: N-acetylgalactosamine 6-sulfatase B: Beta-galactosidase	Keratan/chondroitin/keratin sulfate
MPS type VI (Marateaux-Lamy)	N-acetylgalactosamine 4-sulfatase	Dermatan sulfate
MPS type VII (Sly)	Beta-glucoronidase	Chondroitin sulfate
Metachromatic leukodystrophy	Arylsulfatase A	Sphingolipids
Tay-Sachs disease	Beta-Hexosaminidase A	Sphingolipids
Sandhoff disease	Beta-Hexosaminidase A/B	Sphingolipids
Fabry	Alpha-galactosidase A	Sphingolipids

In Thailand, awareness and diagnostic capabilities for LSDs have been improved, but many challenges remain due to the rarity and complexity of these disorders. The patient support groups for LSDs, including the Thai LSD Foundation and the Thai Rare Disease Foundation, have grown stronger over time and raised public awareness about LSDs, promoting financial and psychosocial support. They collaborate with international organizations, healthcare providers, and policymakers to drive initiatives that improve the quality of life for patients. In Thailand, diagnostic testing for LSDs, including enzyme activity assays and biomarker analysis, is available through specialized laboratories. Treatment with ERT for Gaucher disease was officially approved for reimbursement in Thailand in 2013, marking a significant step forward for patients needing treatment for this rare LSDs. In 2015, Thailand expanded its healthcare reimbursement policy to cover matched-related donor HSCT for Gaucher disease and neuronopathic LSDs; later, in 2020, coverage for matched-unrelated donor HSCT was launched. However, the high costs associated with ERT and HSCT mean that continued advocacy is necessary to maintain and expand access, especially for patients with other LSDs that currently lack approved treatments.

Several studies and experiences on LSDs in Thailand have produced interesting findings, contributing to the understanding of how these diseases manifest specifically in Thai patients. Research has primarily focused on genetic variants, clinical presentations, and treatment outcomes, with some unique trends and variations identified compared to global findings.

- **Specific genetic variants**
  - **Gaucher disease:** Neuronopathic types, including GD type 2 (40.7%) and type 3 (44.5%), were strikingly prevalent among the Thai affected population. The most common variant

among Thai patients is the p.L483P (L444P) in the GBA gene, which accounts for 66% of identified mutant alleles. Other notable variants include IVS2+1G>A, Rec1a, and IVS6-1G>C1.

- **Pompe disease:** The hotspots are in exons 14 and 5 in the GAA gene for Thai infantile-onset Pompe disease patients, accounting for 62% of mutant alleles.
- **Mucopolysaccharidosis type I:** The potential common variant, c.*1T>C in the *IDUA* gene, was identified in 70% of the mutant alleles in five families.
- **Mucopolysaccharidosis type II:** Three family members carry the novel nonsense variant, c.928C>T (p.Gln310*) in the *IDS* gene. The publication exhibited a reduction of IDS mRNA, suggesting its degradation by nonsense-mediated mRNA decay. Moreover, the expression of the mutant in CO7 cells revealed a lack of IDS activity.
- **Sandhoff disease:** The potential common variant in the *HEXB* gene in five patients is c.1652G>A (p.Cys551Tyr), accounting for 90% of mutant alleles.

■ **Treatment Response and Outcomes**

- **Gaucher disease:** A publication focusing on the long-term outcomes of five Thai patients who underwent HSCT concluded that ERT followed by HSCT could be an alternative treatment for patients with GD type 3 who have a high risk of fatal neurological progression. Chronic graft-versus-host disease occurred in one patient. The enzyme activities were normalized at 2 weeks post HSCT. Lyso-Gb1 concentrations became lower than the pathological value and lower than when receiving ERT before HSCT. All of the patients are still alive and physically independent. Most of them (4/5) returned to school. None of the patients with GD type 3 had seizures or additional neurological symptoms after HSCT but showed varying degrees of cognitive impairment
- **Pompe disease:** Our experience: A 9-year-old girl with infantile-onset Pompe disease (IOPD) has received regular ERT since 30 days of age. The patient has not developed respiratory failure or cardiac failure. Proximal muscle weakness in her extremities has been noticed since 4 years of age. The patient goes to school with some limited outdoor activity. Other IOPD patients presented with respiratory failure and ventilator dependence had a poor prognosis.
- **Mucopolysaccharidosis:** Due to the lack of coverage of ERT, HSCT is an option for Thai patients. Parents need to be informed of morbidity and mortality of HSCT. In our experience, three patients with MPS type 1 and two patients with MPS type 2 underwent HSCT. Engraftment was observed in all patients. The enzyme activities were normalized at 2-4 weeks post-HSCT. While the initial results are promising, it is essential to note that long-term outcomes will need to further assessment the sustainability of these benefits and any potential late complications.

The current landscape of LSDs in Thailand reveals a significant gap in the systematic collection of patient data. The number of LSD patients present in Thailand has not been systematically collected. This indicates a need for expanded surveillance and LSD registry efforts to understand better the prevalence and distribution of LSDs in Thailand. This prevalence information aids in targeted screening efforts and public health resource allocation. Despite advancements, significant challenges persist in achieving timely diagnosis and equitable access to treatment. Future directions in research and translational medicine are focused on expanding access to diagnostic tools, refining genetic spectrums, establishing biomarkers, promoting multidisciplinary care, and improving healthcare coverage.

In conclusion, addressing these challenges and focusing on future directions will significantly enhance the management of lysosomal storage diseases in Thailand, ultimately improving patient outcomes. Collaborative efforts among healthcare professionals, researchers, and policymakers will be vital in improving outcomes for patients with LSDs.

ADDUCTOMIC STRATEGIES FOR EMERGING RISK FACTORS IN LIVER CANCER

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Liver cancer causes upwards of 1 million cancer deaths annually and is projected to rise by at least 55% over the next 15 years. An epidemiologic transition underpinning etiology of liver cancer is underway impacting concepts of cancer prevention and control interventions for this nearly always fatal disease. The proportional contributions of traditional etiological factors in HCC - hepatitis B virus (HBV), hepatitis C virus (HCV), alcohol, and aflatoxin - are changing with the emergence of metabolic syndrome, obesity, metabolic dysfunction-associated steatotic liver disease (MASLD), formally called non-alcoholic fatty liver disease (NAFLD), air pollution, and diabetes.

Several recent studies report that ambient air pollution exposure, particularly particulate matter PM2.5, and NO2 increases the risk of liver cancer. Collectively, these reports motivate albumin adductomics technologies to assess air pollution exposures. This approach employing nanoflow liquid chromatography-high resolution mass spectrometry capable of simultaneously monitoring dozens of Cysteine^{34} and other amino acid adducts, in the low femtomolar range and using less than 1 µL of plasma. Significant elevations in oxidation and benzene adduct concentrations of 300% to nearly 700% per doubling of ambient airborne pollutant levels ($p<0.05$) were found. Notably, the ratio of irreversibly oxidized to reduced Cys^{34} rose revealing a dramatic perturbation of serum redox balance; potentially serving as a portent of increased pollution-related risk.

Finally, two of the major risk factors contributing to liver cancer have been well documented by multiple epidemiologic studies and the hepatitis B virus (HBV) and aflatoxin show a synergy that increases by more than 8-fold the risk of liver cancer relative to HBV alone. Using the population-based cancer registry established by the Qidong Liver Cancer Institute in 1972 and aflatoxin specific biomarkers, we document that reduction of aflatoxin exposure has likely contributed to a nearly 70% decline in age-standardized liver cancer incidence over the past 30 years despite an unchanging prevalence of HBV infection in cases. A natural experiment of economic reform in the 1980s drove a rapid switch from consumption of heavily contaminated corn to minimally, if any, contaminated rice and subsequent dietary diversity. Aflatoxin consumption appears to accelerate the time to liver cancer diagnosis; lowering exposure to this carcinogen adds years of life before a cancer diagnosis. Thus, in 1990 the median age of diagnosis was 48 years, whilst increasing to 67 years by 2021. These findings have important translational public health implications since between 3 and 4 billion people worldwide are routinely exposed to dietary aflatoxin, especially in societies using corn as the staple food. Interventions against aflatoxin is an achievable outcome leading to a reduction in liver cancer incidence and years of delay of its nearly always fatal diagnosis.

In summary, despite the recent successes in the prevention and treatment of HBV and HCV through vaccination and chemotherapy, the incidence of liver cancer continues to rise across many different populations. Well annotated epidemiologic studies and concomitant biorepositories will provide the foundational resource for this research.

LIVER CANCER PREVENTION

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HCC is highly preventable.

There are two considerations that support this notion. First, 64.0% of HCC cases (68.8% in males and 49.8% in females) are attributable to five risk factors: chronic infection with hepatitis C virus (HCV), hepatitis B virus (HBV), excess alcohol intake, overweight/obesity associated liver diseases (nonalcoholic fatty liver disease or NAFLD), and tobacco smoking.[1] Second, most risk factors cause cirrhosis, which is the main precursor lesion for HCC. Cirrhosis can be reliably diagnosed using a combination of clinical, laboratory, and radiological findings without the need for invasive testing. Enrolling patients with cirrhosis in HCC surveillance programs can help identify HCC at an early and potentially curable stage.

The preventable burden differs by region, race/ethnicity and by sex. For example, HCC rates in the U.S. have been driven by HCV acquired mostly by a cohort born between 1945 and 1965.1 HCC rates have been stable in each successive birth cohort born between 1970 and 1995, suggesting a potential peak in HCV-related HCC rates.[2] There are also fewer cases due to active HBV infection. However, obesity and NAFLD are emerging as important risk factors for HCC, with an important proportion of HCC cases now attributable to these metabolic risk factors.[3]

Current State in Prevention of HCC and Gaps in Prevention.

Most patients with HCC have chronic liver disease that progresses over decades to cirrhosis, which is the main precursor lesion for HCC.[4] **There are considerable gaps in the prevention of HCC at every step of this cascade** including (1) early recognition people with primary risk factors (HCV, HBV, NAFLD, alcohol use), (2) risk stratification of those with chronic liver disease for their risk of progression to cirrhosis and HCC.[5] (3) linkage and access to disease management.[6]

Identifying and treating the underlying risk factors of liver disease is contingent on a partnership between public health officials, primary and specialty care. For HCV, this means testing for HCV amongst adults in the primary care setting followed by access to oral antiviral therapy with the goal of cure. For alcohol associated liver disease, prevention relies on assessment for alcohol use disorder using screening tools with appropriate support in group or individual setting for those affected, as well as access to specialty care for chronic disease management. Strategies to guide prevention of HCC in patients with MASLD remain unclearly defined resulting in inconsistent care.[7] However, these efforts require systematic identification and risk stratification of people with MASLD, with the goal of identifying the smaller subset at high risk for advanced liver fibrosis for aggressive risk reduction via management of comorbid conditions as well as linkage to specialty care for novel therapeutics.[8] Individuals with advanced fibrosis from any chronic liver disease require structured HCC surveillance programs to ensure regular surveillance.[9]

Systematic identification of high-risk patients among people with risk factors. Care pathways could be useful but remain undefined or untested across integrated healthcare systems. Noninvasive liver disease assessment may play a role in risk stratification but are underutilized. Linkage to care is essential but is frequently lacking or centered around urban centers with rural, low income and underserved population. Finally, access to therapy and resources is spotty. This is true of alcohol use disorder (e.g., access to mental health and addiction resources), MASLD (e.g., nutrition counseling) and viral hepatitis. For example, there is substantial attrition of care at each stage of the HCV care continuum in a safety net

healthcare system. Of 2450 patients screening positive for HCV antibodies, 82.3% received quantitative RNA testing, of whom 53.6% exhibited chronic infection. Providers referred 915 patients (84.6%) to specialty care for evaluation, 540 of these patients (50.0%) received their specialist evaluation, and 299 (27.7%) received a prescription for treatment.[10]

Given the strong association between tumor stage and overall survival, society guidelines endorse **HCC screening in at-risk patients.** The best evidence supporting HCC screening is derived from a large trial in patients with chronic HBV infection.[11] In patients with cirrhosis, several cohort studies also demonstrate a consistent association with improved clinical outcomes, including reduced HCC-related mortality, and acceptable rates of screening-related harms.[12] Continued generation of high-level data evaluating screening benefits and harms are critical to promote national screening policy recommendations.

HCC screening is performed using abdominal ultrasound and a serum biomarker, alpha fetoprotein. This combination is the best validated screening strategy available[13]; however, it may miss over one-third of HCC at an early stage, underscoring a need for novel screening modalities.[14] Although CT or MR imaging can increase sensitivity for early cancer detection, concerns about cost, radiation/contrast exposure, and radiologic capacity limit the wide applicability of this strategy.[15,16] Several efforts are ongoing to evaluate emerging blood-based biomarker panels, including liquid biopsy techniques, although these tests require validation in large cohorts.[17,18] Ongoing efforts should continue to recruit and follow up patients with contemporary risk factors (e.g., MASLD).

HCC screening effectiveness is limited by its underuse in clinical practice, with only 1 in 4 at-risk patients undergoing HCC screening.[19] Patients with MASLD are disproportionately under-screened. HCC screening failures can be attributed to gaps at multiple steps, including under-recognition of cirrhosis, providers failing to order the screening tests, and patients failing to adhere with provider recommendations.[20,21] Survey studies have identified patient barriers (e.g., transportation and costs) and provider barriers (e.g., lack of knowledge or competing interests in clinic) that must be addressed to increase HCC screening.[22,23] Provider education, electronic medical record reminders, and population health outreach invitations, have been demonstrated successful to increase HCC screening, and should be considered for implementation in routine clinical practice.[19]

Risk Assessment for HCC Prevention

Patients with chronic liver disease at the highest risk for developing HCC can be identified using risk stratification algorithms (the simplest of which is presence/absence of cirrhosis). Several validated models have been developed both for people with a specific risk factor as well as irrespective of the etiology.

For wider testing and implementation of algorithms for early detection and risk stratification of liver disease, EHR-based methods are required. Thus, if healthcare systems can properly recognize those with chronic liver disease, especially those at the highest risk for liver failure or cancer, surveillance processes using easily accessible, actionable and transparent dashboards can be created to help monitor compliance with quality indicators.[9,24] Further, communication within the EHR can help providers and patients so that they can mutually engage validated care pathways, thus ensuring a higher level of valued care.

However, it is critical that healthcare systems go beyond patient identification and care pathway tracking by using process improvement methods (such as Plan-Do-Study-Act cycle). These approaches would establish periodic evaluations of disease algorithms and care pathways as well as incorporating response feedback from all stakeholders, to continuously refine and optimize the process. Incorporation of artificial intelligence will likely play an important role in the future of high valued healthcare delivery. The time is ripe for large scale testing of effectiveness and cost-effectiveness of these methods before wider implementation.[25,26]

Community/Population Programs

Hepatitis C Elimination. Globally an estimated 58 million persons are living with hepatitis C virus (HCV). The World Health Assembly has set the goal to eliminate viral hepatitis as a major health threat by 2030. which includes a 90% reduction in HCV incidence and prevalence, treatment of 80% of eligible individuals, a 65% reduction in HCV-related mortality, and universal access to preventive and therapeutic services. To prevent HCV-related HCC, it is critical to treat and cure all individuals infected with HCV. This requires strong national policies and rigorous infection control measures. However, a significant challenge remains in the large number of individuals who remain undiagnosed or untreated for HCV infection. Universal HCV screening is recommended for all adults and pregnant individuals in regions with an HCV prevalence of at least 0.1%. Public health education focused on reducing HCV transmission is crucial, emphasizing the importance of avoiding the sharing of personal items, refraining from blood or organ donation, covering bleeding wounds, and properly disinfecting contaminated objects.

The introduction of direct-acting antivirals (DAAs) has revolutionized the therapeutic landscape for HCV infection, enabling sustained virologic response (SVR) in most cases. Achieving SVR has been shown to reduce HCC risk by 50%–80%, particularly when treatment is initiated prior to the onset of advanced liver fibrosis or cirrhosis.

However, the rate of treatment for chronic HCV has declined recently.[27] The Coalition for Global Hepatitis Elimination helps nations put in place seven essential components of effective elimination programs 1) mobilizing political support; 2) creating evidence -based action plans; 3) leveraging sustainable financing; 4) setting policies to guide prevention, testing and treatment; 5) implementing hepatitis care within existing health systems; 6) focusing on health equity for marginalized populations; and 7) evaluating and improving programs until targets are achieved. With these components in place, Egypt, Georgia, and Rwanda are examples of countries dramatically reducing HCV –related mortality. Although the goal is global elimination, regional and micro elimination programs are needed to achieve this overarching goal.

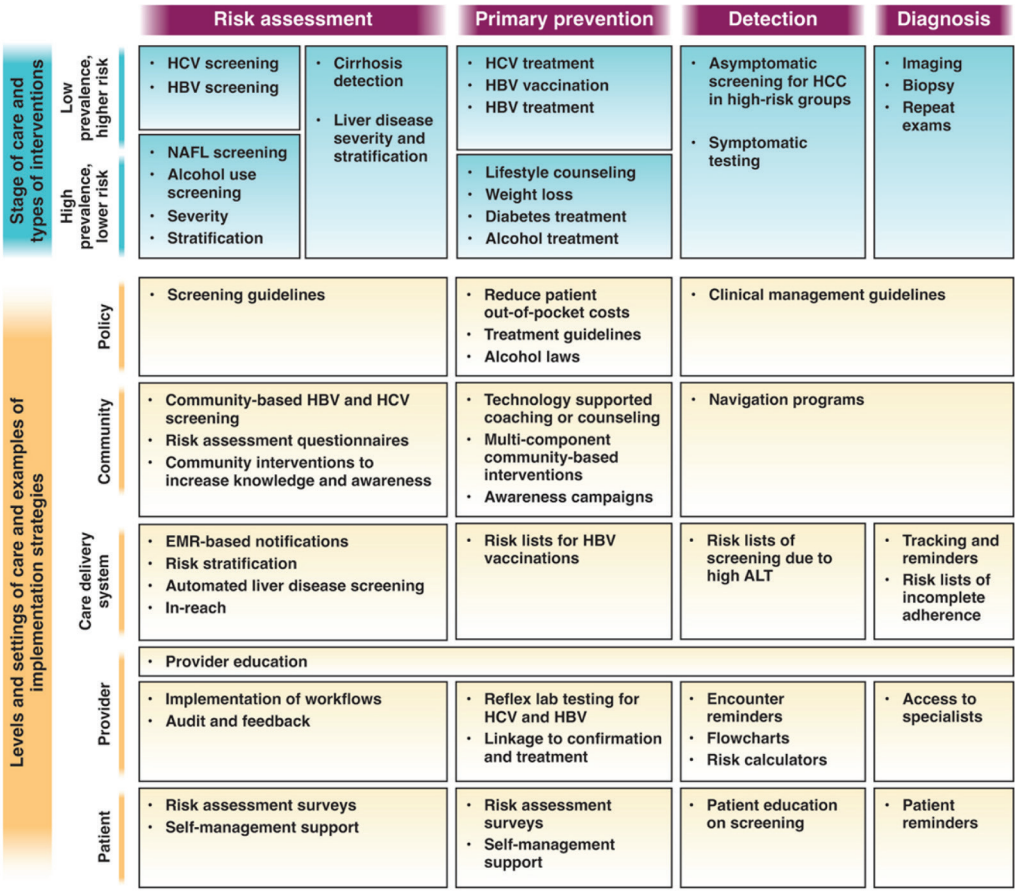
Useful implementation frameworks include the Reach, Effectiveness, Adoption, Implementation, and Maintenance (RE-AIM) framework that has five dimensions to guide HCV continuum of care including: Reaching eligible subjects; Adopting the intervention with support in primary care; Implementing the intervention with fidelity; Effectiveness assessment; and Maintaining the intervention long-term. Key components are community-based education about HCV; electronic medical record modification to facilitate screening; reflex testing to quantify HCV viral load; a low literacy, mobile app offering personalized patient education about HCV; case management to coordinate/promote adherence to care; and specialist teleconference to guide HCV management.[28]

Hepatitis B Vaccination and Risk Reduction

Universal HBV vaccination. The introduction of universal HBV vaccination programs in the 1980s significantly reduced mother-to-child transmission of HBV over the past several decades[29] followed by HCC reduction in children and young adult.[30] However, the COVID-19 pandemic has disrupted universal hepatitis B vaccination efforts in many regions, threatening global progress towards HBV elimination by 2030.

Antiviral therapy for HBV. Data from long-term follow-up studies indicate that 90% of patients achieve sustained suppression of HBV DNA with oral nucleos(t)ide analogues and pegylated interferon, resulting in significant reductions in necroinflammation and regression of liver fibrosis and cirrhosis.[31,32] This leads to a significantly decreased risk of HCC.[33] Despite the benefits of long-term antiviral therapy, only 20%–40% of individuals with chronic HBV meet the current eligibility criteria for treatment, and of those, only 40%–55% ultimately receive it. Several factors contribute to delays in treatment initiation in medically eligible patients, including patient refusal, physician decisions to continue observation, and patient loss to follow-up. To enhance care, key strategies include increasing public awareness, providing targeted education for

healthcare providers, and implementing streamlined referral systems. Recent evidence suggests that individuals with minimally active HBV infection who are currently ineligible for treatment face a considerable risk of developing HCC, and future guidelines is likely to broaden the population eligible for antiviral therapy.



MASH. MASLD is the most common cause of chronic liver disease worldwide. Between 2010 and 2019, NASH was the fastest growing etiology of incident liver cancer cases (+39%) as well as liver cancer deaths in most world regions, with the greatest increase in the Americas (APC: 1.33%). The incidence of liver cancer due to NASH is projected to increase further in the next decade in the United States, Europe, and Asia.

Lifestyle interventions, particularly dietary modifications and physical activity, are the cornerstone of managing MASLD. Both the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD) recommend the Mediterranean diet as the optimal dietary pattern for individuals with MASLD. However, cultural, ethnic, and dietary preferences may limit the universal applicability of the Mediterranean diet. Therefore, tailoring dietary modifications to accommodate individual cultural, religious, and personal preferences is essential to improving long-term adherence and compliance.

Regular physical activity, irrespective of its form, is associated with a reduced risk of HCC. Among patients with MASH and obesity, bariatric surgery has shown superiority over lifestyle interventions in resolving MASH.[34] Furthermore, bariatric surgery has been linked to a subsequent reduction in the risk of HCC.[35] Recent studies suggest that glucagon-like peptide-1 receptor agonists (GLP-1RAs) are associated with MASH resolution in phase 2 randomized trials and possibly a reduced risk of incident HCC and hepatic decompensation in observational studies[36-38].

Several professional societies have developed Clinical Care Pathways that explicate the high-risk groups of patients who may benefit from screening; these Pathways also include practical steps to assist clinicians in diagnosing and managing MASLD based on the best available evidence.[39] Data also show significant gaps in knowledge among clinicians about who to screen and how to diagnose and treat patients at high risk from MASH.

Alcoholic Liver Disease. Alcohol had the second fastest rising cause of liver cancer ASDRs globally (APC: 0.23%), with the highest increase in the Americas (APC: 1.78%). Globally, the alcohol per-capita consumption rose from 5.5 litres in 2005 to 6.4 litres in 2016 and is projected to increase further to 7.6 litres in 2030. Measures are required to curb heavy alcohol consumption to reduce the burden of alcohol-associated cirrhosis and HCC. Implementing policies such as an increased price and taxation for alcohol may be considered at a national level to reduce the burden of alcohol-associated liver cancer in countries with a high alcohol-per-capita consumption. There is also a need for health care models that integrate alcohol associated liver disease treatment and treatment for alcohol use disorder. The benefit of standardized validated alcohol use screening tools such as AUDIT-3 or −4 for screening, and full AUDIT-10 for diagnosis of alcohol use disorders has been shown. The harm associated with heavy alcohol use is also well documented, however, the best practices for treatment of alcohol use disorders in patients with alcohol associated liver disease is unclear and therefore research is needed in this area especially in multidisciplinary clinic setting.

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EMERGING STRATEGIES OF EARLY DETECTION OF LIVER CANCER

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The current diagnostic approach for liver cancer, like many other cancers, relies heavily on imaging and the detection of circulating tumor-specific markers, such as alpha-fetoprotein (AFP) for hepatocellular carcinoma (HCC) and CA19-9 for intrahepatic cholangiocarcinoma (iCCA). Imaging-based methods face technical limitations in detecting small tumors, and smaller tumors do not always indicate an early stage of cancer. Moreover, early-stage tumors release very few biomarkers, which are often diluted in the bloodstream or cleared by the kidneys. Tumor heterogeneity further complicates detection, as the molecular characteristics of early lesions can differ significantly from those of advanced cancers, making it difficult to create universal detection methods that work across cancer types and stages. Accordingly, identifying stable biomarkers for early-stage HCC or iCCA remains challenging. Despite decades of cancer surveillance programs aimed at identifying individuals at risk for HCC, their effectiveness in detecting early-stage HCC and improving survival outcomes has been inconsistent. Most liver cancer cases are still diagnosed at advanced stages, which limits the opportunity for curative treatments and contributes to poor survival rates. There is a critical need for an improved biomarker-guided surveillance strategy for early liver cancer detection. Several alternative strategies have been proposed to address these challenges, including the development of synthetic biomarkers, the use of multiplexed biomarker panels, machine learning applications, and sensors designed to detect tumor-specific signals, all of which aim to improve the signal-to-noise ratio for early detection. Given that cancer may arise from failures in immunosurveillance, we hypothesize that a history of viral exposure, reflecting virus-host interactions, could indicate the status of host immunity and serve as a stable biomarker for early liver cancer onset. To explore this, we utilized a phage-display immunoprecipitation sequencing (PhIP-seq) approach to profile human serological responses to human virome as potential synthetic biomarkers for early HCC onset. Using a gradient-boosting machine learning model, we identified an optimal viral composition that could distinguish HCC patients from controls in our discovery cohort, as a part of the NCI-UMD study. We developed an HCC-specific viral exposure signature (HCC-VES) and validated it in a longitudinal cohort of at-risk individuals who were followed for HCC development. To extend this study, we analyzed sequentially enrolled 2,944 Thai patients with HCC and iCCA as a part of the TIGER-LC consortium. Through machine learning techniques, we developed viral scores for HCC and iCCA, finding that both scores were positively associated with several liver function markers in two distinct at-risk populations, independent of viral hepatitis status. Notably, the HCC score predicted all-cause mortality over an eight-year period in patients with chronic liver disease at risk of HCC, whereas viral hepatitis status did not predict survival. These findings suggest that non-hepatitis viral infections may play a role in the development of HCC and iCCA and could serve as potential biomarkers in at-risk populations. Collectively, these findings provide proof of concept that a viral exposure signature could serve as a predictive biomarker for HCC in at-risk populations, potentially enhancing current surveillance programs.

ONE HEALTH SELECTION AND TRANSMISSION OF ANTIBIOTIC RESISTANCE IN THAILAND, ARGENTINA AND THE UNITED KINGDOM

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Antibiotics have been in clinical use for more than 70 years and are responsible – perhaps more than any other drug type – for increasing human life expectancy world-wide. This is due to their ability to cure potentially life-threatening bacterial infections, and their utility in preventing infections that can result following life-extending healthcare interventions. Antibiotics are derived from naturally occurring chemicals, produced by microbes to kill their competitors. Hence many families of environmental bacteria have been exposed to antibiotics for millions of years and mechanisms have therefore evolved that overcome the actions of these chemicals. The most common antibiotic resistance mechanisms produced by bacteria are enzymes that modify or destroy the antibiotic when it enters a bacterial cell. The most important examples are the β-lactamase enzymes, of which hundreds are known.

The first antibiotic used clinically in a controlled manner was penicillin, a β-lactam antibiotic, which was deployed primarily to treat infections caused by Gram positive pathogens such as Staphylococcal wound infections and Streptococcal pneumonia. The first β-lactamase enzyme to be discovered – before penicillin was clinically used – came from the Gram negative bacterium *Escherichia coli*, but since penicillin was not used to treat E. coli infections this was more of a curiosity. Finding this enzyme – later called AmpC – was portentous, however, because a few years after the first use of penicillin, another β-lactamase enzyme, called BlaZ, emerged in *Staphylococcus aureus*, which within a decade was causing penicillin resistance in 90% of *S. aureus* from human infections in the UK. Indeed we now know that BlaZ did not evolve in *S. aureus* but millennia ago in another bacterium now lost to time. The DNA encoding BlaZ has been pasted onto a plasmid – a self replicating, self mobilizing circle of DNA that can move between bacteria – meaning that pre-evolved penicillin resistance rapidly spread among *S. aureus* strains.

After taming some Gram positive pathogens using penicillin, antibiotic research moved on to try and address the rising tide of Gram negative pathogens such as the family Enterobacterales, particularly *E. coli, Salmonella* spp. and *Klebsiella* spp., which collectively now cause a majority of sepsis deaths globally, numbering in the tens of millions per year. The development of the third generation cephalosporins (3GCs), another β-lactam class, was one of the most significant advances in this regard. 3GCs were stable against plasmid-encoded β-lactamase enzymes then known and whilst *E. coli* AmpC can destroy 3GCs, *E. coli* does not normally produce enough of this enzyme to confer 3GC resistance in a clinically meaningful sense; Salmonella spp and Klebsiella spp do not naturally produce AmpC at all. Another β-lactam class introduced was the fourth generation cephalosporins (4GCs) which are not normally broken down by AmpC.

As has so often been the case in the history of antibiotic discovery, early optimism following the introduction of 3GCs and 4GCs that the days of bacterial infection were numbered proved naive in the extreme. Instead, first, 3GCs were rendered useless in some E. coli by promoter mutations in the ampC gene leading to over-expression, so that AmpC enzyme abundance became great enough to confer 3GC resistance. Secondly, ampC genes from other Enterobacterales species became embedded onto plasmids, which could then circulate into *E. coli* and *Klebsiella* spp. The most important enzymes of this type are CMY-2 and DHA-1. Thirdly and most importantly was the emergence of extended spectrum β-lactamases (ESBLs), and particularly the enzyme CTX-M, which can confer 3GC resistance and 4GC resistance in

E. coli, Salmonella spp and *Klebsiella* spp, as well as many other less common pathogenic Enterobacterales. CTX-M enzymes are distributed into 5 groups based on their bacterial origin; all originate from species of *Kluyvera*, which are very rarely pathogens, but are found in the human gut, and in faecally contaminated sites alongside *E. coli* and the other pathogens. The two dominant CTX-M groups in most of the world are Groups 1 and Group 9, though in South America, Groups 2 and 8 are most common. These different CTX-M groups emerged from different species of *Kluyvera*, likely in different parts of the world.

Today, 3GC resistance in *E. coli* and other Enterobacterales is considered one of the most pressing threats to human health. With 3GC resistant Enterobacterales included among the "critical" group of priority pathogens by the World Health Organization. This is partly because 3GC resistance forces doctors to use last resort antibiotics, which will inevitably drive the emergence of resistance to them. Moreover, in many lower and middle income countries 3GCs are the de facto last resort, meaning that there is an unequal burden of 3GC resistance globally. Indeed, this issue is exacerbated because in many parts of the world the 3GC ceftriaxone is the only antibiotic active against Gram negative pathogens that can be given intravenously, and particularly in outpatient clinics. Hence it is relatively over-used in lower and middle income countries, compared with regions having access to a wider range of antibiotics. Another area of concern is the use of 3GCs to treat and prevent infections in farmed and companion animals, which is common in large parts of the world. This is because 3GC resistance could then result in increased morbidity and mortality in farmed animals, and reduced rates of food production. But also because it is possible that 3GC resistant *E. coli* and related species that emerge on farms might transfer to humans – through the consumption of meat and milk, plants fertilized with animal manure, or through human interaction with the environment, particularly water contaminated with animal manure. This possibility reduces food security and collectively these issues mean that not only is antibiotic resistance, and particularly 3GC resistance, a threat to human health, but to a number of United Nations Strategic Development Goals.

As well as releasing 3GC resistant *E. coli* (and those resistant to other antibiotics) into the environment and food chain, farms may also release antibiotics as chemical waste because antibiotics are generally excreted unchanged in feces and urine. Antibiotic residues in the environment may then further select for the emergence – or at least the maintenance – of antibiotic resistant *E. coli.* But of course resistant bacteria and antibiotics could also contaminate the environment from human sewage, hospital outflows, pharmaceutical factories, etc., and human 3GC resistant *E. coli* could colonize animals. Hence, the selection and transmission of antibiotic resistant fecal bacteria such as *E. coli* in humans, is a compelling focus of One Health research. The primary challenges are to quantify the degree (and direction) of transmission of resistant bacteria between One Health compartments, the degree of selection elicited by antibiotic use relative to incidental contamination in different One Health compartments, and the relative impacts of different human behaviors, and of geographical or meteorological conditions on the transmission and selection drivers defined as most important. Finally, it is essential to consider the socio-cultural, economic and political drivers of any relevant human behaviors. Through this complex and interconnected analysis it may be possible to propose changes that reduce the burden of resistant infections on human health and other relevant development goals in a particular region. This work is complex, and is likely to yield different outcomes when considering different pathogens, different antibiotics and different regions. A "one size fits all" solution may not be possible.

In this talk I will describe our work considering the selection and transmission of antibiotic resistant bacteria in a One Health context. And to do this I will use 3GC resistant E. coli as an exemplar. I will refer to three large projects: in the UK, the "One Health Selection and Transmission of Antibiotic Resistance" (OH-STAR) project; in Thailand, the "One Health Drivers of Antibiotic Resistance in Thailand" (OH-DART) project; and in Argentina, the "Future-proofing Antibacterial resistance Risk Management Surveillance and Stewardship in the Argentinian Farming Environment" (FARMS-SAFE) project. In each project, we have established study

regions, within which we have collected thousands of samples from humans (not FARMS-SAFE), animals and the environment, and tested these samples for 3GC resistant *E. coli.* Alongside, we have collected medicine usage and behavioral surveys and other meta-data, which allow epidemiological risk factor analysis to be completed. Our goal is to identify variables associated with the increased odds that a sample is positive for 3GC resistant *E. coli.* We have also used whole genome sequencing (WGS) of the 3GC resistant *E. coli* present to allow core-genome alignment, which gives evidence of sharing bacteria between samples, and so potential transmission between One Health compartments.

In the OH-STAR project, based in South West England, we have WGS data for 3GC resistant *E. coli* from 54 dairy cattle farms – a common form of farming in this region – and also from hundreds of human urinary tract infections. Samples were collected in parallel in a 50 x 50 km study region. Phylogenetic analysis confirmed that there is no phylogenetic overlap suggestive of recent sharing of 3GC resistant *E. coli* between these compartments. However, since there is a time lag – which can be many years – between ingestion of *E. coli* by a person and the emergence of an opportunistic urinary tract infection, our analysis likely underestimates the real impact of 3GC resistant *E. coli* from farms on human infections. This is also a problem for other published studies with similar conclusions. In the talk, I will discuss analysis of an index called “Farm Animal Specific Resistance”, because by using this, we have identified that there is a proportion of human urinary tract and bloodstream infections in the OH-STAR study region that have been caused by *E. coli* that evolved within farmed animals during the lifetimes of the people infected. This is particularly true of phylogroup A and B1 *E. coli*, which are commonly carried by farmed animals, and cause around 15% of human urinary an bloodstream *E. coli* infections in the OH-STAR study region. In contrast, phylogroup B2 *E. coli* are the dominant form of *E. coli* from these human infections, and have never been seen on farms in our study. Hence whilst the impact of antibiotic resistant bacteria from farmed animals on human infections is limited, it is not negligible, and it is therefore important to reduce the levels of antibiotic resistance in *E. coli* on farms, and particularly 3GC resistance, which can pose a major barrier to treatment of human infections. Our work on dairy cattle farms in OH-STAR identified antibiotic usage risk factors that are associated with the proportion of samples positive for 3GC resistant *E. coli* on farms. Specifically, the use of 4GCs was associated with increased sample level positivity for *E. coli* carrying the CTX-M β-lactamase, an enzyme that confers 4GC resistance as well as 3GC resistance (but not amoxicillin-clavulanate resistance). However, amoxicillin-clavulanate usage was associated with sample-level positivity for E. coli hyper-producing AmpC, which are 3GC resistant and amoxicillin-clavulanate resistant (but not 4GC resistant). Immediately after our 2-year survey of 3GC resistant *E. coli* on the 54 OH-STAR study farms, the use of 3GCs and 4GCs (but not amoxicillin-clavulanate) was banned in all but very rare circumstances. We hypothesized that this would result in a reduction in CTX-M positive *E. coli* but not AmpC hyper-producing *E. coli* due to changing patterns of on-farm selection. Resampling the farms three years later allowed us to test our hypothesis and the results will be discussed in the talk.

In the FARMS-SAFE project in Central Eastern Argentina, we have sampled from 70 farms over a one year period, including dairy cattle and pig (swine) farms. This survey showed 33.9% sample-level prevalence for 3GC resistant *E. coli* dominated by CTX-M. WGS analysis confirmed that the isolates are highly variable, suggesting long-term circulation across our study regions. However, the specific mechanisms of resistance seen among 3GC resistant *E. coli* different significantly between pig and dairy farms, suggestive of different antibiotic usage practices in each. For example there was more plasmid-encoded AmpC (CMY-2) mediated 3GC resistance in pig farms and more amoxicillin-clavulanate resistance among 3GC resistant isolates, suggesting that amoxicillin-clavulanate selection is higher in pigs than dairy cattle on our study farms. Similarly, florfenicol resistance was more common in 3GC resistant *E. coli* from pig rather than dairy cattle farms and again this suggests imbalances in the use of this antibiotic in these two systems. We are currently investigating antibiotic usage practices and performing detailed management practice analyses. One key risk factor for the

presence of 3GC resistant *E. coli* on farms is temperature, with resistant bacteria being found less often on dairy cattle farms during the colder months in Argentina. This was also seen in the dairy cattle farms sampled for the OH-STAR study in the UK, and the implications of this for routine surveillance will be discussed in the talk.

Another aspect of our OH-STAR work was to consider the risk factors associated with an increased odds that domestic pet dogs excrete 3GC resistant *E. coli*. This is important because it is know that dogs and their owners can share resistant bacteria. In two studies, one of 224 puppies (<16 weeks of age) and one of 600 adult dogs the key risk factor for excretion of 3GC resistant *E. coli* identified is the dog being fed raw (uncooked) meat. This was particularly true for *E. coli* sequence types commonly found in farmed animals. Further work that will be discussed in the talk is our investigation of the contamination with resistant *E. coli* of meat sold for human consumption, and of raw dog food in our study region, and the relationships between these *E. coli* and those causing human infections and those colonizing dogs fed raw meat. It seems therefore that ingestion of 3GC resistant *E. coli* from farmed animals has a limited impact on human health, but practices such as eating undercooked meat, or poor hygiene when preparing and cooking raw meat will increase the risk of transmission of resistant *E. coli* from farmed animals to humans and domestic pets.

Our study area for the OH-DART project is in central Thailand and includes semi-urban and rural areas in which we have collected almost 10,000 samples from humans, farmed animals, food sold in local markets, and water and soil samples in the wider environment. Samples have also been tested for the presence of antibiotics, heavy metals and pesticides, but the results of this analysis will not be discussed. In terms of 3GC resistant *E. coli,* sample-level positivity across the OH-DART study region was high; almost 60% of fecal samples from people were positive. 3GC resistance was predominantly caused by CTX-M and these data will be discussed in more detail during the talk. Overall our results tend to support the findings of OH-STAR, that 3GC resistant *E. coli* from animals play a minor, but not insignificant role in the emergence of 3GC resistant infections in humans. So, for example, during a mathematical modelling study parameterized by levels of 3GC resistant *E. coli* found among multiple surveillance studies in Thailand, we were able to test the impact on 3GC resistance in human infections of reducing antibiotic usage in human medicine, or those given to farmed animals, and the impacts of reducing human to human or farmed animal to human transmission. Overall the model predicted that by far the greatest impacts would be found when reducing antibiotic usage by humans and human-human transmission. However, the impact of farmed animal derived 3GC resistant *E. coli* is not minimal, as we have shown when considering risk factors for people in the OH-DART study area associated with the odds of them excreting 3GC resistant bacteria. One risk factor that was very difficult to model was the usage of antibiotics in the community in our study region in Thailand. This was largely due to the problems of obtaining an accurate assessments of the frequency and magnitude of antibiotic consumption in a community setting where antibiotic use is frequently “silent”, as we have shown using ethnographic analysis. In hospitalized patients, in contrast, it was clear that 3GC usage was directly associated with an increased odds of a patient excreting 3GC resistant *E. coli.*

Overall, the work that will be discussed in this talk concerning 3GC resistant *E. coli* carriage, which is considered critically important as a threat to human health by the World Health Organization, demonstrate that antibiotic usage practices do play a significant role in the selection of 3GC resistance, and particularly the mechanism of 3GC resistance and the types of co-resistance present; both in humans and farmed animals. But overall the work shows that the emergence of 3GC resistant *E. coli* in farmed animals has a relatively small impact on the number of 3GC resistant human infections compared with those 3GC resistant *E. coli* transmitted between humans. Indeed, when 3GC resistance is already common among humans, the impact of additional 3GC resistance coming from farmed animals is likely to be minimal. Our work does show, however, how resistance mechanisms present in bacteria carried in farmed animals that are rare in humans might enter the human population. One clear case was the emergence of

colistin resistance among pigs, which later transferred to humans, and another is the presence of nitrofurantoin resistant *E. coli* in poultry, which is a key discovery of OH-STAR. In both cases, the fact that antibiotics were used extensively in agriculture, and subsequently found an important place in human medicine, where resistance was rare seen, left animals as a key source of zoonotic transmission of resistance. The work presented will give insight into the interventions that might be deployed to minimize this zoonotic threat. For example, food preparation, storage and cooking practices are likely very important when considering the risk of ingestion of resistant *E. coli* derived from farmed animals.

Acknowledgements

There are many colleagues who have been involved in the work that will be reported in this talk and their names will be listed there. But particularly I would like to acknowledge my key co-investigators Kristen Reyher, Tristan Cogan, Katy Turner, David Barrett, Helen Lambert, Ed Feil, Henry Buller, Philip Williams, Alasdair MacGowan, Alastair Hay, Walasinee Sakcamduang, Visanu Thamlikitkul, Luechai Sringernyuang, Jutamaad Satayavivad, Andrew Singer, Luzbel De La Sota, José Giraudo, Fabiana Moredo, and Nora Mestorino. This work has been funded by the Medical Research Council, the Biotechnology and Biological Sciences Research Council, the Natural Environment Research Council, the Welsh Government, the Medical Research Foundation, the UK Department of Health and Social Care, UK Aid and the UK National Institute for Health Research.

LESSONS FROM WASTEWATER SURVEILLANCE: IMPACT OF COVID-19 ON AMR

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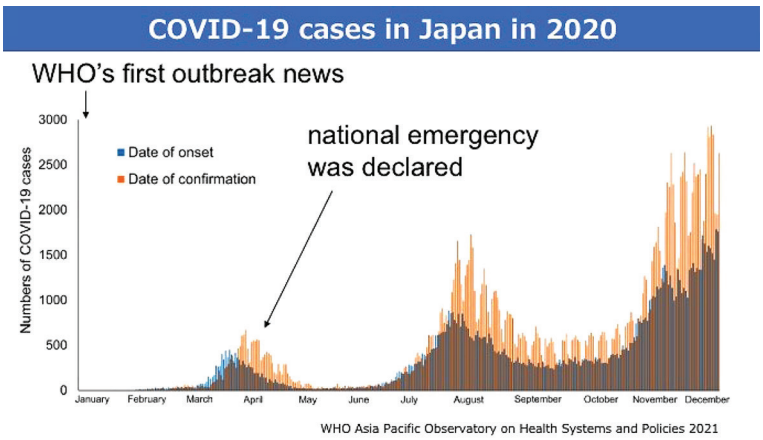
Antimicrobial resistance (AMR) represents one of the most critical global health threats of the 21st century. A comprehensive analysis of the Global Burden of Disease study revealed that over 7 million deaths were attributed to bacterial infections, representing over 10% of all global deaths and the second leading cause of mortality globally. It is therefore essential that antibiotics are readily available and used properly to prevent and treat bacterial infections. AMR infections are challenging to treat, resulting in longer hospital stays and less favorable clinical outcomes. The silent pandemic of rising AMR pathogens is driven by multiple factors, including the overuse and misuse of antibiotics and the lack of infection prevention and control measures in healthcare settings. These factors are undermining the efficacy of existing treatments and jeopardizing all medical practices in modern medicine, including the treatment of cancer patients. The globalization of antimicrobial supply systems, international travel, and climate change further complicate the issue, creating a challenging environment for effective management. In the Western Pacific Region, AMR is estimated to result in 5.2 million deaths and 172 million additional hospital days between 2020 and 2030. Moreover, the projected economic impact is estimated at US$148 billion over the specified period.

This year, the 79th United Nations General Assembly High-Level Meeting on AMR was held on September and political declaration has been approved to accelerate the fight against AMR. In the declaration, the Quadripartite was expected to play central role in supporting the global response to AMR through One Health approach. The One Health concept is based on the understanding that human, animal, and environmental health are deeply interconnected. The Quadripartite is a newly established consolidation mechanism including the Tripartite (the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (WOAH)) plus the United Environment Programme (UNEP). Based on this, it was recognized the need to strengthen cross-sectoral data sharing through innovative surveillance approach, and an efficient multi-sectoral surveillance system has been eagerly awaited to collect cross-sectoral data to monitor AMR trends.

Based on the idea of Dr. A. Andremont, a member of the Advisory Group on Integrated Surveillance of Antimicrobial Resistance (AGISAR), AGISAR and the WHO Surveillance Prevention and Control of AMR team have developed the One Health surveillance system, Tricycle protocol for global surveillance on extended-spectrum β-lactamase producing *Escherichia coli* (ESBL-Ec). This is a simplified integrated, trans-sectoral surveillance protocol for AMR and focuses on the single key indicator: the frequency rates of ESBL-Ec. The Tricycle is named after its three-wheel configuration, which reflects One Health approach towards three aspects of AMR in sectors of human health, food chain, and the environment. Tricycle surveillance is linked with the WHO Global Antimicrobial Resistance and Use Surveillance (GLASS) and can be expanded to include satellite surveillance and research project of AMR.

In Japan, we started discussing the implementation of Tricycle surveillance in 2018 and a pilot study on wastewater surveillance was initiated in 2019, one step ahead, as we have no prior experience. Wastewater sampling was conducted twice a year, in June and November each year. Then, in December 2019, the first reports of a new respiratory disease from Wuhan, China began to surface but the initial response of Japan was cautious because the extent of virus spread was not clear. In January 2020, the first case of COVID-19 was confirmed in Japan and the outbreak began to intensify in February 2020, when the Diamond Princess cruise ship docked in Yokohama became a daily news hotspot for a large outbreak. In early April, Japan declared a state of emergency due to the widespread spread of the virus. The government encouraged voluntary social distancing, school

closures, and the cancelation of major events. Japan experienced a second wave of COVID-19 in the summer of 2020 and this surge strained the healthcare system, which faced challenges in accommodating the increasing number of patients.



We had the privilege of collecting wastewater samples taken before and after the onset of the COVID-19 pandemic started in Japan, and analyzing the data based on the Tricycle surveillance protocol. Cefotaxime (CTX)-resistant and susceptible *E. coli* were isolated from untreated wastewater collected from four large wastewater treatment plants in Hiroshima Prefecture, Japan, between June 2019 and October 2020. The percentage of ESBL carriers (ESBL-Ec/all isolated *E. coli*) in each sample was calculated, and all isolates obtained in this study were conducted whole genome sequencing and standardized quantitative antimicrobial susceptibility testing. After the COVID-19 pandemic, a significant reduction (from 9.8% to 4.9%) of the ESBL-Ec rate in wastewater samples was observed, although the concentration of *E. coli* was stable. We have a surveillance system that collects all the ESBL-Ec isolates from major regional hospitals in Hiroshima but the surveillance data indicated that the detected number of ESBL-Ec did not change significantly in 2020 compared to 2019. On the other hand, the local antibiotic consumption in terms of number of patients decreased by 27.1% and the consumption of third-generation cephalosporins decreased by 17.7%. We examined the impact of COVID-19 on the use of healthcare facilities. The number of consultations for upper respiratory tract infections in healthcare facilities and the number of patients visiting local healthcare facilities decreased by approximately 37.3% and 38.4%, respectively in 2020 compared to 2019.

The significant decrease in the percentage of ESBL-ECs in wastewater in 2020 was not merely accompanied by a decrease in the number of ESBL-ECs, but also by a clonal change of the isolates. The number of certain ESBL-Ec clones (ST38 $bla_{\text{CTX-M-14}}$ and ST131 $bla_{\text{CTX-M-27}}$) remained stable but other ESBL-Ec clones decreased or disappeared completely in 2020. Our *in vitro* study showed that the entire blaCTX-M-14 gene in ST38 was located on its chromosome, and 89% (24/27) $bla_{\text{CTX-M}}$ gene in ST131 was located on its chromosome or on the high stability (F1:A2:B20) plasmid, suggesting that these ESBL-Ec were highly stable even after antimicrobial pressure was reduced or removed. Most of ESBL genes are often carried on plasmids, which plays a crucial role in the rapid dissemination of antimicrobial resistance. However, plasmids are known to be a burden to bacteria, and the extra genetic material can reduce the bacterial fitness, particularly in environments where antibiotics are not present. Under condition of reduced selective pressure, other ESBL-Ec clones in the wastewater may have dropped the ESBL plasmid.

Our data suggest that the reduced antimicrobial pressure after the onset of the COVID-19 pandemic began in the community likely contributed to the decrease in the number of ESBL-Ec in wastewater. The observation that the decrease in the prevalence of ESBL-Ec in wastewater preceded that in clinical settings is highly significant and provides valuable insights into how environmental monitoring and AMR dynamics are intertwined. It suggests that environmental monitoring, especially wastewater monitoring, is a powerful tool for tracking trends in AMR and could help predict future clinical resistance patterns.

MINING THE EPITRANSCRIPTOME FOR FIRST-IN-CLASS ANTIMICROBIALS

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The Central Dogma defines the "what" of biology: genes are transcribed into mRNAs that are translated into proteins. But it says nothing about the "when" or "how much" of expressing 20,000 genes in humans or 5000 genes in bacterial pathogens. Convergent technologies have uncovered information-rich scheduling systems for gene expression involving the dozens of chemical modifications of DNA, RNA, and histone proteins in every cell – the epigenome and epitranscriptome. Using multi-omic and informatic approaches, we discovered and validated a mechanism of translational control of cell response in all forms of life, from RNA viruses to humans. This mechanism involves stress-induced reprogramming of dozens of tRNA modifications and tRNA copy numbers to facilitate selective translation of codon-biased mRNAs critical for cell adaptation and survival. Emerging evidence suggests that this fundamental system serves as a rich source of new drug targets. Corrupt expression of tRNA-modifying enzymes in many cancers and other diseases as well as distinctly different enzyme structures in humans and pathogenic microbes support their validity as targets for drug discovery.

Here we describe recent efforts to develop and apply tools to facilitate drug discovery and development against the tRNA epitranscriptome. Among these is a high-throughput platform for screening microbial and mammalian gene knockout libraries for target identification and for screening compound libraries against purified proteins and cells. Automation of next-generation and mass spectrometry-based RNA-seq on the platform allows quantitation of small RNAs and mapping modifications in specific tRNAs to validate drug targets and identify diagnostic biomarkers. We demonstrate the utility of the platform for developing antibiotics and resistance-reversing antimicrobial adjuvants and whole-cell phenotypic screens of curated libraries of tRNA-modifying enzyme inhibitors for anticancer and antimicrobial drug discovery.

AMASS: AUTOMATED TOOL FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE SYSTEM - HOW THAILAND UTILIZES TIMELY DATA FROM 127 PUBLIC HOSPITALS FOR ACTIONS AT THE FACILITY AND NATIONAL LEVELS

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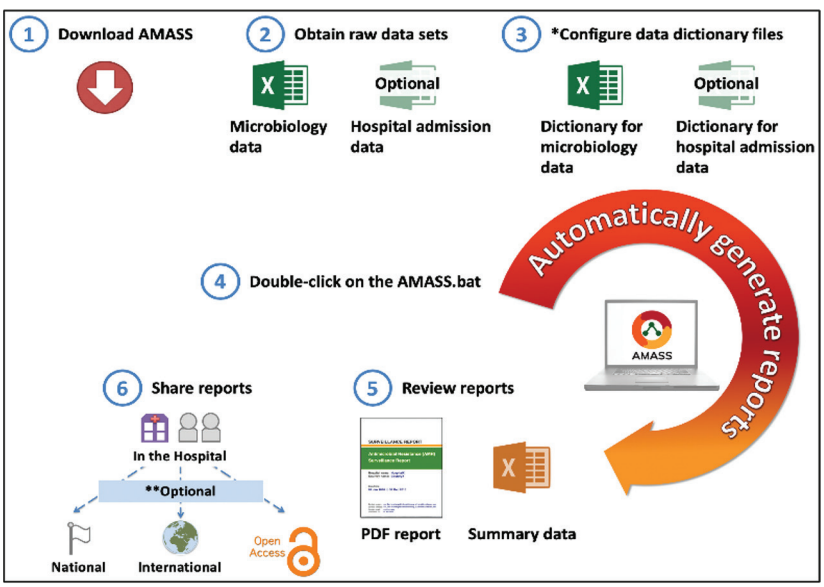
AMASS (AutoMated tool for Antimicrobial resistance Surveillance System)

In 2019, we developed AMASS (AMASSv1.0), an open-access, offline, and easy-to-use application that enables hospitals to independently and automatically analyse their own microbiology and hospital admission data files to generate facility-specific AMR surveillance reports (Fig. 1) (www.amass.website) (PMC7568216). AMASS categorizes infections as community-origin when the first specimens culture positive for a pathogen are obtained within the first two calendar days of hospitalization. Infections are categorized as hospital-origin when the first culture-positive specimens are obtained after two calendar days of hospitalization as defined by the WHO GLASS. AMASS overcomes important limitations (e.g. lack of experts, data complexity, and data security) by using a Microsoft Excel-compatible, data dictionary and onsite approach. The generated report (in PDF) and summary data (in Excel) are easy-to-use and easy-to-share. Our tool thus improved our understanding of AMR in six LMICs.

Fig 1. Conceptual framework of AMASS (JMIR, 2020, CC-BY 4.0, PMC7568216).



In 2021, we expanded AMASS to AMASSplus, which additionally analyzes and generates notifiable bacterial disease reports (e.g. *Burkholderia pseudomallei* infection and *Streptococcus suis* infection. Using AMASSplus, we showed that, between 2020 and 2021, the number of cases in the national notifiable disease surveillance system was severely underreported in six hospitals in Thailand (PMC11229635).

In 2022, we expanded AMASSplus to AMASSv2.0, which additionally analyzes and reports on quality indicators of microbiology data (e.g. percentage of unusual resistance profiles). Using AMASSv2.0, we showed that, between 2012 and 2015, the frequency of AMR bloodstream infection (BSI) in tertiary-care hospitals was higher than that in secondary-care hospitals (PMC11104583).

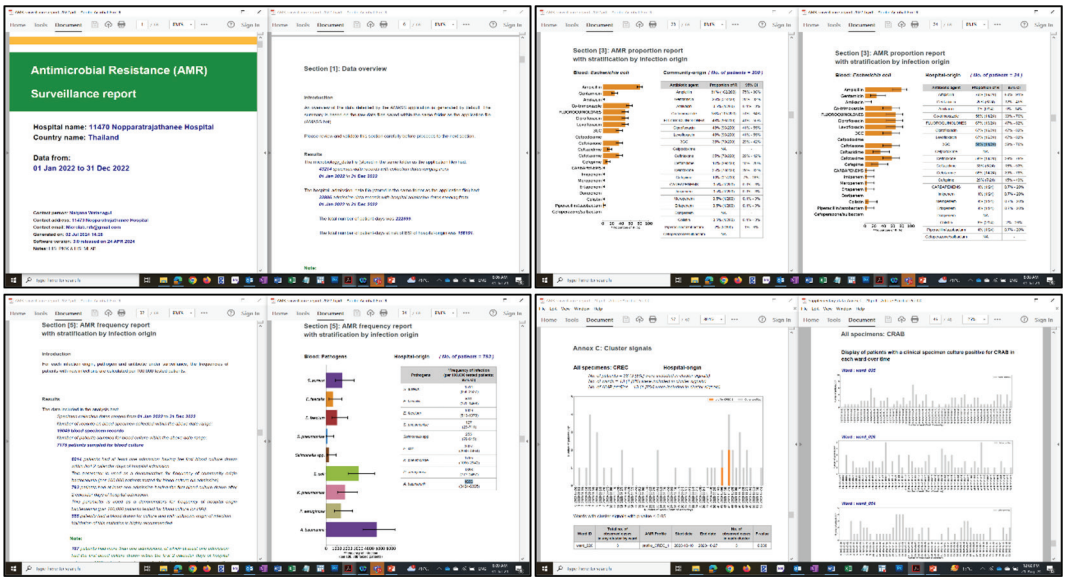
In 2023, the Ministry of Public Health (MoPH) Thailand implemented AMASSv2.0 in 127 public hospitals for data from 2022 (PMC10349292 PMC7568216). We have been collaborating with the MoPH by training the trainers, leading workshops, validating reports, and supporting two-way communications between the hospitals and the MoPH.

Local and timely AMR data are crucial for local and national actions. We strongly suggest that all hospitals in LMICs that have electronic data records should analyse and utilize their data for immediate actions using any appropriate analytical software or tools.

In 2024, we expanded AMASS to AMASSv3.0, which additionally analyzes and reports on clusters of hospital-origin AMR infections by integrating open-access SaTScan codes into AMASS. In 2024, MoPH implemented AMASSv3.0 for data from 2023.

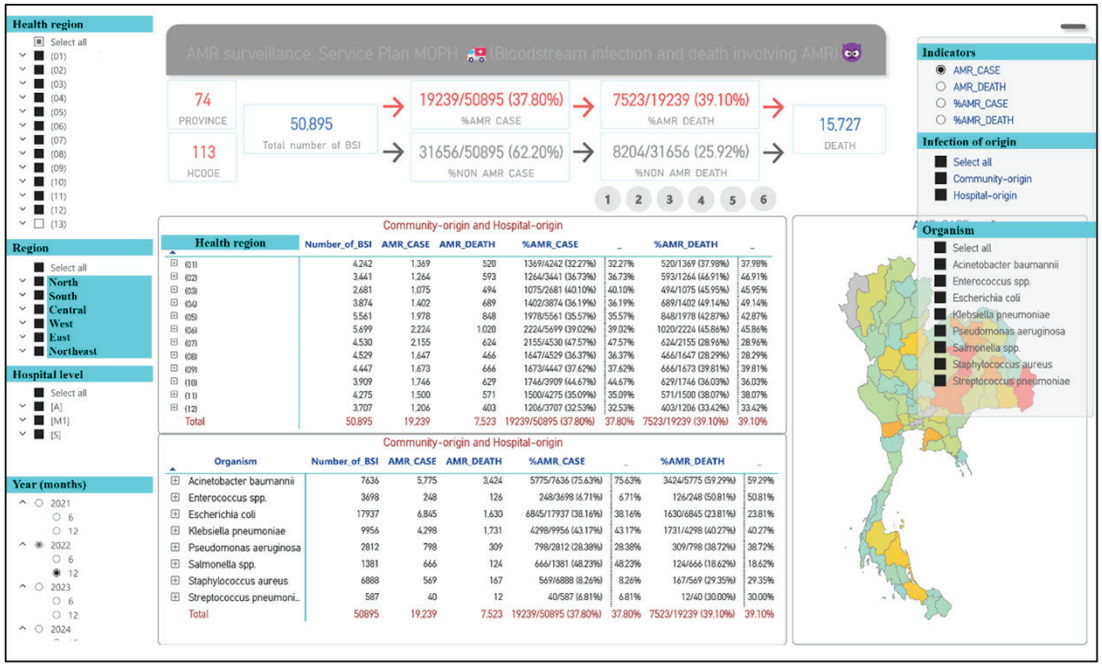
At the hospital level, AMS/IPC teams agree that they can utilize AMASS reports for their programmes. Examples of each individual AMASS report is available in the appendix and data depository of PMC7568216 PMC11229635 PMC11104583 PMC10349292 (Fig 2.)

Fig 2. An example of AMASS report of a hospital (in PDF format)



Using AMASS summary data (in Excel) submitted by the hospitals, the MoPH launched a new open-access dashboard presenting proportion, frequency, mortality and deaths following AMR BSIs in each hospital and nationwide (Fig. 3).

Fig 3. New open-access interactive Thailand AMR dashboard (full link is also available at J Infection , 2024, CC-BY 4.0, PMC7568216).

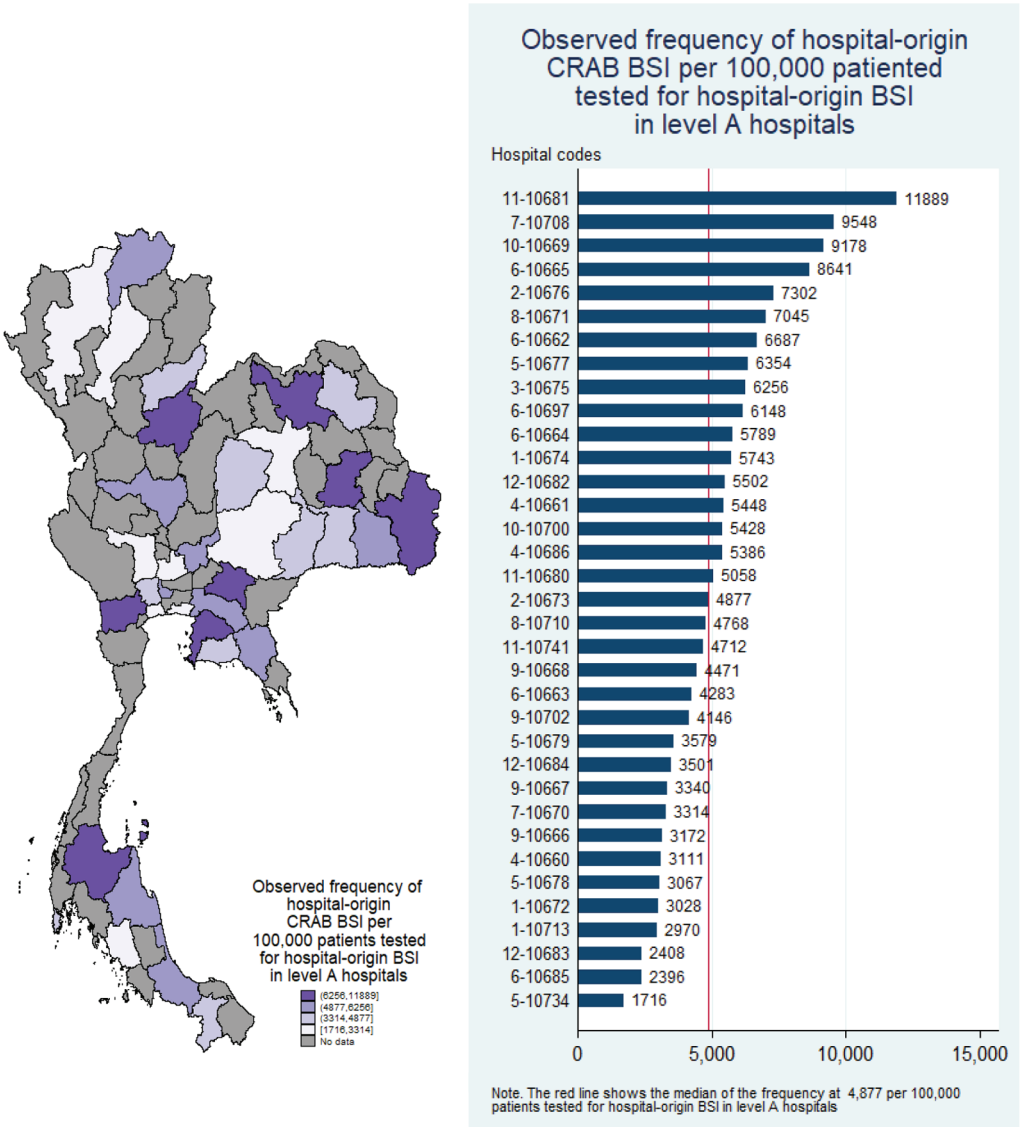


We show that, in 2022, the most common cause of community-origin AMR BSI was third-generation cephalosporin-resistant Escherichia coli (3GCREC, 65.6%; 5,101/7,773 patients) and of hospital-origin AMR BSI was carbapenem-resistant Acinetobacter baumannii (CRAB, 51.2%, 4,968/9,747 patients) (PMC7568216). The total number of deaths following community-origin 3GCREC BSI was 1,048, representing 54.1% (1,048/1,936) of all deaths following community-origin AMR BSI caused by pathogens under evaluation. The total number of deaths following hospital-origin CRAB BSI was 2,955, representing 58.7% (2,955/5,036) of all deaths following hospital-origin AMR BSI caused by pathogens under evaluation.

Hospitals in health regions 4 (lower central region) had the highest frequency of community-origin 3GCREC BSI (adjusted incidence rate ratio, 2.06; 95% confidence interval: 1.52-2.97). Health regions were not associated with the frequency of hospital-origin CRAB BSI, and between-hospital variation was high even adjusting for hospital level and size. The high between-hospital variation of hospital-origin CRAB BSI suggests the importance of hospital-specific factors (such as AMS and IPC programmes).

At the country level, in 2023, the MoPH announced that the AMR key performance indicators in the country's operation plan (called AMR service plan) were changed to frequency of hospital-origin BSI caused by CRAB, and carbapenem-resistant Klebsiella pneumoniae (CRKP) and Escherichia coli (CREC) per 100,000 tested patients. The data and new indicators enable the MoPH to prioritize interventions (e.g. re-evaluation of AMS and IPC programmes being implemented) at the hospitals with the highest frequency of CRAB, CRKP or CREC BSI (Fig 4).

Fig 4. Observed frequency of hospital-origin CRAB BSI (per 100,000 tested patients) among 35 level-A hospitals in Thailand (J Infection, 2024, CC-BY 4.0, PMC7568216)



In the future, we aim to expand AMASS to additionally analyze pharmacy data and reports on conventional and new indicators of AMS and diagnostic stewardship.

The development of AMASS is funded by the Wellcome Trust. The implementation of AMASS in Thailand is supported by the MoPH Thailand.

Please note that AMASS is an open-access tool. Any hospitals and policy makers in any countries are more than welcome to test and use AMASS as they see fit. Some countries have tried AMASS (PMC9117993 PMC10021607). We are more than happy to train the trainers, and advise how to use and implement the tool for actions against AMR at both hospital and national levels.

More information is also available at www.amass.website and amass@tropmedres.ac.

PROTECTING ANIMALS AND HUMAN LIVES FROM RABIES PROJECT: ONE HEALTH APPROACH FOR RABIES CONTROL AND ELIMINATION IN THAILAND

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Rabies is an important zoonotic viral disease in humans and other mammals. Thailand has faced persistent rabies outbreaks, prompting national efforts to align with the World Health Organization's goal of zero human rabies deaths by 2030. This study highlights the implementation of the Protecting Animals and Human Lives from Rabies Project initiated by HRH Princess Chulabhorn Mahidol. Operating under the One Health approach, the project integrates strategies across animal and human health sectors, encompassing animal and human rabies surveillance, prevention, and control, as well as legislative measures, public awareness campaigns, and community engagement.

The project's first phase (2017–2020) achieved a notable reduction in rabies cases among animals and humans, with significant milestones reached by the third year. However, challenges such as the COVID-19 pandemic and vaccine shortages during the second phase (2021–2025) temporarily impeded progress, leading to a resurgence in outbreak trends. In response, intensified measures have been implemented, including immunization efforts, population control of dogs and cats, comprehensive pet populations management, improved disease surveillance, and strengthened community engagement. Preliminary results indicate the successful establishment of rabies-free zones in some areas of Thailand, with promising potential for expansion nationwide.

This integrated approach demonstrates the effectiveness of One Health concept in addressing zoonotic diseases, providing a model for other nations seeking to elimination rabies. Sustained collaboration across sectors remains critical to achieving long-term success and ensuring a rabies-free future in Thailand and beyond.

ONE HEALTH IN ACTIONS ON ZOONOSES AND AGRIFOOD SYSTEMS: PERSPECTIVES FOR GLOBAL HEALTH AND FOOD SECURITY

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The One Health concept emphasizes the interconnectedness of human, animal, plant, and environmental health and is crucial for addressing global challenges such as zoonotic diseases, antimicrobial resistance (AMR), and food security. Zoonoses—diseases that spread between animals and humans—pose significant risks to global health, animal and food production, and ecosystems. Addressing these threats requires a One Health approach that integrates health measures across species and environments.

The Food and Agriculture Organization of the United Nations (FAO) plays a leading role in applying One Health principles to agri-food systems. By focusing on the health of animals, plants, and ecosystems, FAO seeks to enhance food safety, productivity, and environmental sustainability. This approach is central to achieving the FAO Strategic Framework 2022-2031, which aims to build more efficient, inclusive, sustainable, and resilient agrifood systems, supporting global health and food security.

Managing zoonotic diseases like highly pathogenic avian influenza (HPAI), Rabies, Anthrax, Brucellosis, Ebola, Nipah Virus, Middle East Respiratory Syndrome (MERS), COVID-19 (SARS-CoV-2), Mpox, etc and reducing AMR are key focus areas of FAO's One Health initiatives. These efforts involve multisectoral collaboration between human, animal, plant, and environmental health sectors. Through measures such as integrated surveillance, early warning system, biosecurity, laboratory capacity building, animal health workforce development, and responsible antibiotic use, FAO helps countries prevent disease transmission, improve food safety, and protect biodiversity.

This abstract highlights the importance of One Health in transforming agrifood systems, addressing zoonotic diseases, and promoting global health and food security. FAO's leadership and collaboration with WHO, WOAH, and UNEP through the Quadripartite collaboration strengthen coordinated responses to health threats, ensuring a sustainable and healthy future for all species and ecosystems.

SUCCESSFUL ONE HEALTH APPROACH TO REDUCE ZOONOTIC DISEASE FROM PIGS

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Effective One Health strategies for preventing zoonotic diseases must incorporate capacity building, communication, collaboration, and coordination at the community level. Addressing zoonotic diseases linked to pigs requires community engagement from all stakeholders in the pig value chain, including farmers, buyers, butchers, market sellers, government inspectors, processors, home cooks, and consumers. One Health research also necessitates interdisciplinary collaboration and coordination among agencies, with teams working together to gather data on animal and human health and disease. A successful One Health response can be assessed using tools like outcome mapping, which highlights both knowledge acquisition and long-term behavior changes throughout the entire pig value chain.

The presentation will describe how a one health approach was used to reduce the prevalence of *Cycticercus cellulosae* in pigs, a leading cause of epilepsy in people. The *Taenia solium* tapeworm is linked to 30% to 70% of epilepsy cases (neurocysticercosis) in regions with poor hygiene and where pigs are allowed to roam freely (1). It ranks as a major cause of foodborne fatalities. The larval stage of this tapeworm develops in pig muscle (*C. cellulosae*) and individuals who eat undercooked, infected pork can contract the adult tapeworm. To ensure pork is safe, it should be cooked to 60°C for 15-30 minutes, which will eliminate the larvae. Epilepsy results from the ingestion of tapeworm eggs. To combat the prevalence of this parasite, educating farmers is essential.

The presentation will describe how this one health approach was used to impact behaviour changes across the pig value chain to reduce the likelihood of epilepsy due to the tapeworm.

Methods

This multi-year, prospective, longitudinal observational study employed a transdisciplinary approach (Table 1) whereby an interdisciplinary team of academics worked together with community members across the pig-value chain and the local, district and provincial government personnel. The project had three main areas of focus; small-holder farmers, pig butchers and pig nutrition.

Farmers: A longitudinal observational study included a random sample of 163 smallholder pig farmers from rural Western Kenya (2). In year one, the research began by engaging with the community. This included farm visits, focus group meetings with farmers, and key informant interviews with village chiefs and elders, government extension officers, veterinary inspectors, and district government officials. The community engagement provided researchers with an understanding of the farmers' challenges and opportunities and the concerns of those supporting the local pig industry. Farms were visited every 5 months for three separate visits to survey the farmers about baseline farmer management practices and knowledge. In subsequent surveys, data were gathered about changes to pig management. Each pig on the farm was examined for presence of lingual cysts. A farm was considered positive if any pig was positive for cysticerci, the larval cysts of *T. solium* (2). The age, source, gender and weight of the pig was recorded. One-on-one trainings were provided to these farmers at each visit. Village-level trainings were conducted; in year 1 and year 3 (3). In total, 451 farmers attended one or both trainings.

A train-the-trainers model was used for farmer training. The two lead authors (Dewey and Mutua) wrote a pig-keeping training manual and provided 2, one-day training sessions for government extension workers, and key community leaders such as teachers and village chiefs (4). The extension workers included agronomists, veterinarians, social workers, public health specialist, and adult-education specialists. They were facilitated to provide training to local farmers (3). The training in year one provided information on the cycle of sustainable pig production, reproduction, feeding, housing, lifecycle of T. solium, identification and control of common pig diseases and parasites. In year 3, the training focused on research results that were shared with the village farmers. Details of the trainings are described elsewhere (15, 16).

In year 5, two years after the second workshop, an outcome mapping program was conducted to determine whether farmers made long-term management changes and if they changed behaviours to prevent the lifecycle of T. solium. A new random sample of 50% of pig-farmers in each village was selected at this stage. Farmers were asked two open-ended questions, What have you changed in your pig keeping since the research team came to the community four years ago? and What are you doing to prevent epilepsy caused by the tapeworm? (4) In years 6 to 10, the work expanded to include swine nutrition. The purpose of the research was to develop complete pig feeds using mostly weeds and freely available or inexpensive food from the market. Feed trials were completed to compare growth rate using commercial feed versus homemade forage diets.

Butchers: In year two, the research project expanded to include all 16 of the butchers who purchased pigs from these villages and sold pork. The researchers conducted community engagement with butchers, people working in abattoirs, restaurant owners, and consumers of pork. Butchers were surveyed to determine their challenges and opportunities as business owners, the variable and fixed costs of the butchery, what they paid for live-weight of pigs, price of pork per kilogram, what they did to ensure pork safety, and if they were aware of C. cellulosae (the larvae of Taenia solium).

In year three, 3-hour workshops were given for the butchers that focused on business training, accounting, and pork safety. The business training emphasized the importance of co-operation and trust between farmers and butchers to ensure a sustainable pig industry. The butchers were encouraged to discuss possible solutions to the challenges described by butchers the previous year (5). These included seasonal variation in consumer demand for pork and pig availability, finding pigs for sale, financial requirements for purchasing pigs, capital investments and annual business and health licenses. The group workshops were followed by one-on-one business training based on the butcher's own fixed and variable costs of their business. In year five, the butchers were included in the outcome mapping project mentioned above.

Statistical analyses: McNemar chi-square test was used as a paired analysis to compare changes in behaviour in farmers over time. Logisitic regression was used to determine changes in percent of farmers using a specific management style or behaviour from one measurement year to the next (covariates included gender, age, and education level as less than grade 8 or completed at least grade 8). Multiple regression was used to compare average daily gain and cost of feed per kg gain of pigs fed commercial versus forage based diets.

Results and Discussion:

Most farmers made multiple behavior changes in response to the trainings (Table 2). Importantly, whereas only 34% of farmers kept their pigs confined in year 1, this increased to 51% in year 3 and to 85% in 2010. Farm-level prevalence of positive pigs decreased over time. Most consumers only purchased inspected pork (87%) and no longer purchase 'home slaughtered' pork (81%). Both consumers and butchers said they cooked the pork longer than they had previously (Tables 2 and 3). Most (80%) of the butchers sold both raw and cooked pork. All

butchers interviewed made changes to enhance pork safety (Table 3). Management changes they made included buying tethered pigs, keeping pork behind fly screens, and cleaning the butcher shop. Business training enabled the butchers to set aside income from one pig to pay for inspection of the next pig. Some of the butchers (25% - 33%) knew inspection, cooking, and tethering prevented the human tapeworm from pork.

The transdisciplinary approach used was crucial for achieving positive, long-term outcomes in this One Health program (Table 1).Traditionally, much research has been conducted within specific disciplinary boundaries, which is vital for gaining deep insights into particular issues, such as understanding cancer cell functions or the genome of a new virus. Some research is multi-disciplinary, where researchers collaborate side by side on the same problem without integrating each other's findings into their work. In contrast, interdisciplinary research involves teams that span multiple disciplines, collaboratively shaping research questions, methodologies, analyses, and interpretations of data. This collective effort allows the team to tackle problems from various perspectives, such as those of an epidemiologist, economist, anthropologist, and swine nutritionist. Transdisciplinary teams enhance this process by incorporating community input into the research. Instead of imposing research findings on the community, team members engage with community voices to shape research questions and approaches, ensuring that the community's needs are prioritized. An essential aspect of transdisciplinary research is the return to the community to share findings and build capacity for improved health and sustainable behavior change. Researchers who collect baseline and follow-up data can effectively measure the impact of their work within the community.

Table 1: Transdisciplinary teams included multiple academic disciplines and many members of the local communities where the research was conducted.

Academic Disciplinary Expertise	**Community Members**
Agronomist	Butchers
Anthropologist	Consumers
Computer scientist	Farmers
Data scientist	Government extension workers
Economist	Meat inspectors
Epidemiologist	Meat sellers at market
Gender specialist	Para-vets
International development	Pig buyers
Livestock feed specialist	Restaurants owners
Parasitologist	Slaughter slab workers
Public health specialist	Teachers
Sociologist	Village Chief, assistant chief
Veterinarian	Village Elders
Veterinary public health	Women who cook pork

Table 2. Preventing the life cycle of Taenia solium. Farmers' behaviour changes measured two years after farm visits and farmer training were completed.

Pig farmer behaviour changes	**Year 1**	**Year 3**	**Year 5**
Pigs regularly confined (p=0.008)	34%	63%	85%
Built a pig barn (p<0.0001)	2%	--	27%
Pigs weighed before sale	0%	100%	100%
Built or repaired a latrine	--	--	5%
Percent of pigs with a positive pig (p=0.07)	15%	15%	9%
Question: What are you doing to keep your family safe?			
Did not buy 'home slaughtered pork'	--	--	76%
Bought only inspected pork	--	--	81%
Cooked pork longer	--	--	87%

Table 3. Pig butcher behavior changes one year after providing pork safety training at both group and individual levels.

Change	**Percent of butchers indicating this change**
Question: Did you change anything in response to the training we provided to butchers?	
Only purchases pigs that are tethered	33% a
Uses slaughter slab and ensures all pigs are inspected	58%a
Enclosed pork behind a fly screen	41% a
Stores meat in a better location	17% a
Improves cleanliness and hygiene	75% a
Question: Which of these specific behaviours have you added to your butcher shop / business?	
Cleans the countertop	64%b
Disinfects the knife used to cut pork	50% b
Cleans the floor of the butchery	50% b
Shares pork with another butcher to sell while still fresh	50% a
Wearing a white coat	33% b

[a] Proportion of butchers who mentioned changes without prompting (in an open-ended question) about what they changed in their business since the training sessions

[b] Proportion of butchers who responded affirmatively when asked about specific changes

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COMPLEXITY, CONTEXT, AND COMMUNITY ENGAGEMENT: CENTERING ANTHROPOLOGY WITHIN THE ONE HEALTH PARADIGM

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Anthropological approaches are necessary for moving One Health research and policy agendas forward. Long term engagement with communities, which is core to most anthropological research, allows for deeper understandings of the lived experiences of people across diverse cultural, ecological, and political contexts. As such, centering anthropology, and the related social sciences, means incorporating diverse community perceptions and experiences into One Health work, while elucidating community priorities for addressing the intersections of human, animal, and environmental health. This includes taking seriously local conceptualizations of health and wellbeing, which in turn contributes to the development of meaningful and effective health-related programs and resources.

Anthropology as a discipline has a long history of conducting research that considers a combination of cultural, economic, environmental, and political factors in shaping the health experiences of people. This includes the development and refining of theoretical frameworks such as a biocultural approach and medical ecology. Central qualitative research methods include participant observation (living and working with research communities), semi-structured interviews, focus groups, oral and life histories, and participatory research methods such as participatory mapping.

Conducting this kind of research also means developing in-depth understandings of and respect for the ways that people define health, which may include the health of the other-than-human world: the environment and the organisms that live within it. In some cases, the spiritual world may also be encompassed within a broader understanding of health. We must therefore recognize that the idea of 'one health', while an important and valuable research framework, may be redundant in some cultural contexts. That is, there may be an already existing wholistic understanding of health that recognizes that humans, animals, and the environment are interdependent, and that when one element is out of balance and unhealthy, so are the other elements. Recognizing the complexities in the ways people think about what it means to be healthy, or unhealthy, and notions of what constitutes health risk is therefore crucial to ensuring that research, intervention, and policy initiatives are truly community engaged and meaningful.

Case illustrations from long-term anthropological fieldwork that draws on various data collection methods in different geographical and cultural contexts can demonstrate the value of centering anthropological methods and theory in order to guide One Health research and policy agendas. Here, I present three case illustrations: Two that emerge from research with smallholder farmers, and a third that demonstrates the ways that anthropological research approaches can be built into One Health research agendas from the ground-up.

Case Illustration #1: Smallholder farmers, environmental change, and livelihoods in rural India

Core anthropological research methods: Participant observation, semi-structural interviews, focus groups, participatory community mapping, dietary recalls, food and wealth ranking.

This research revealed the ways that people think about the health of the environment and the health of their bodies, related to changes in access to traditional crops. Specifically, increasingly unpredictable rainfall patterns contributed to a move away from growing traditional millet crops to growing cassava as a cash crop. In the process, purchased rice became the

staple grain in household diets. While purchased rice is faster and easier to prepare than millets, the increasingly rare access to millets affected people's perceptions and experiences of health. Millets were described as giving strength, as preventing a feeling of hunger, as and being tastier; rice was seen as less healthy. This everyday understanding of the nutritional benefits of millets versus rice is borne out in nutritional analyses of different grains. But people did not feel they had other options other than to eat more rice. Moreover, changes in rainfall patterns that affected the ability to grow millets were discussed in terms of the health of people and animals. As the environment was increasingly perceived as dry, so too were individual bodies; as one woman put it, people were becoming 'dry husks', a parallel to the drying out of millet plants before they were ready to be harvested. The shift to cassava production also affected animal health, as land was converted from forest and pasture to cassava fields. This reduced livestock access to grazing, which increased the perceived risk of disease. Overall, increasing environmental unpredictability and resultant agricultural changes were discursively mapped onto human and animal bodies, contributing to an overall sense of ill health and loss that was about more than simply nutritional changes.

Case Illustration #2: Agricultural livelihoods and the connections made among healthy food, healthy land, and healthy animals in a peripheral farming region in Canada

Core qualitative research methods: Participant observation, semi-structured interviews

This research explores what it means to be a 'good farmer' and how farmers enact their agricultural values while defining and producing what they consider to be 'good food' that is healthy for their families and their customers. This includes thinking about how to best ethically care for animals and the land on which they grow and raise their products. For example, livestock farmers argued that good, healthy food can only come from healthy animals, and key factors for healthy animals are feed, living conditions, and stress levels. Livestock farmers identified the importance of pasture time for livestock, uncrowded living conditions, and tended to limit any use of pesticides. Ensuring that the environment was healthy – both in terms of agricultural and non-agricultural spaces and organisms – was also key for many participants. Essentially, for farmers in this study, agricultural and ethical priorities highlighted the interconnections among healthy animals, healthy environments, and healthy human bodies. This points to an everyday practice of 'one health'.

Case Illustration #3: Emerging research in Madagascar - People, lemurs, and zoonotic disease: Conservation through an interdisciplinary One Health lens

Core interdisciplinary research methods: participant observation, focus groups, semi-structured interviews, lemur behavioural and spatial ecology, spatial mapping of pathogen transmission routes, fecal sampling and microbiological analysis.

Building on anthropologist and PI Dr. Travis Steffens' long-term research agenda in Madagascar, and his well-established relationship with communities who share their living environments with lemurs and livestock, this research examines zoonotic disease transmission in rural contexts among lemurs, humans, and livestock animals to improve human and animal health, and to support lemur conservation. Focusing on disease transmission in the context of human and other-than-human shared living environments allows for the consideration of both acute and chronic illnesses (e.g., gastrointestinal infections, chronic undernutrition) in terms of spatial and behavioural risk factors.

At the same time, our project takes a novel approach to conservation and the potential for species extinction by recognizing the burden that zoonotic pathogens place on already fragile lemur populations. This project has been designed from the ground-up, taking community priorities and concerns into account, and integrating interdisciplinary researchers with complementary areas of expertise including primate behavioural and spatial ecology, qualitative and participatory research, microbiology, and sanitation. This kind of research requires merging qualitative

anthropological methods with core methods for studying non-human primates, including full-day animal follows to record behaviour (focal and instantaneous sampling methods), home range mapping, habitat assessments (tree size and phenology), and pathogen assessment (microscopy and molecular methods).

Such an approach means that ultimately, the research results will be more meaningful for community members, and more robust in terms of developing, testing, and implementing interventions that have positive implications for human lives, lemur conservation, livestock health, and the health of the environments in which they all live. The project design is made possible due to long-term community engagements which have built trust and shared research agendas over 15+ years.

VACCINE MANUFACTURING CONSIDERATIONS FOR PANDEMIC PREPAREDNESS

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Access to timely, affordable, and equitable vaccine supply for populations of all economic backgrounds is essential for dealing with future pandemics. A review of the Covid-19 pandemic experience showed that while the speed and variety of vaccine development was unprecedented during this pandemic, equitable access to the vaccines was not achieved (Figure 1).

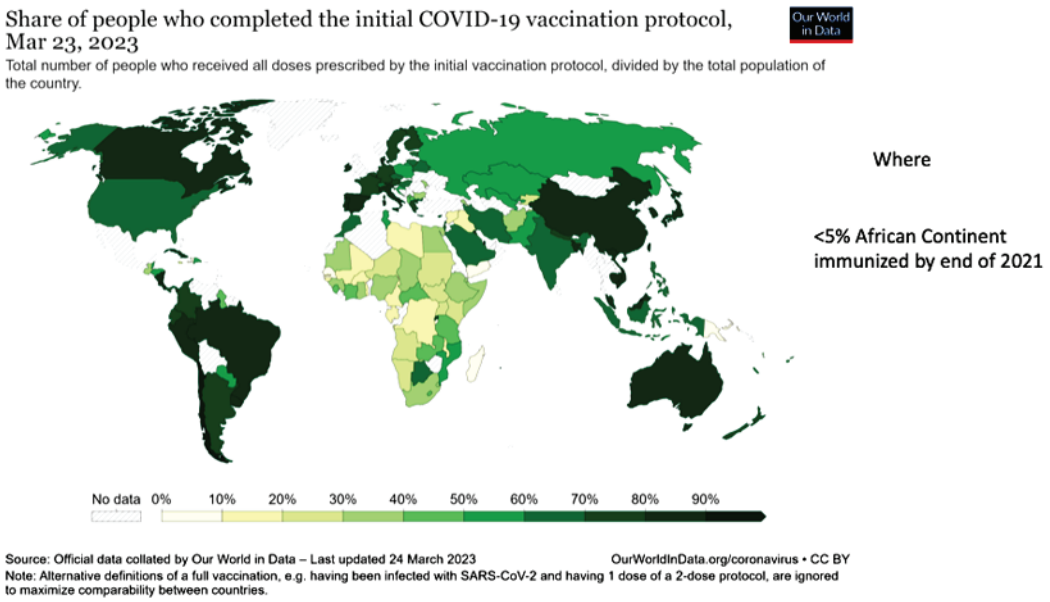

Figure 1.

There were 5 vaccines that had the greatest impact in saving lives during this pandemic – those developed by Pfizer/BioNtech, Moderna, AstraZeneca/Oxford, Sinovac, and Sinopharm. From a vaccine supply standpoint*, there were several success factors that led to the success of these vaccines – prior experience with commercialization and regulatory approval, robust quality systems, rigorous process development strategies, flexible manufacturing facilities with a skilled workforce, collaboration, access to consumables, reagents, and adjuvants (if relevant), and an equitable distribution of the global vaccine manufacturing network.

While incredibly helpful, these insights have limited value for most countries to become self sufficient in pandemic vaccine supply. Compliance with these success factors require deployment of large amounts of capital to build a large manufacturing infrastructure, train a large work force with sophisticated expertise, and have sufficient work during times of no pandemic related manufacturing to ensure economically sustainable operations. These requirements are not practical for most countries even if they can muster the initial capital to set up vaccine facilities. In this talk, we will discuss alternative approaches to create vaccine supply that do not require these very difficult considerations. Our solution involves technologies that enable point of care production and quality control of vaccines ensuring every country has the means and access to timely life savings vaccines and nobody is at the mercy of a company or a country to allocate their vaccine supply.

DESIGN AND PATTERNS OF OPTIMIZED DRUG COMBINATIONS FOR TB

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Motivation:

Tuberculosis (TB) remains a significant cause of death worldwide. There is a critical need for shorter and more effective multidrug regimens using a new set of antibiotics that are active against drug-resistant strains. Lengthy therapy is required to overcome the variation in bacterial response to drug therapy. This develops via innate differences in growth behaviors and adaption to different microenvironments encountered during infection. Treatment of TB requires multidrug regimens to ensure the killing of a heterogeneous bacterial population present in different lesion types because adaptation to these different microenvironments gives rise to differential drug susceptibilities. Design of more effective, treatment-shortening therapies should realize the vast drug-combination space's potential early in development.

Approach:

We have developed a platform based on systematic, efficient in vitro measurement of the drug combination space that uses a geometric optimization of the standard checkerboard assay[1]. This method, called DiaMOND (diamond measurement of n-way drug interactions), is generally applicable and can be used in many other cell types and disease systems (Figure 1). Using DiaMOND, we have generated a drug combination response dataset for *Mycobacterium tuberculosis* (Mtb), the causative agent of TB[2]. Having a dataset of this depth and breadth has enabled us to evaluate patterns in drug combination responses in Mtb as they relate to dependence on growth conditions and the ability to predict treatment outcomes in pre-clinical animal models.

Results:

Our initial drug combination response dataset for Mtb included data from eight distinct growth conditions that were designed to model different aspects of the host environment. We observed major discrepancies in drug interaction profiles across different growth conditions (Figure 2). To understand how to resolve these context-driven synergies versus antagonisms, we tested the hypothesis that some of the measured metrics (Figure 1C), but not all, could be used to predict treatment outcomes in the BALB/c relapsing mouse model. We used a machine learning approach to develop accurate classifier models to predict treatment outcomes relative to the benchmarked drug combinations including the standard of care. Furthermore, we found that *in vitro* potency and synergy metrics of pairwise drug combinations can be used as building blocks to articulate principles of designing optimized combinations of three and four drugs (Figure 3)[3].

Conclusions:

Using these tools, we have established a platform to efficiently search the drug combination space for TB treatments. We have used this platform to determine which growth conditions are predictive of *in vivo* outcomes and how to rationally design drug combinations based on pairwise drug combination behaviors that can be readily measured *in vitro*. This platform is

expandable and can be used to design drug combinations for other hard-to-treat bacteria, including nontuberculosis mycobacteria, MRSA, and ESKAPE pathogens[4-6]. We propose that this empirical approach for early drug combination design can be used for iterative model improvement and as a resource to understand the principles of drug combination effects.

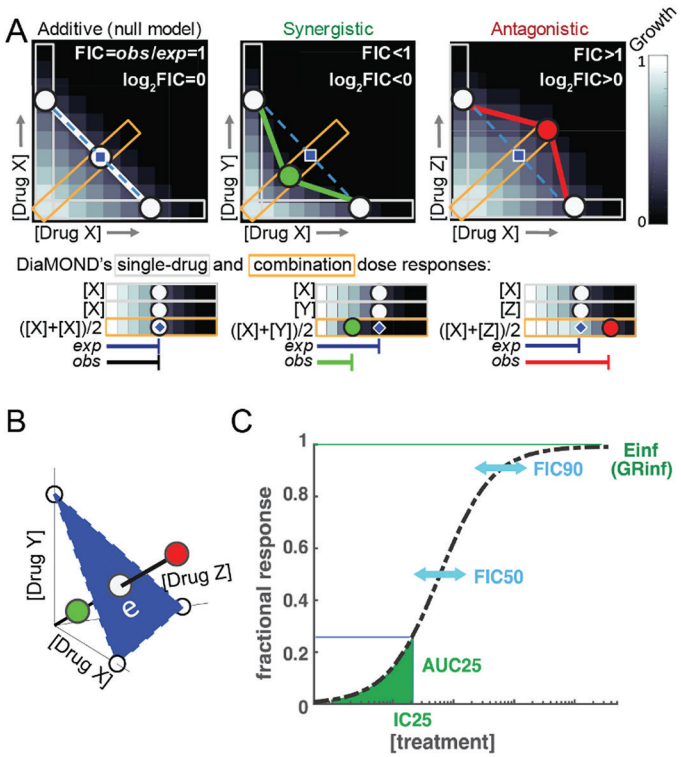

Figure 1. DiaMOND measurement and analysis. (A) DiaMOND efficiently measures drug combinations using equipotent combination drug dose responses (the diagonal of the checkerboard). The expected (exp) and observed (obs) concentrations (such as IC50) in the combination dose responses are marked by a blue square and a circle, respectively. The fractional inhibitory concentration (FIC) is calculated by obs/exp. (B) To measure n-way interactions, we measure equipotent n-drug combination dose responses and project this combination dose response onto an expected plant or hyperplane, shown in blue, to give the expected concentration based on single drug responses. (C) Metrics from DiaMOND dose response curves. IC50 and IC90 are used to calculate drug interactions at the 50% and 90% levels of growth inhibition (FIC50 and FIC90, respectively). Three potency metrics are derived: AUC25 = normalized area under the curve until 25% inhibition, Einf = theoretical maximum inhibition, and (not shown) GRinf = theoretical maximum normalized growth rate inhibition. Figures are adapted from [1, 2].

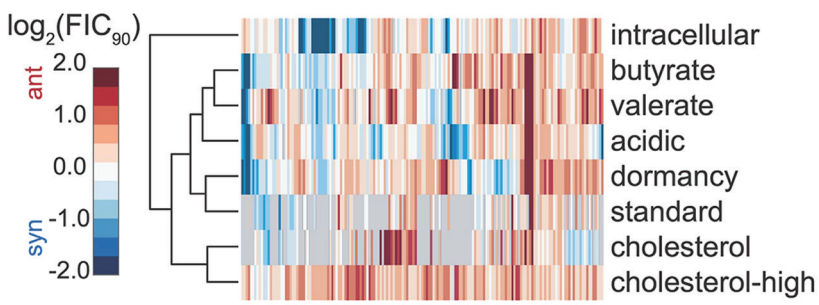

Figure 2. 10-drug DiaMOND compendium. Drug interaction profiles of 2- and 3-drug combinations among the ten compendium drugs across eight *in vitro* models, as described in Larkins-Ford et al[2]. This figure is reproduced from [2].

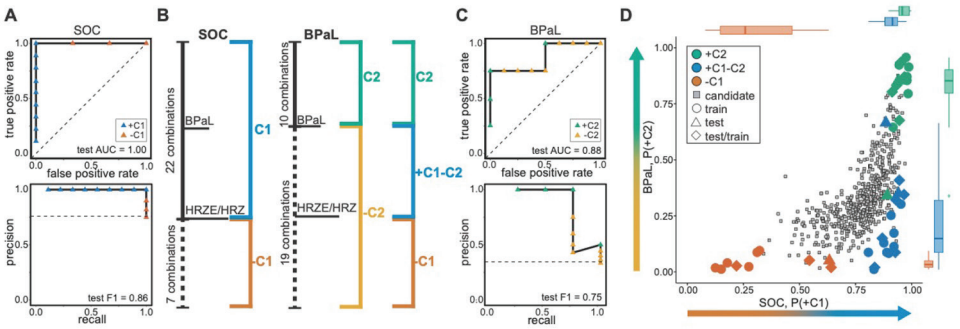

Figure 3. ***In vitro*** **pairwise data are predictive of treatment improvement** ***in vivo*****.** (A) Receiver operator characteristic (ROC) and precision-recall (PR) curves associated with a standard of care (SOC) random forest classifier trained using all summary pairwise features from seven in vitro growth conditions. The model was trained on 70% of annotated combinations and tested on the remaining 30%. Test combinations are colored by outcome (blue = +C1, better than SOC; orange = -C1, SOC or worse). (B) Schematic of combinations in the training set with annotations indicated by color and brackets. (C) ROC and PR curves associated with a random forest classifier around the three-drug regimen bedaquiline+pretomanid+linezolid "BPaL" (green = +C2, better than the BPaL; yellow = -C2, BPaL or worse). (D) Probability scatter plot for SOC model predictions (+C1 probability) and BPaL model predictions (+C2 probability). Marginal box plots show the annotated combination probability distributions. Annotated combinations are colored as in panel B. Figure is reproduced from [3].

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DEVELOPMENT OF MULTI-EPITOPE SUBUNIT VACCINES AGAINST *ACINETOBACTER BAUMANNII*

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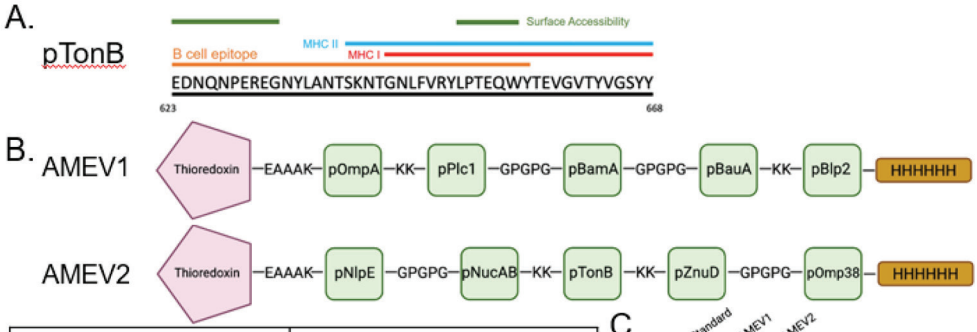
Acinetobacter baumannii (Ab) is a Gram-negative bacterium emerging as a major cause of nosocomial infections. The CDC has identified *Ab* as a "Serious" multi-drug resistant (MDR) threat to human health. The most frequent clinical manifestations of Ab infection are ventilator-associated pneumonia and catheter-related bloodstream/urinary infection, in addition to that of wounds. Due to the MDR nature of *Ab*, empiric treatment is generally ineffective and results in poor clinical outcomes. If untreated, rampant bacterial growth can ensue and give rise to TLR4-mediated septic shock and death. Immunotherapeutics alone or in combination with antimicrobials can be a solution to prevent and treat MDR *Ab* infection. Currently, there is no licensed vaccine for *Acinetobacter.* We have previously shown that a thioredoxin-deficient *A. baumannii* (ΔTrxA) provided robust protection against systemic Acinetobacter infection. However, due to the safety concerns associated with live attenuated vaccines, particularly among immunocompromised individuals, effective subunit vaccines are more desirable for clinical use.



AMEV1		AMEV2	
OmpA	Outer membrane protein A	NlpE	Copper resistance protein
Plc1	Phospholipase C	NucAB	Extracellular endonuclease
BamA	Outer membrane assembly factor	TonB	Siderophore receptor
BauA	Ferric acinetobactin receptor	ZnuD	Zinc piracy receptor
Blp2	Ig-like repeat protein	Omp38	Outer membrane protein

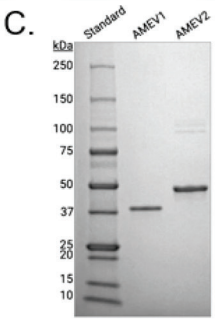


Fig. 1. Generation of polypeptide vaccine. (A) Diagram of a putative immunogenic peptide (pTonB, aa623-668) predicted to be surface exposed and have B cell epitope and binding sequences for MHC I, MCH II. (B) Diagram of *Acinetobacter* Multi-Epitope Vaccines (AMEV1 and AMEV2), each consisting of *Ab* Thioredoxin linked to 5 predicted immunogenic peptides and a C-terminus His tag. (C) SDS-PAGE analysis of the purified AMEV1 and AMEV2.

Development of novel *Acinetobacter* polypeptide vaccines: We applied a reverse vaccinology approach to identify peptide antigens for constructing novel subunit Acinetobacter vaccines. Specifically, 32 selected Ab virulence factors important for bacterial pathogenesis

were subjected to immunoinformatic analysis using EigenBio's proprietary epitope prediction software to identify putative B and T cell epitopes. Initial analysis was to identify short peptides (approximately 30-60 amino acids) each comprising putative B-cell linear epitopes, MHC-I and MHC-II binding peptides. This antigen design aims to enhance T cell-dependent antibody response which has shown to be important for vaccine protection against Ab infection. Identified peptides were filtered by their predicted localization in the outer membrane. Additionally, each protein was analyzed for surface accessibility to ensure that the predicted B cell epitopes are surface exposed. A graphical representation of an *in silico* predicted peptide, TonB, is shown in Fig. 1A. Lastly, peptide candidates were screened against all identified Ab strains for high conservancy as well as mouse and human genomes with BLASTP to limit the potential for autoimmune reaction. A total of 10 peptide antigens were identified from this selection platform. Subsequently, two novel *Acinetobacter* Multi-Epitope Vaccines, AMEV1 and AMEV2, were constructed with a leader *Ab* TrxA protein (a virulence factor and might improve recombinant protein production and solubility) linked with 5 identified peptide antigens in tandem (with GPGPG/KK linker) and a C-terminal 6-histidine tag to aid in protein purification (Fig.1B). The AMEV encoding nucleotide sequences were synthesized using optimized codons for protein expression in *E. coli*. The recombinant AMEV1 and AMEV2 proteins were purified to high homogeneity with cobalt affinity chromatography (Fig. 1C) and its protein sequence was confirmed by standard liquid chromatography tandem mass spectrometry (LC-MS/MS).

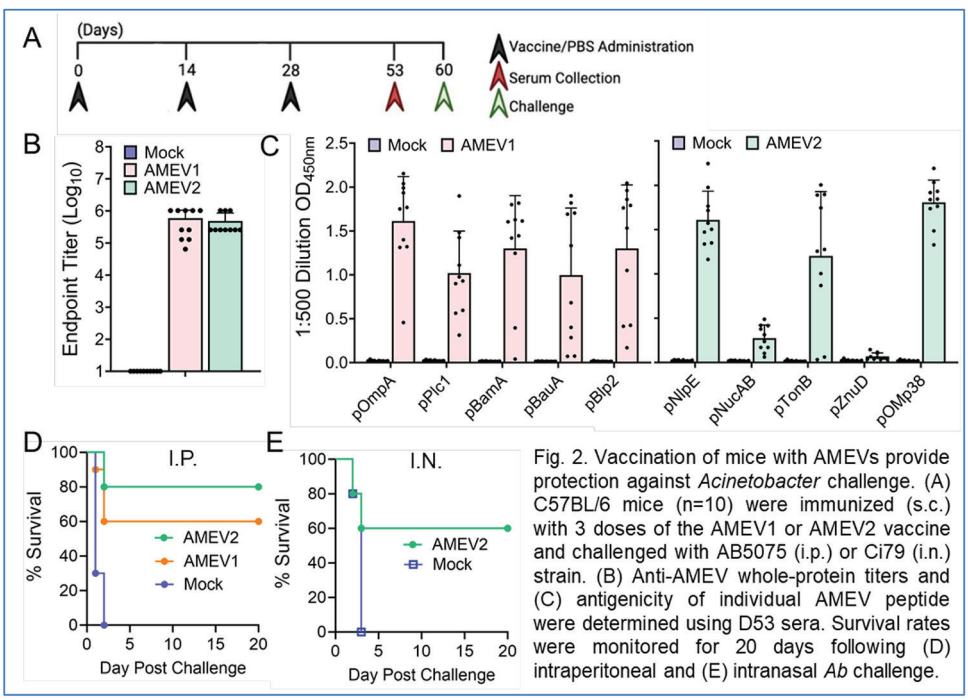


Fig. 2. Vaccination of mice with AMEVs provide protection against *Acinetobacter* challenge. (A) C57BL/6 mice (n=10) were immunized (s.c.) with 3 doses of the AMEV1 or AMEV2 vaccine and challenged with AB5075 (i.p.) or Ci79 (i.n.) strain. (B) Anti-AMEV whole-protein titers and (C) antigenicity of individual AMEV peptide were determined using D53 sera. Survival rates were monitored for 20 days following (D) intraperitoneal and (E) intranasal *Ab* challenge.

Evaluation of vaccine antigenicity and protective efficacy: Vaccine efficacy of AMEV1 and AMEV2 was first evaluated in a mouse model of systemic Ab infection. The subunit vaccine was formulated with a TiterMax adjuvant (Sigma-Aldrich). Mice (C57BL/6) were primed (Day 0) with either PBS (Mock), AMEV1 (5 µg/mouse), or AMEV2 and boosted twice (2.5 µg/mouse at days 14 and 28; Fig. 2A). Sera were obtained at day 53 to measure vaccination induced antigen-specific antibody production. These analyses revealed that mice vaccinated with TiterMax adjuvanted AMEVs elicited robust anti-AMEV1 and anti-AMEV2 antibody responses (Fig. 2B). Additionally, peptide ELISA's revealed antigenicity of most peptide components (Fig. 2C). Five weeks after final AMEV vaccination, mice were challenged with 2x106 CFUs (~ 4 LD50) of AB5075 strain (an MDR clinical isolate) by the intraperitoneal route. All PBS mock treated mice succumbed to acute bacterial infection within two days while 60% and 80% of the AMEV1 and AMEV2 vaccinated mice survived the lethal dose challenge, respectively (Fig.

2D). AMEV2, the superior protective vaccine against systemic Ab infection, was further tested for protection against intranasal Ab challenge with another MDR clinical isolate Ci79. AMEV2 vaccine (with AddaS03 adjuvant) provided partial (60%) protection against pulmonary Ab infection (Fig. 2E). These results demonstrated the effectiveness of immunoinformatic-based vaccine design against Ab infection.

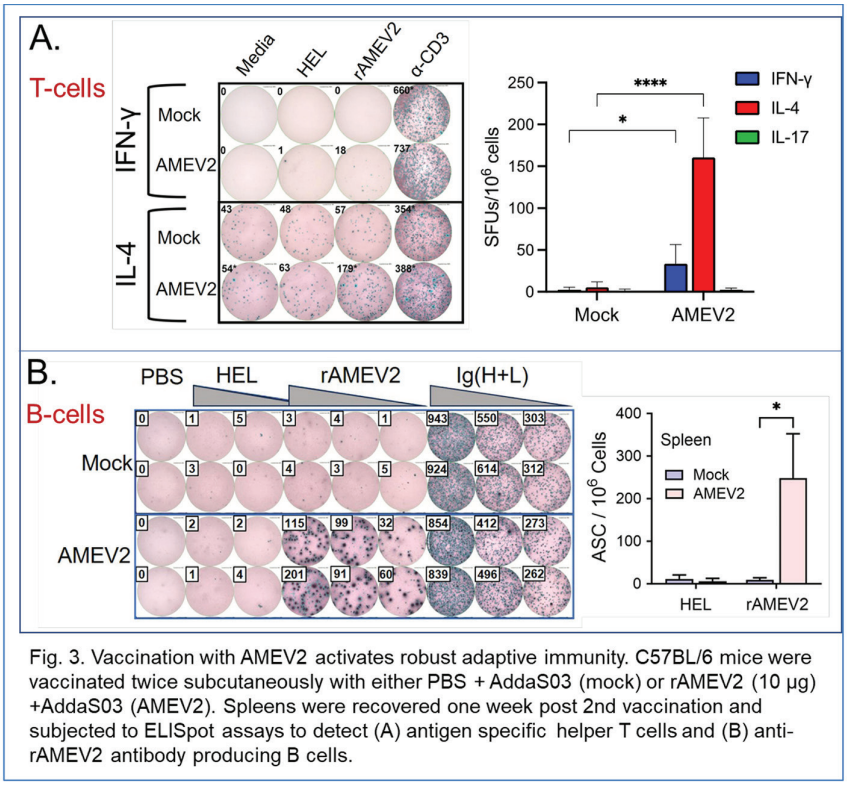


Fig. 3. Vaccination with AMEV2 activates robust adaptive immunity. C57BL/6 mice were vaccinated twice subcutaneously with either PBS + AddaS03 (mock) or rAMEV2 (10 µg) +AddaS03 (AMEV2). Spleens were recovered one week post 2nd vaccination and subjected to ELISpot assays to detect (A) antigen specific helper T cells and (B) anti-rAMEV2 antibody producing B cells.

Assessment of vaccine induced protective immunity: An effective vaccine activates antigen specific adaptive immunity. To assess induction of T- and B- cell immune responses, C57BL/6 mice (n = 3 per group) were vaccinated subcutaneously with either PBS+AddaS03 (mock) or rAMEV2 (10 µg) +AddaS03 (AMEV2) on days 0 and 14. Splenocytes were prepared at one-week after final vaccination. For T cell ELISpot assay, splenocytes were stimulated with media, HEL (an unrelated antigen as negative control), rAMEV2, and α-CD3 (a positive control). IFNγ, IL-4, or IL-17 spot forming units (SFU) were measured as indicative of a Th1, Th2, or Th17 response, respectively. As shown in Fig. 3A, while mock splenocytes had minimal to no reactivity to HEL and rAMEV2, the AMEV2 vaccinated mice showed a significant increase in both IFNγ and IL-4, but not IL17, secreting cells upon restimulation with rAMEV2. This robust type 2 helper T-cell activation can further facilitate humoral immunity response that is required for protection against Ab infection. Generation of rAMEV2-specific antibody-secreting cells following AMEV2 vaccination was confirmed by a B-cell ELISpot assay using the splenocytes described above. As shown in Fig. 3B, the B cell ELISpot revealed specificity (no reaction to HEL) and high frequency of rAMEV2-specific antibody-secreting cells in spleen. These data demonstrate that vaccination with AMEV2 mounted robust antigen-specific adaptive T- and B- cell immunity. We further demonstrated the protective role of humoral immunity by passive anti-AMEV2 serum vaccination (Fig. 4A). Naïve C57BL/6 mice (n = 6 per group) were injected intraperitoneally with 100 µL of either mock (adjuvant only) or AMEV2 vaccinated serum. Twenty-four hours later, the mice were challenged intranasally with $1x10^8$ CFUs of Ci79. Four of the six mice passively vaccinated with AMEV2 antisera survived the pulmonary challenge, while only one of the mock mice survived. The potential protective mechanisms by AMEV2 immune sera were investigated using an in vitro opsonophagocytic killing assay (OPKA). Ci79

bacteria were opsonized in the presence of heat-inactivated pooled naïve, mock or AMEV2 sera and fresh baby rabbit serum as complement source for 30 minutes at 37 °C. Following the opsonization, bacteria were incubated with bone marrow derived macrophages (BMDMs) at 0.1 MOI. Bacterial uptake and killing were measured. The results showed a significant increase of Ci79 uptake by BMDMs when the bacteria were opsonized with AMEV2 sera compared to that with mock sera (Fig. 4B). Furthermore, bacteria that had been opsonized with AMEV2 sera showed a roughly 50% reduction in viable Ci79 compared to naïve serum. In contrast, the mock sera opsonized bacteria showed no increase in bacterial killing (Fig. 4C). This AMEV2 immune serum-mediated opsonophagocytic killing is significantly reduced by BMDMs lacking FCγR (Fig. 4D) suggesting that AMEV2 antibodies opsonized the bacteria, allowing their Fc region to be recognized by macrophages and subsequently enhancing uptake and killing of *A. baumannii.*

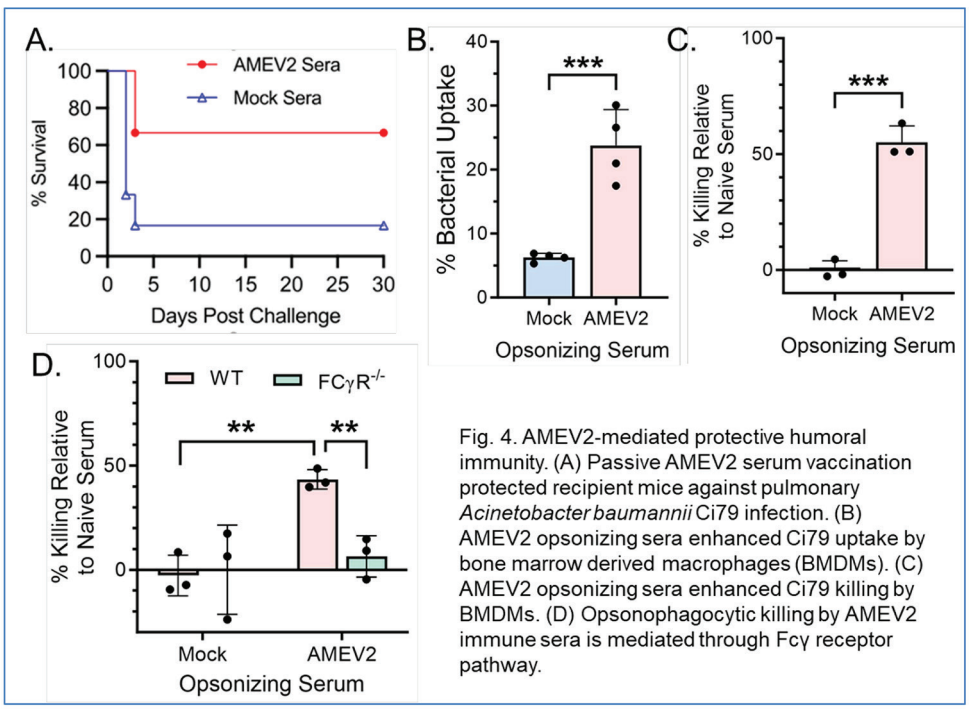


Fig. 4. AMEV2-mediated protective humoral immunity. (A) Passive AMEV2 serum vaccination protected recipient mice against pulmonary *Acinetobacter baumannii* Ci79 infection. (B) AMEV2 opsonizing sera enhanced Ci79 uptake by bone marrow derived macrophages (BMDMs). (C) AMEV2 opsonizing sera enhanced Ci79 killing by BMDMs. (D) Opsonophagocytic killing by AMEV2 immune sera is mediated through Fcγ receptor pathway.

Summary: Despite efforts over the past two decades to develop vaccines against Acinetobacter baumannii, none have advanced to clinical evaluation. Using a novel immunoinformatic approach, we identified antigenic peptides that contain both putative B and T cell epitopes from Ab proteins associated with pathogenesis. Subunit vaccines were constructed by expression these identified peptide antigens in tandem. Overall, these in silico selected peptides demonstrate the ability to remain immunogenic, generating effective antibodies when linked together in a multi-peptide construct. These AMEV vaccines were shown to be effective for preventive intervention and therapeutic treatment against Ab infection. Protection associated with AMEV2 vaccination seems to be mediated by humoral immunity that aligns with most experimental Ab vaccines demonstrating the effectiveness of antibody-mediated protection with passive serum transfer, and enhancement of opsonophagocytic killing. These study results provide insights into the effectiveness of immunoinformatic-based vaccine design to combat the rise of MDR Acinetobacter infection.

TOWARDS ONE HEALTH: TOXICOKINETICS AT THE INTERSECTION OF HUMAN AND ECOLOGICAL HEALTH

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The evaluation of chemical safety is crucial for protecting both human health and the environment. The One Health concept represents a paradigm shift in chemical risk assessment by recognizing the interdependence of health across species and ecosystems. It calls for integrated approaches that address human, animal, and environmental health collectively. However, one of the key challenges in evaluating chemical risks is the limited availability of data and methods, a problem amplified when working toward a holistic One Health approach to chemical management.

These challenges have spurred scientific advancements in both exposure assessment and toxicity prediction, particularly through the development of New Approach Methodologies (NAMs). NAMs are innovative tools and techniques that integrate advanced methods for toxicity and exposure assessment. Examples include in silico models, in vitro methods, computational modeling, 'omics technologies, alternative test species, and microphysiological systems. In toxicology, NAMs often refer to approaches that reduce reliance on animal testing while providing more relevant and accurate data. For exposure assessment, NAMs can include innovative experimental, informatics, and in silico approaches that offer valuable insights to chemical risk assessments.

Toxicokinetics (TK), which encompasses the absorption, distribution, metabolism, and excretion of chemicals, is central to chemical risk assessment. It connects critical elements like exposure assessment, bioaccumulation, and species sensitivity with cross-species extrapolation and the application of uncertainty factors. TK provides the framework for interpreting toxicity data and designing studies, while also facilitating the use of NAMs and *in vitro* to *in vivo* extrapolation. TK is the crucial link between exposure and Adverse Outcome Pathways (AOPs) and is key to advancing their application in chemical risk assessment. Additionally, a deeper understanding of TK is essential for identifying the links and differences between human, animal, and ecological responses.

This presentation will highlight the role TK plays in realizing the vision of a One Health approach to chemical risk assessment, emphasizing the common needs and challenges across human, animal, and ecological health. Although human and ecological risk assessments are often addressed in separate silos, measurements or predictions for TK properties—such as biotransformation rates—are required inputs for all Physiologically-Based Pharmacokinetic (PBPK) models, whether for humans, rats, fish, or birds. This information is also essential for performing *in vitro* to *in vivo* extrapolation (IVIVE), a critical step for applying NAMs data to real-world organisms or populations.

The importance of understanding chemical fate and behavior, and the link between exposure and toxicity, is further illustrated by bioaccumulation assessment. Bioaccumulation, the net result of competing rates of chemical uptake and elimination in an organism, is a key endpoint in national and international chemical regulatory programs and treaties. Potential health risks for humans and the environment from bioaccumulative chemicals can be evaluated using various approaches and metrics, with TK playing a central role.

While One Health is an ambitious goal requiring broad coordination across sectors and disciplines, there are focused opportunities to improve the foundational science necessary to achieve this vision. One such area is the need for coordinated and integrated approaches to TK and bioaccumulation research. Addressing uncertainties in TK, particularly regarding biotransformation, will require fostering collaborations that enhance data collection and model development. Improved interspecies and inter-taxa comparisons will also be vital for extending research applicability across different biological systems.

FOOD SAFETY FROM A ONE HEALTH PERSPECTIVE

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Food Safety is an essential pillar of Food Security, and, hence, an important prerequisite for sustainable development. Provision of adequate, nutritious and safe food to the world population is a major challenge and requires efforts by various sectors of society to ensure sustainable food systems that respect environmental integrity, are mindful of available resources, and assure the safety of food from farm to table. This larger consideration already demonstrates the need for a holistic One Health approach to Food Safety.

The safety of food requires the prevention of risks of microbiological, chemical or physical risks. Such hazards could emanate at any point during the production, storage, transport or preparation of food. In the case of chemicals, for example, pesticide residues from crop protection applications, veterinary drug treatments of farm animals, supplementation of animal feed or food intended for human consumption with additives, or contamination resulting from the presence of certain, often persistent, chemical in the environment represent potential exposure to chemicals from food. The fact that residues can migrate from crops to farm animals and humans, and from farm animals to humans show that a One Health approach would be appropriate.

A number of major incidents have shown that outbreaks of foodborne diseases and major toxic outbreaks required a back-tracing that identified the source either in certain crops or food commodities (partly processed) or in animal feed. The presence of persistent organic pollutants and heavy metals due to anthropogenic activity in crops and animal tissue also shows that primary holistic interventions may be the most efficient.

Current regulatory approaches to assessing chemical risks in the field of Food Safety are organized in silos. Risks to plant health, animal health and human health are evaluated independently, even though there are approaches to identify the interrelations between the different sources of migration of chemicals. Some promising holistic models have been developed but still require refinement and testing for their applicability to a wider range of chemicals.

Micro- and nanoplastics also provide a good example of the complex nature of food contamination that would benefit from a One Health view. Plastic waste ends in various environmental compartments, including land, rivers, lakes and oceans. Degradation of plastics via different microorganisms results in the formation of micro and nano particles which pose a risk to terrestrial and aquatic species, including farm animals and affecting aquaculture. Ultimately, they are found in foods for human consumption, posing a potential health risk to various organs. From a One Health point of view, a holistic impact assessment of plastic use and plastic waste disposal, covering all pathways that may pose a risk to the environment, crop production, animal and human health would have pre-empted the damages we see today.

Linking the risks of individual food chemicals or mixtures across species within the food production chain requires knowledge of toxicokinetic (TK) and toxicodynamic (TD) processes. The use of historical in vivo data as well as data from in vitro cellular models and in silico models is a promising avenue to integrate New Approach Methodologies (NAMs) in food and feed safety using holistic approaches. These include generic and refined physiologically-based kinetic (PBK) models for humans, test species (rat, mice, rabbit, dog) and farm animals for the TK dimension, TD data to integrate within the adverse outcome pathway framework while TK and TD data can be integrated within the mode of action. It is important to note that all these frameworks involve using a weight of evidence approach for which the evidence is assembled, weighed and integrated. For environmental risk assessment, dynamic energy budget models as TK-TD models for the environment taking into account life cycle parameters enable assessing the impact of chemicals on species of ecological relevance on individuals and populations.. This approach will be outlined including the illustration of modelling aspects developed at the European Food Safety Authority (EFSA) and the formulation of future perspectives, in particular to refine such holistic One Health approaches.

PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS): THE COMPLEXITY IN TOXICITY AND RISK ASSESSMENT

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Presence of PFAS in food and environment:

The presence of per- and polyfluoroalkyl substances (PFAS) in the environment and food is a complex and evolving field. The number of PFAS congeners that have already been identified in the environment is enormous, with estimations that range from thousands to nearly ten thousand. This overview will delve into the current understanding of PFAS identification in the environment, drawing from recent research findings.

A 2018 OECD/UNEP report identified thousands of PFAS congeners. However, only a few hundred of these congeners were identified as commercially relevant These numbers highlight the complexity of the risk assessment and management of this diverse group of persistent organic pollutants.

In addition, dozens of PFAS congeners have already been identified in human food, and as a result these can now also easily be detected in human milk with at least 39 different PFAS already been identified (Zheng et al., 2021).

In this presentation the difficulties arising from the complex toxicological and biological mechanism will be further addressed and at the end will be put in the context that are faced when viewed from a risk assessment perspective.

Mechanisms of PFAS toxicity:

The toxicity of per- and polyfluoroalkyl substances (PFAS) in human cells is a complex process involving multiple biochemical pathways. These pathways include interactions with nuclear receptors, oxidative stress mechanisms, metabolic disruptions, and immune system modulation. Understanding these pathways is crucial for assessing the health risks associated with PFAS exposure and developing strategies to mitigate their effects. At present at least the following mechanistic pathways are known for these persistent organic pollutants.

Nuclear Receptor Interactions: PFAS have been shown to interact with various nuclear receptors, which are critical for regulating gene expression for many essential biological processes. Notably, PFAS can activate peroxisome proliferator-activated receptors (PPARs) and estrogen receptors, which may directly influence lipid metabolism and endocrine functions (Houck et al., 2021). Furthermore, extensive Tox21 screening in vitro data revealed that these compounds inhibit cytochrome P450 enzymes, particularly CYP2C9, which plays a significant role in drug metabolism. This inhibition suggests a potential for PFAS to interfere with the metabolism of other compounds, e.g. pharmaceuticals (Ooka et al., 2024)

Oxidative Stress and Metabolic Disruption: PFAS exposure can also lead to oxidative stress by generating reactive oxygen species (ROS), which can damage essential cellular components and processes. This has been shown by decreased activities of antioxidant enzymes in human neuronal cells (Obiako et al., 2024). Metabolomics studies identified alterations in metabolic pathways, including lipid, amino acid, and carbohydrate metabolism. It has been suggested that these metabolic disorders may contribute to the well know hepatotoxicity of these compounds (Alijagić et al., 2024).

Neurotoxicity and Neurotransmitter Modulation: PFAS can be transported across the blood-brain barrier and affect neurotransmitter systems, such as dopamine and glutamate related processes, which play an essential role in brain function. These interactions can lead

to neurotoxic effects and potential neurological disorders (Brown & Cannon, 2023). In addition, the impact on neurotransmitter systems involves changes in calcium signaling, mitochondrial function, and protein expression. These complex neurological interactions highlight the multifaceted nature of PFAS-induced neurotoxicity (Brown & Cannon, 2023)

Immune System Modulation: PFAS have clearly been associated with immunotoxicity, including altered antibody responses and modulation of immune cell populations. Again, these effects are mediated via pathways that involve some nuclear receptors like NF-κB and PPARs, but also oxidative stress could be of importance too (Ehrlich et al., 2023). This immune system's vulnerability to some common PFAS is particularly concerning during developmental stages, where exposure can lead to adverse effects later in life (Neagu et al., 2021).

Metabolic Pathway Alterations: PFAS exposure has been linked to disruptions in key metabolic pathways that are crucial for cellular redox balance and biosynthesis (Beale et al., 2022). Moreover, combined exposure to PFAS with other endocrine-modulating compounds can lead to interactive effects, which may complicate the metabolic impact and shows the importance of considering combined exposures in risk assessments (Yang et al., 2024)

Although, the mechanistic pathways involved in PFAS toxicity are becoming clearer, there are still significant gaps in understanding the full extent of PFAS effects, especially at relevant environmentally exposure of human and wildlife. Further integration of modern molecular technologies, like multi-omics and metabolomics, can further unravel the complexities of PFAS-toxicities.

Toxic Equivalency factors:

The mixture toxicity of complex groups of compounds have been successfully approached by using relative potency factors (RPFs) or toxic equivalency factors (TEFs). Most notable, this approach has been used for risk assessment of the dioxin-like compounds (Van den Berg, 2005, DeVito, 2023). TEFs, which are used to compare the toxicity of different chemicals, have also been considered for PFAS due to their widespread presence in mixtures and potential health risks. However, the application of TEFs to PFAS remains complex due to their diverse chemical structures, different toxicokinetic properties and multiple (nuclear) receptor interactions. Several methodologies have now been proposed to approach the mixture toxicity of PFAS for risk assessment.

Internal Relative Potency Factors (RPFs): Recent studies have developed RPFs for PFAS, to estimate the relative toxicity of different PFAS based on internal serum levels rather than external doses. Notable, this approach accounts for differences in bioaccumulation and toxicokinetics, as well as toxicodynamic factors among PFAS. As such it should provide a more accurate assessment of the potential health risks of mixtures of PFAS in the body (Schmidt, 2022; Bil et al., 2022).

Application in Human Biomonitoring: These RPFs have been applied to human biomonitoring data, allowing for the calculation of PFOA-equivalent doses from PFAS mixtures. This method facilitates the assessment of combined exposure risks and can be used to inform regulatory decisions (Schmidt, 2022; Bil et al., 2022).

Challenges in using RPFs or TEFs: PFAS usually occur as mixtures in food and the environment. A perquisite for the RPF/TEF approach is the assumption of additivity in toxicity, which may not always be valid due to potential interactions among PFAS and congener specific differences in modes of action (Bil et al., 2021; Goodrum et al., 2021)

Furthermore, the use of TEFs, like those used for dioxin-like compounds, is problematic for PFAS due to their diverse mechanisms of action and subsequent potential for species-specific responses. This known diversity may impede a straightforward application of TEFs and possible need for yet undetermined alternative approaches (Peters & Gonzalez, 2011)

While the development of RPFs and the use of computational models represent significant

advances in PFAS risk assessment, challenges remain. The diversity of PFAS and their complex interactions in mixtures pose significant hurdles to the application of TEFs for these compounds. Moreover, the lack of comprehensive data on the RPFs or TEFs of many PFAS compounds limits the robustness of current risk assessments.

The complex mixture toxicity of PFAS:

The assessment of toxicity of PFAS mixtures is complex and evolving. Several methodologies have been proposed to understand their impact on human health, ranging from computational models and in vitro assays to in vivo studies using model organisms. Obviously, understanding mixture toxicity of PFAS is crucial for interpreting experimental results and for guiding future research and regulatory decisions.

Agent-Based and Physiologically-Based Kinetic Models: These models, like the Universal Immune System Simulator (UISS) and Physiologically Based Kinetic (PBK) models, will help to simulate the immune system's response to PFAS. These include among others biokinetics aspects of individual PFAS in the human body. As such, these models can e.g. predict time-concentration profiles of individual PFAS from birth to old age, while at the same time giving insights into potential immunotoxic effects (Iulini et al., 2024). However, it should be recognized that such models require extensive data and may not fully capture the complexity of PFAS interactions.

Cell Culture Metabolomics: This approach integrates metabolomics with molecular responses in specific cell cultures that are induced by PFAS mixtures and individual congeners. It could help in identifying alterations in metabolic pathway, while at the same time identifying potential biomarkers of toxicity and reducing the need for animal testing of PFAS mixture toxicity (Trang & Kumar, 2024). An obvious limitation of these approaches lies in the extrapolation of the *in vitro* results to the *in vivo* situation, especially in humans.

Moreover, the results obtained from these *in vitro* experiments may vary significantly between different cell lines and experimental conditions.

Tox21 Screening and Nuclear Receptor Profiling: This efficient method involves high-throughput screening to profile PFAS bioactivities on various mechanistic pathways, including those with nuclear receptors and stress responses. It should also help in to identify the overall PFAS mixture toxicity that may assist future hazard and risk assessments (Ooka et al., 2024). However, the complexity of PFAS mixtures and the variability in their biochemical, molecular and toxicological effects pose challenges in interpreting and prioritizing the obtained results.

Despite the advancements in novel molecular, biochemical and in vitro methodologies, several limitations persist. The complexity of environmental PFAS mixtures, with hundreds of compounds present, makes it difficult to assess their combined effects fully. Unfortunately, many in vitro and in vivo studies have focused only on a small number of well-known PFAS compounds, leaving us still with a significant knowledge gap for less-studied compounds (Gkika et al., 2023) Additionally, the variability in methodologies and endpoints used in different studies can lead to inconsistent results, further complicating risk assessments (Bil et al., 2022). Future research should focus on bridging the gaps in data and understanding the interactions within PFAS mixtures to improve risk assessments and inform regulatory decision making.

Risk Assessment of PFAS :

The risk assessment methodologies for per- and polyfluoroalkyl substances (PFAS) face several limitations and biases. These limitations stem from the complex toxicological and biological mechanisms of PFAS, their widespread presence at varying concentrations, and the difficulties encountered with chemical analysis. Addressing these challenges for adequate risk assessments requires a multidisciplinary approach incorporating advanced toxicological and molecular techniques, comprehensive congener specific data analysis, and advanced regulatory frameworks.

Limitations in Current PFAS Risk Assessment Methodologies: Current risk assessments focus usually on a limited subset of PFAS compounds, neglecting the majority of congeners present in food and the environment. This bias is most notable in environmental studies that frquently analyze less than 1% of known PFAS compounds (Gkika et al., 2023).This limited focus certainly lead to incomplete data set causing insufficient assessment of the overall risks of PFAS.

From a global point of view there is an obvious lack of consensus on appropriate health protection levels, with regulatory thresholds varying significantly across regions and exposure settings. This inconsistencies and regional differences clearly can undermine effective risk management of PFAS (Reinikainen et al., 2024)

Moreover, there are significant challenges in toxicokinetic modeling, as estimation of PFAS half-lives in humans is burdened with uncertainties because of differences in exposures and variability in congener specific characteristics. Obviously, this variability complicates the insights of PFAS persistence and toxicity in human populations (Rosato et al., 2023)

The complicated mechanisms of action of PFAS also hamper a single endpoint directed risk assessment. In view of the tremendous number of PFAS congeners that have already been introduced into the environment without proper toxicological testing it is also clear the traditional animal studies are not feasible anymore.

Therefore, the only way to go forward is the use of both in vitro and in silico testing to determine congener specific toxicological potencies. This may also support the mixture toxicity identification of this complicated group of compounds. These methods can provide prioritization of PFAS for further study and inform regulatory decisions without relying solely on animal testing (Tsai et al., 2024; Ford et al., 2024).

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THE AIR WE BREATHE – CHEMICAL CHALLENGES TO HEALTH, A UK-THAILAND PERSPECTIVE

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It is known that poor air quality can have a detrimental effect on human health, and that urban environments can experience very high levels of potent chemicals. High numbers of vehicles are an unwanted feature of urban environments, being a source of nitrogen oxides (NO and NO_2, collectively known as NOx) that can lead to ozone (O_3) formation, a toxic gas at the Earth's surface but also a potent Greenhouse gas. Vehicles can also generate particles, through exhaust emissions, brake wear and tyre wear. Particles of around a μm in diameter are known to penetrate the human respiratory system and enter the body, where they can cause short- and long-term health impacts. The chemicals embedded on these particles can themselves exacerbate health responses. The latter two sources of particles (brake wear and tyre wear) will persist whether vehicles use fossil fuels or are electrically powered.

On the one hand, recent trends in vehicles employed in urban centres in both Thailand and the UK suggest that NOx emissions are reducing as a direct result of adoption of Euro 4-6 vehicles. Figure 1 shows the downward trend in OX ($[O_3] + [NO_2]$) in London (Marylebone Road (2000-2019)) and at three sites in Bangkok (Din Daeng (2005–2014), Chokchai (2005–2014) and Thonburi (2005–2018)) respectively (Khan et al., 2021). The study of Khan et al. (2021) demonstrates that the abatement of pollution emissions has had an impact on the trends of local and background oxidants, [OX]L and [OX]B, decreasing by 1.4% per year and 0.4% per year, respectively from 2000 to 2019. The pollutant levels at the Marylebone Road site (0.21[NOx] and 32 ppbv) are comparable with the roadside sites of Bangkok, Thailand, Din Daeng, Chokchai and Thonburi (0.12[NOx] to 0.26[NOx] and 29 to 32 ppbv). The seasonal variation of [OX]B levels displays a spring maximum for London, which is due to the higher northern hemispheric ozone baseline, but a maximum during the dry season is found for Bangkok which is likely due to regional-scale long-range transport from the Asian continent. However, the diurnal variations of [OX]L for both London and Bangkok roadside sites confirm the dominance of the oxidants from road transport emissions, which are found to be higher throughout the daytime.

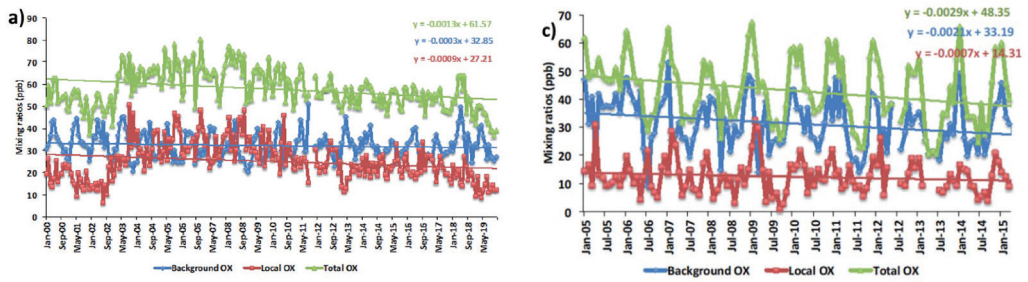

Figure 1. Changes in OX ($[O_3] + [NO_2]$) levels measured at the Marylebone Road in London (a) and at three sites in Bangkok (Din Daeng, Chokchai and Thonburi), over the period ca. 2010-2019 (Khan et al., 2021)

On the other hand, despite reductions in pollutant emissions from vehicles, it is known that pollution from vehicles penetrates deeper into the urban environment than from stationary

sources. In the study of Shallcross et al. (2009), comparing inert gas-phase tracers released from stationary and moving sources, during the DAPPLE field experiment, it was shown that dose scales with 1/distance from a moving source, compared with 1/distance2 from a stationary source. Hence, the impact of vehicles on concentration-time profiles of pollutants in urban environments and as a consequence exposure, is a dominant factor in both the UK and Thailand. Studies in Thailand show that outdoor workers (Vattanasit et al., 2014; Hinhumpatch et al., 2013; Arayasiri et al., 2010; Buthbumrung et al., 2008) and people walking, cycling or travelling on motor bikes will be particularly susceptible to inhalation of toxic particles and known carcinogenic gases such as benzene and polyaromatic hydrocarbons (PAHs). In similar exposure studies in London (Kaur et al., 2005) during DAPPLE, cyclists were exposed to the highest levels of particles and toxic gases, but it was noted that cleaner routes through urban areas, taking less busy roads or travelling through green spaces on foot or by bicycle could reduce exposure by up to 50%. The Thai studies showed through the use of biomarker analysis of blood and urine of exposed cohorts, that DNA damage was accelerated, and the rate of DNA repair was retarded, that correlated well with exposure to benzene, 1-3 butadiene and PAHs. Therefore, vehicle use in urban environments of both countries must continue to be reduced. A recent study of modes of transport and exposure to particles in Bangkok (Matthews et al, 2023) showed a distinct difference between the BTS Skytrain and MRT underground, which were much less than the railway and bus (see figure 2).

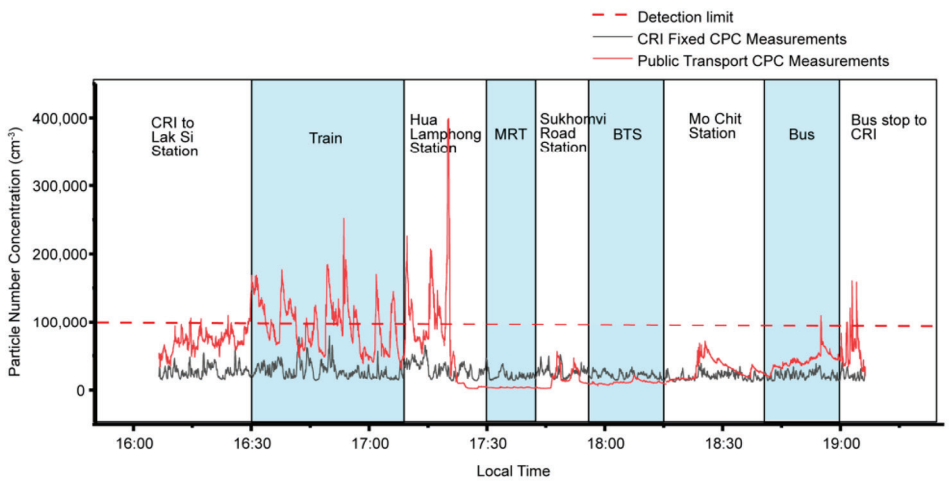

Figure 2. Particle number concentration measured on train, MRT, BTS and bus in Bangkok, compared with measurements at a fixed site. Walking and waiting to board each mode of transport are coloured in white, while mass transit modes are highlighted in light blue (Matthews et al., 2023).

Whilst use of mass transit is preferable, reduction in overall vehicle numbers in both countries are needed to make mass transit travel times (buses) lower and more reliable. The COVID-19 pandemic was a devastating incident, but it has challenged the need to travel to work every day and to travel to meetings that could be conducted on-line, such reductions in travel have significant impacts on pollutant exposure (e.g. Holland et al., 2024).

It is well known that meteorology and building geometry can affect the concentration-time profiles of pollutants in the urban environment (e.g. Wood et al, 2009). Street canyons can bias exposure on one side of the road compared with the other, whilst pollutant concentrations at a fixed point can vary significantly with wind speed and direction, even when moving and stationary emissions are the same. Hence, effective pollution control measures may need to change on seasonal and even on weekly bases.

Work in both countries have shown that analysis of metals (Matthews et al., 2022), trapped

on particles can provide significant insight into pollution sources and facilitate estimates on health impacts. Strong correlations between metals have been found, e.g. Cu and Zn, which is consistent with brake wear and tyre wear sources. Furthermore, the transformation to more crustal metals in dry seasons, lends weight to the usefulness of such analysis. Recent work has seen evidence of high levels of Chromium in Bangkok air whilst in Bristol in the UK, elevated levels of Nickel are associated with industrial activity near Avonmouth.

Fires, whether natural, accidental or deliberate are a strong source of toxic chemicals in the environment. Whilst forest fires are loss common in the UK, biomass burning resulting from agricultural clearage is an annual problem in Thailand and a potential future project. However, in the UK, in early November every year, bonfire night is celebrated (Priestley et al, 2018) and using a TOF-CIMS to measure pollutants during bonfire day and the following day, high levels of isocyanates, amides and nitrates were detected (see figure 3) in addition to PAHs and other toxic chemicals. The evolution of these compounds as bonfires were lit and eventually left to smolder showed over an approximate 12 hour period how pyrolysis temperatures changed the mix of compounds emitted. Air quality on bonfire night is typically the worst in the UK each year (Harrison and Shallcross, 2011). In January 2022, there was a major fire caused by vehicles in Bristol, not only did this give rise to high levels of particles, but metal deposits were also observed, some at very high levels. Therefore, not only was the particle level itself a problem to human health, the material embedded on it raised the toxicity considerably. The meteorology at the time of the fire exacerbated its impact, through a very slow-moving high-pressure system, that trapped pollutants in the Bristol basin near to the surface, high particle levels persisted for days.

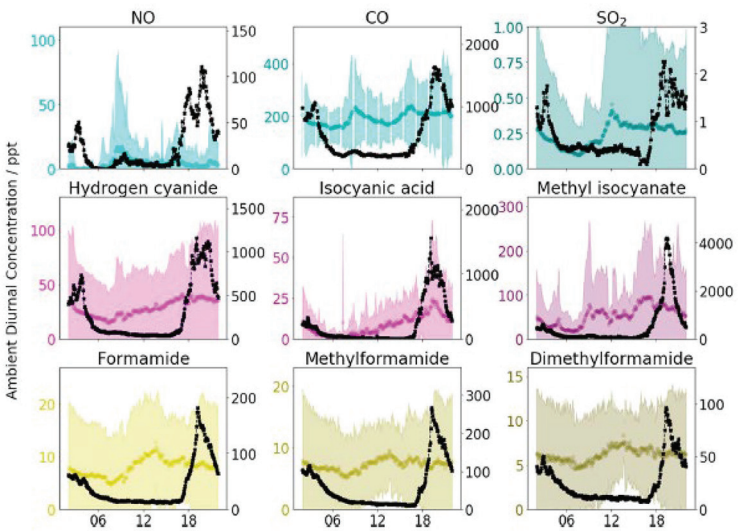

Figure 3. Five-minute averaged ambient diurnal profiles of identified species throughout the campaign with the bonfire period removed. The black line and right-hand axis show the concentration profile for species during the bonfire burn period, starting at 16.30 on November 5th (nightfall) and extending to 16.30 on November 6th (Priestley et al., 2018).

Another major pollution environment is that of the indoors. Here, emissions from indoor sources such as smoking, cooking, household furnishings and cleaning products can all contribute significantly to exposure. Oxidative transformation of indoor air is usually very slow and often requires infiltration of outdoor air, with elevated levels of ozone (O_3). Once in the indoor environment, ozone can react with alkenes to yield Criegee intermediates e.g. Welz et al., 2012), these biradical species can yield OH radicals that further accelerate oxidation, leading to secondary organic aerosol formation. In addition, through recently established chemistry (Caravan et al., 2024), Criegee intermediates can insert into O-H bonds to produce higher molecular weight more oxygenated species that can condense onto existing particles

or potentially seed new particles indoors. Many indoor spaces, are naturally ventilated in both countries and extreme heat or extreme cold tend to make these indoor spaces places of low ventilation, allowing the buildup of pollutants. Schools, meeting spaces for the elderly and many hospitals are naturally ventilated and despite every effort are often places where poorer indoor air quality resides. Recent studies (Mattews et al., 2017; 2024) using inert and reactive tracers have demonstrated that significant amounts of outdoor air can penetrate into the indoor environment, where residence times can then be very long.

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$PM_{2.5}$ TOXICITY AND ADVERSE HEALTH EFFECTS

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Ambient air is a complex mixture of chemical compounds, and WHO has estimated that roughly 7 mill. people globally will die prematurely due to the exposure.

Numerous studies on the link between air pollution and adverse health effects have been conducted in different populations and locations with focus on particulate matters (PM), especially $PM_{2.5}$ (particles with a geometric diameter of 2.5 μM). These particles consist of a core of elementary carbon to which polycyclic hydrocarbons, metals, volatile organic compounds and sulphates are associated. The composition and amount of the compounds depend on the source of the particles, e.g. biomass or fuel type used, driving conditions.There are both a spatial and a temporal variation in the chemical composition of the particles and thus their toxicity. Global meta-analysis studies have shown an association between $PM_{2.5}$ and overall mortality (HR 1.O39 per 5 $\mu g/m^3$ increase).

Due to the small size $PM_{2.5}$ are easily inhaled and can reach the lower part of the lung, e.g., the alveoles from where they can translocate into systemic circulation. $PM_{2.5}$ particles have been detected in blood, placenta and fetal tissues. The inhaled particles are taken up by alveolar macrophages that release proinflammatory cytokines TNF-alpha, IL-6 and promotes local inflammation, and influence the expression of many signal transmission genes, that aggravate respiratory and cardiovascular diseases. Both epidemiological and experimental studies have shown that the particles cause systemic inflammation and induce reactive oxygen species, and furthermore have an antioxidant effect by inhibiting MnSOD2, reduce GSH and causes mitochondrial dysfunction.

Epidemiological studies have shown that both acute and chronic $PM_{2.5}$ exposures are associated with increased mortality and morbidity as well as various pathologies in different organ systems e.g., respiratory, cardiovascular, cancer, neurological and reproductive effects (Tabel 1).

TABLE 1. HEALTH EFFECTS and $PM_{2.5}$ Chronic Exposure

	RR per 5 $\mu g/m^3$
Term low birth weight	1.21
Preterm birth	1.09
Asthma at age 8	1.20
Respiratory infection	2.58
Hypertension	1.09
Stroke	1.19
All lung cancer	1.19
Adenocarcinoma	1.58

ESCAPE Project (Bert Brunekreff

Several adverse outcome pathways (AOP) have been develop for the link between $PM_{2.5}$ exposure and adverse health effect. Central to some of the these pathways are the molecular initiation events (MIE), excessive generation of reactive oxygen species, and activation of the AhR receptor by the PAH associated with particles. The subsequent key event relationships (KER) are DNA damage, inflammation and induction of various cell death pathways, e.g. apoptosis.

Apoptosis is one of the cell death pathway induced by particulate matters. Two different pathways have been identified, one linked to inflammation and the TNF-alpha pathway, while others are linked to reactive oxygen species either as an direct effect on mitochondria or activation of the KEAP1/Nrf2 pathway.

$PM_{2.5}$ exposure aggravates respiratory and cardiovascular diseases. It is proposed that the uptake of the particle in macrophages promotes local inflammation, and activation of the NRLP 3 with subsequent production of TNF-alfa and Il-1 beta protein levels and caspase 1 activity, resulting in apoptosis.

Apoptosis plays an important role in the biological maintenance of cellular homeostasis and aberrant apoptosis is involved in the development of various pathologies, e.g. cardiovascular, immune and neurodegenerative diseases. Apoptosis could be induced by inflammation through the TNF-alpha pathway and ROS induction via Keap1/Nrf2 or an direct effect on the mitochondria.

Telomere is a region of repetitive DNA sequences at the end of the chromosome, which protects the chromosome to be frayed or tangled. Telomere has been shown to be shortened by oxidative stress and has been shown to be associated with $PM_{2.5}$ exposure, and will as a consequence influence chromosomal stability. Experimental studies showed that short term exposure had no effect on telomere length, but did increase hTERT activity, whereas long term exposure decreased both telomere length and hTERT protein levels. In human studies only chronic and high $PM_{2.5}$ exposure were linked to leucocyte telomere length shortening and was associated with an increased risk for developing lung diseases, e.g. asthma, and with increased risk of some cancers.

Air pollution has been classified by IARC as a Human Group 1 carcinogen. The exposure for $PM_{2.5}$ has been associated with increased risk of various types of cancer, including lung cancer, especially adenocarcinoma in contrast to tobacco induced lung cancer (Tabel 2), i.e., Small Cell Carcinoma, though many of the carcinogenic compounds are similar. It has been suggested that the $PM_{2.5}$ leads to an influx of macrophages that triggers the release of interleukin.

TABEL 2. Cancer Risk – Danish Diet and Cancer Risk Study
(Exposure assessment GIS based models)

Cancers identified (20 sites)

# Cases		RR
592	Lung Cancer	1.3
57	Liver	1.6
987	Breast Cancer	1.2
35	Cervix Cancer	2.5
221	Bladder	1.3
95	Brain	2.3

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$PM_{2.5}$ toxicity play many roles in development of cancer through initiation, progression and angiogenesis. The latter is mediated by the HIF-1 alpha transcription factor.

IARC has defined different pathways of $PM_{2.5}$ toxicity as potential mechanism for cancer development: genotoxicity, oxidative stress, chronic inflammation, alteration in DNA resulting in genomic instability, epigenetic changes, immunosuppression and modulation of receptor mediated effects.

Different pathways have been proposed for the organic material associated with the particles, e.g. PAH and the particle itself. The PAHs are procarcinogen and are activated by Cytochrome P450 enzyme into metabolites that react with DNA forming adducts that may result in mutations.

A high level of DNA adducts are associated with an increase risk of cancer. The organic material can also mediated its effect via the ER-beta and AHR pathways, while the metals may influence cell oxidative stress and may damage DNA.

The progression of lung cancer is promoted by activation of the the AhR –TMPRSS 2 pathway and upregulates the IL-18 expression.

Many human and experimental studies have demonstrated the induction of oxidative stress, e.g. oxidative damage to DNA, 8-OH Gua, and a positive association between $PM_{2.5}$ exposure and oxidative DNA damage has beenreported. Exposure for $PM_{2.5}$ has also been monitored by the comet assay for DNA damage and malondialdehyde in serum as well as upregulation of the expression of proinflammatory markers.

Genetic factors have also been shown to play a role in PM2.5 induced carcinogenesis, in some tissues, e.g. ER- Breast cancer RR 2.57 and ER+ 1.2.

$PM_{2.5}$ exposure has both chronic and acute effects on the cardiovascular system. An adverse outcome pathway (AOP) for the link between chronic $PM_{2.5}$ exposure and cardiac development toxicity has been proposed. The molecular Initiating events involve excessive generation of reactive oxygen species resulting in cellular oxidative stress and endoplasmic reticulum stress, DNA damage and inflammation. Activation of the aryl hydrocarbon receptor followed by inhibition of the Wnt/beta-catenin pathway and as a consequence inhibits cardiomyosite differentiation, which results in abnormal cardiac structure and function, and subsequently increased morbidity of congenital cardiovascular heart defect. The organic materials from the particles induce senescence in cardioblast and induce cell cycle arrest.

$PM_{2.5}$ may be neurotoxic to the brain and cause central nervous system damage, contributing to neurodevelopment disorders such as autism spectrum disorders and neurodegenerative diseases such as Alzheimer's, Parkinson and dementia. The particles can reach the brain through thevblood-brain barrier or the olfactory system, and induce oxidative stress, neuroinflammation and neurotoxicity. Furthermore, inflammatory mediators released into the blood can cross the blood brain barrier into the brain. It has been suggested that $PM_{2.5}$ induced changes in the gut and lung microbiome may play a role in this process.

An association between $PM_{2.5}$ exposure and epigenetic changes has been observed in connection with neuropsychiatric disorders.

Maternal $PM_{2.5}$ exposures alter placental gene expressions related to aminoacid transport and cellular respiration pathways, and pregnant women experience a higher risk of infant mortality, preterm delivery. and increased risk in their offspring for neurological effects autism. Prenatal and postnatal exposures have an effect on neurodevelopmental skills, like global intellective function and attention/executive functions memory and ADHD.

There are consistent epidemiological studies to show that $PM_{2.5}$ exposure is a general health concern.

Most of the epidemiological studies have used different approaches to estimate $PM_{2.5}$, exposure. A major concern with the studies is that particles is not a uniform entity and their toxicity depend on the source, and show seasonal variation. In spite of the weaknesses of the human studies, the observation is supported by experimental studies *in vitro* and *in vivo* to conclude that exposure to $PM_{2.5}$. are of a major global health concern.

Based upon key epidemiological studies, WHO has established a guidance value for $PM_{2.5}$ concentrations, annual 5 $\mu g/m^3$ and 24 hrs 15 $\mu g/m^3$, and recommend that national value should be established based upon socio-economic values of the country.

HOW A SINGLE MUTATION IN CFTR CAUSES THE SYSTEMIC DISEASE CYSTIC FIBROSIS: INTERACTIONS, PTMS, AND STRUCTURE

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Protein conformation is dynamic as it is influenced by post-translational modifications (PTMs) and interactions with other proteins, small molecules or RNA. The ΔF508 mutation of the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) is the major cause for Cystic Fibrosis (CF), the most common inherited childhood disease. The mutated anion channel is not fully glycosylated and has little to no activity in bronchial epithelial cells of CF patients. These cellular processing defects can be partially rescued by low temperature or by inhibition of histone deacetylases (HDACi). A favorable change in protein-protein interactions for ΔF508 CFTR was proposed as a mechanism of rescue, however interactome dynamics during temperature-shift and HDACi rescue are unknown. We reported the first comprehensive analysis of the wt and ΔF508 CFTR interactome and its dynamics during temperature shift and HDACi. By using a novel deep proteomic analysis method (CoPIT) (Figure 1), we identified 638 individual high-confidence CFTR interactors with 208 specific for ΔF508 that constitute a mutation-specific interactome, which is extensively remodeled upon rescue. A detailed analysis of the interactome remodeling identified key novel interactors, whose loss promotes enhanced CFTR channel function in primary CF epithelia or which were critical for normal CFTR biogenesis (Figure 2). Our results demonstrated that global remodeling of ΔF508 CFTR interactions is crucial for rescue, and provide comprehensive insight into the molecular disease mechanisms of CF caused by deletion of F508.

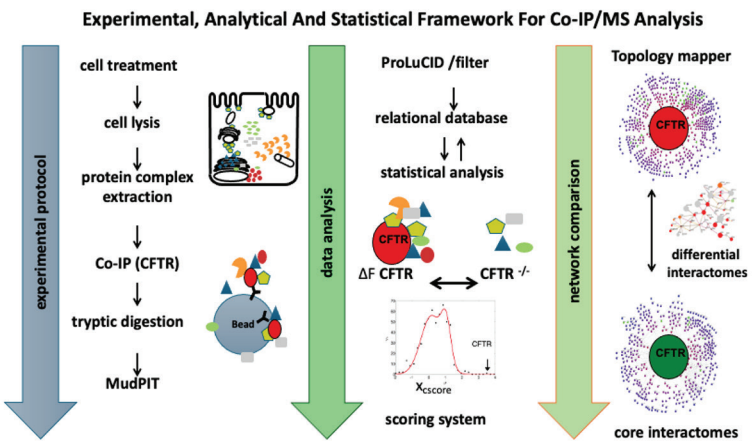

Figure 1: The co-immunoprecipitation workflow used to identify the interactomes of the wild type CFTR and ΔF508 CFTR.

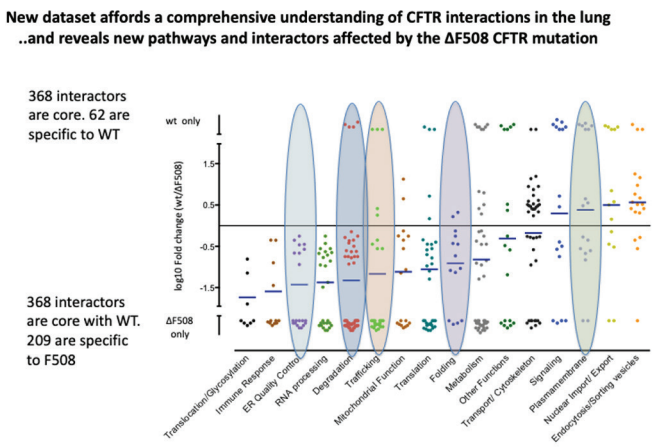

Figure 2: Differences in the interactions of wild type CFTR and ΔF508 CFTR based on gene ontologies. The results show there is a disease specific interactome.

The multistep process regulating the maturation of membrane proteins, in the endoplasmic reticulum (ER) and through the secretory pathway, is disrupted in protein misfolding disorders. Mutations in the ion channel CFTR that impairs its folding and subsequent localization to the plasma membrane causes cystic fibrosis (CF), an inherited, severely disabling, and eventually lethal disease that impairs the function of multiple organs, mostly the lungs. There is currently no cure for CF. Here, we found that proper maturation of CFTR is dependent on crosstalk between phosphorylation and methylation events in the regulatory insertion (RI) element of the protein (Figure 3). Manipulating these posttranslational modifications (PTMs) prevented the maturation of wild-type CFTR and instead induced its degradation by ER quality control systems. Deletion of Phe508 (ΔF508), the most prevalent mutation in CF, and other mutations in CFTR that impair its trafficking, such as N1303K, also led to quantitative and qualitative PTM changes that prevented maturation of misfolded CFTR. Further quantitative analysis revealed that a wild-type CFTR-like PTM pattern is restored at permissive temperature and is essential for the rescue of ΔF508 CFTR function. Furthermore, the ability to replicate this PTM pattern predicted the efficacy of treatments in restoring ΔF508 CFTR activity. Accordingly, bioinformatics analysis revealed that point mutations of several of the modification sites are associated with clinical CF diagnosis. The findings identify a minimal quantitative and qualitative PTM code for CFTR maturation that distinguishes correctly folded from misfolded CFTR.

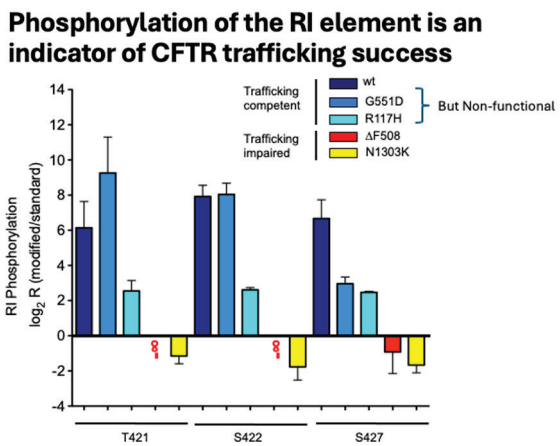

Figure 3: Phosphorylation at one of three sites is essential for the protein to mature. Trafficking impaired proteins are not phosphorylated.

Protein conformation is dynamic and is influenced by post-translational modifications (PTMs) and interactions with other proteins, small molecules, or RNA. However, in vivo characterization of protein structures and protein structural changes after perturbation is a major challenge so experiments to characterize protein structures are typically performed in vitro with highly purified proteins or protein complexes, revealing a static picture of the protein. To identify the true conformational space occupied by proteins, we developed an quantitative protein footprinting method (Covalent Protein Painting (CPP)), a novel low-resolution method that allows the characterization of protein conformations *in vivo*. Here, we report how an ion channel, the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR), is conformationally changed during biogenesis and channel opening in the cell (Figure 4). Our study led to the identification

of a novel opening mechanism for CFTR by revealing that the interaction of the intracellular loop 2 (ICL2) with the nucleotide binding domain 2 (NDB2) of CFTR is needed for channel gating, and this interaction occurs concomitantly with changes to the narrow part of the pore and the walker A lysine in NBD1 for wt CFTR. However, the ICL2:NBD2 interface, which forms a “ball-in-a-socket” motif, is uncoupled during biogenesis likely to prevent inadvertent channel activation during transport. Mutation of K273 in the ICL2 loop severely impaired CFTR biogenesis and led to accumulation of CFTR in the Golgi and TGN. CPP further revealed that, even at permissive temperature or upon treatment with approved drugs such as Trikafta, the uncoupled state of ICL2 is a prominent feature of the misfolded CFTR mutants ΔF508 and N1303K that cause Cystic Fibrosis (CF). Although Trikafta treatment reduced the amount of uncoupled ICL2:NBD2 interfaces, more than 75% of F508 CFTR remained in the uncoupled state, suggesting that stabilization of this interface could produce a more efficient CF drug. CPP can characterize a protein in its native environment and measure the effect of complex PTMs and protein interactions on protein structure, making it broadly applicable and valuable for the development of new therapies.

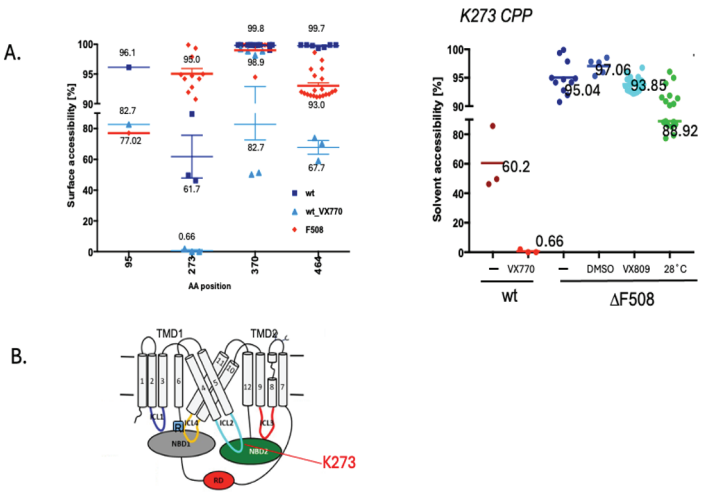

Figure 4: A. The lysine at K273 in ICL2 is partially buried in the NBD2 domain in the WT protein but when treated with the drug VX-770 which activates the protein K273 is completely buried in NBD2. Rescue of DF508 with temperature shift moves the K273 to be partially buried versus mostly exposed. B. Schematic of the structure shows ICL2 and K273 interfacing with NBD2 after treatment with VX-770.

ULTRASENSITIVE PROTEOMICS FOR PRECISION ONCOLOGY

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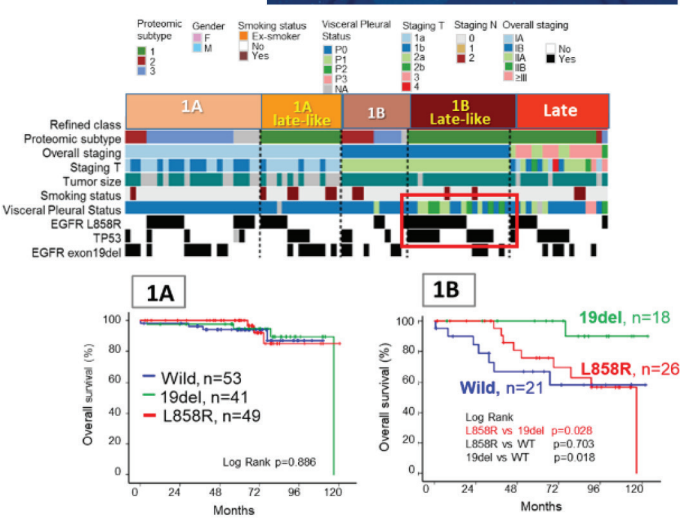
Proteomics is the study of functions, structures, and interactions of the entire set of proteins that plays fundamental roles to shape and regulate the life. With great passion to reveal disease network, we have devoted our efforts to developing mass spectrometry-based methodologies towards genome-wide profiling of membrane proteomics and post-translational modificome with sensitivity to explore clinical specimen. In this talk, I will share our experiences and the long-term journey from the development of proteomics techniques to their utility in discovering new biology and opportunity for cancer diagnosis and precision medicine. Taking cancer research that requires multi-disciplinary efforts to cross the borders as an example, we also learn that team work will create higher impact in science. I will share the challenges and cross-border process how we assembled a multi-organization and multi-disciplinary team to launch a Cancer Moonshot project in 2016. Starting from scratch, the joint and highly multi-disciplinary efforts delineated molecular signatures of pathogenesis and progression in non-smoking lung cancer.

1. Taiwan Cancer Proteogenomics Moonshot

With the aim of accelerating the progress toward prevention, control and treatment for cancer, we were invited to join the global effort of International Cancer Proteogenome Consortium (ICPC) in 2016. The first proteogenomics study of non-smoking lung cancer in East Asia delineates molecular signatures of pathogenesis and progression (***Cell, 2020, Cover Story,*** *highlighted by* ***Nature Review Clinical Oncology, Cancer Discovery***). Mutational signature analysis revealed age- and gender-related mutagenesis mechanisms, characterized by high prevalence of APOBEC mutational signature in younger females and over-representation of environmental carcinogen-like mutational signatures in older females. Most excitingly, we developed a proteomics-informed classification that was able to distinguish the clinical characteristics of early stage patients with EGFR-mutation, answering the previously observed yet unresolved question on the poor survival of L858R mutation patients compared to Del-19 type. Thus, the proteomics has identified a new subtype, terms “late-like” subtype, who are in the clinically early stage yet their molecular profiles resemble the ones from late stage patients. After this publication, a new follow-up data in 2022 confirmed that this group of patient has extremely high recurrence (unpublished). The



findings demonstrated the power of proteomics for identification of high risk patients for early treatment. Furthermore, integrated protein network analysis revealed the cellular remodeling underpinning clinical trajectories and nominated candidate biomarkers for patient stratification and therapeutic intervention. This multi-omic molecular architecture suggested strategies for management of early stage never-smoker NSCLC.

Nevertheless, the translation of proteomic discovery fasces multiple challenges in both technical robustness and sensitivity, as well as large-scale validation and assay development. I will also share how these scientific findings inspire the development of precision medicine strategies and our efforts to translate research assay into clinical laboratory.

New strategies of Ultrasensitive Proteomics from Microscale to Single Cell Resolution

The Taiwan Cancer Moonshot Project shows that personalized proteomics starts to inspire new strategies for precision medicine by facilitating discovery of disease biomarkers, molecular signature of disease subtypes and druggable pathways of treatment of individual patients. Due to the high complexity of human proteome and disease, however, clinical proteomic still faces challenges to achieve high sensitivity for limited specimens in different stage of a patient's journey and sufficient profiling depth to "see" the disease biology. With the rapid advancement of instrumentation, various types of data-independent acquisition mass spectrometry (DIA-MS) have been reported and gain popularity in recent years (**Mass Spectrom. Rev., 2022 May 29, Figure 1**).

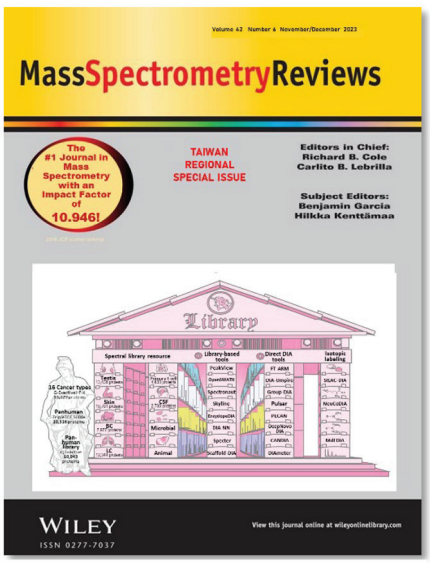


Figure 1: Advances in Data-Independent Acquisition Mass Spectrometry towards Comprehensive Digital Proteome Landscape", Reta Birhanu Kitata, Jhih-Ci Yang, Yu-Ju Chen*, Mass Spectrom. Rev. 2022.

To further enhance the sensitivity and proteome coverage for microscale sample, **a global phosphoproteomics strategy (GPS** in navigating proteome analysis) based on data-independent acquisition (DIA) mass spectrometry and high-quality mass spectral libraries was developed ***(Nat. Commun. 2021, Figure 2)***. We use lung cancer cell lines and patient-derived tissue to construct a hybrid phosphoproteome spectral library covering 88,107 phosphosites). Our single-shot DIA workflow provides one of the deepest coverage of 36,350 phosphosites in cell lines within 2 hours. Application to drug-resistant cells and patient-derived lung cancer tissues delineates site-specific phosphorylation events associated with resistance and tumor progression (Nat, Commun, 2021).

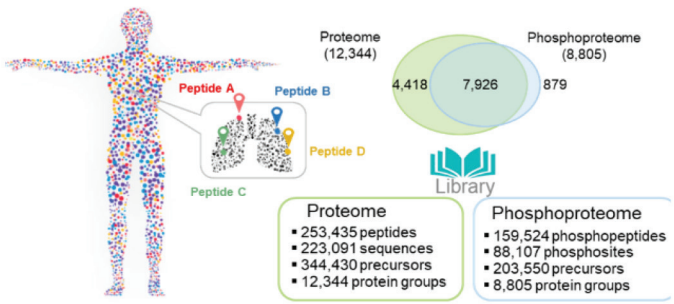


Figure 2: Proteome and phosphoproteome libraries composition of Global Phosphoproteomics System (GPS)

Single Cell Proteomics and Phosphoproteomics To further push the extreme sensitivity of human proteome profiling, I started a thematic program to collaborate with my colleagues, Dr. Hsiung-Lin Tu, who is an expert in fabrication of microfluidics. Though microfluidics device was considered as an out of fashion tool in the proteomics community, we succeeded to develop a highly streamlined microfluidics coupled with data-independent acquisition mass spectrometry to achieve 1500 proteins at the single cell resolution (***Nat. Commun.* 2022, Figure 3**). In addition to launch a thematic program since 2019, my team is responsible of developing the protocol as well as data-independent acquisition mass spectrometry to push the analysis sensitivity. We found that sample-size comparable mass spectra library established from high quality of small cell dataset can effectivity enhance 1.5-2 fold in number of proteins (*Anal. Chem.* ***2022***). This microfluidics platform can be fully automatic to minimize sample loss and successfully identified ~1500 proteins from a single cell, covering a 5-order dynamic range of with good reproducibility and <16% missing values between runs.

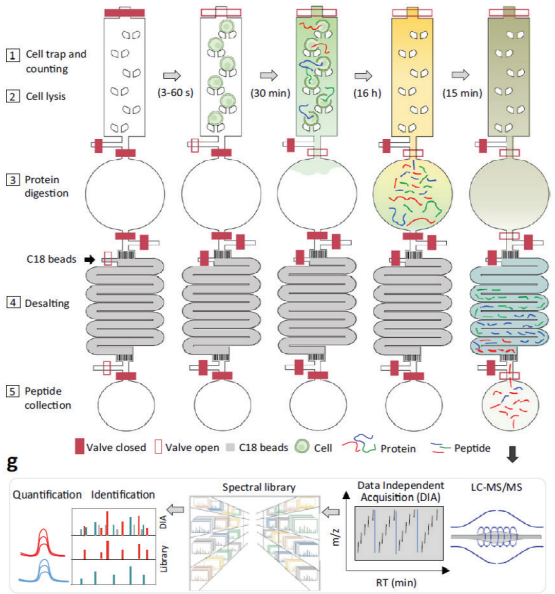


Figure 3: Streamlined single-cell proteomics by an integrated microfluidic chip and data-independent acquisition mass spectrometry

Nanoscale-to-Single Cell Phosphoproteomics

To further implement DIA into the more challenging PTMomics, using phosphoproteomics that has lower LC-MS/MS detectability, extremely low abundance and stoichiometry as a model, we developed a simple and rapid one-pot phosphoproteomics workflow (SOP-Phos) for microscale phosphoproteomic samples (1000 – 10 cells) (*J. Proteome. Res.* ***2024***). With an ultra-streamlined workflow combining integrated proteomics chip (iProChip), we ultimately demonstrated nanoscale-to-single cell phosphoproteomic profiling (Adv. Sci. accepted, **Figure 4)**. The strategy successfully reveals heterogeneity of patient with acquired resistance to third-generation EGFR therapy. Notably, the sensitivity and coverage enable illumination of heterogeneous cytoskeleton remodeling and cytokeratin signatures among patient-derived cells, stratifying mixed-lineage adenocarcinoma-squamous cell carcinoma subtypes and identifying alternative next-line therapy for late-stage patients.

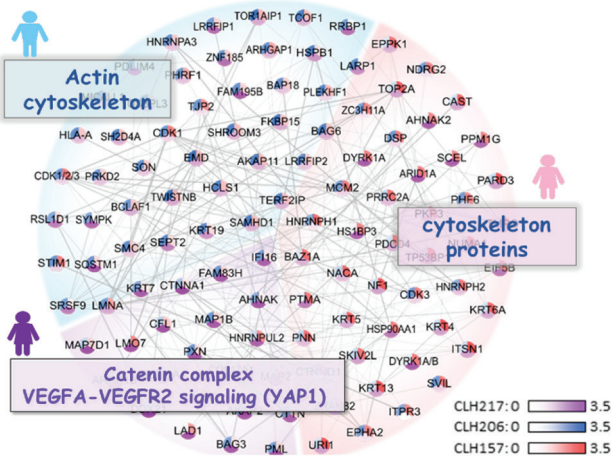


Figure 4: Nanoscale single-cell phosphoproteomics reveal Cytoskeleton remodeling associated with tumor progression and metastasis in late-stage NSCLC

To our knowledge, these tools represent one of the most sensitive (phospho) proteome profiling technologies at the single cell resolution.

In summary, these new tools are realizing our dreams to allow mapping towards whole proteome typing the cancer signaling pathway for clinical specimens of individual patient.

NAVIGATING DRUG-TARGETOME-PHENOTYPE INTERACTION AND ITS TRANSLATIONAL IMPLICATIONS

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Navigating the protein targets of drugs (hereafter, targetome) and deciphering the specific mechanisms of action at the molecular level of these interactions are critical steps in the development of drugs to treat human diseases. We have developed target protein identification methods including conventional affinity chromatography using labeled small molecules as well as recent methods using label-free small molecules such as Drug Affinity Responsive Target Stability (DARTS) and Cellular Thermal Shift Assay (CESTA) in combination with LC-MS/MS analysis to identify the targetome of drugs (1). The direct interaction between drug and the target protein is validated using biophysical, and bioinformatics tools. In addition, the biological relevance of this 'drug-targetome-phenotype' interaction is verified by genetic modulation, facilitating structure-based better drug design. In this presentation, our recent studies on 'drug-targetome-phenotype' interaction for navigating new mechanisms of small molecules, target proteins, and their translational impact will be presented by introducing our three case studies of protein target identification and validation of small molecules that enhance autophagy (2-4).

First, a natural compound, Kaempferide (Kaem) was identified as an autophagy inducer through phenotype-based screening. Kaem enhanced autophagy through translocation of transcription factor EB (TFEB) without perturbation of MTOR, accelerated autophagy-induced lipid droplets (LDs) degradation, and ameliorated metabolic dysregulation in obese mice. To elucidate the mechanism underlying the biological activity of Kaem, a combination method of label-free drug affinity responsive target stability (DARTS) technology with a liquid chromatography/ tandem mass spectrometry (LC–MS/MS) readout was used. Among 10 protein candidates, mitochondrial elongation factor Tu (TUFM) was identified as a key binding protein of Kaem. Absence of TUFM reversed Kaem-induced autophagy and lipid degradation. Kaem also induced mitochondrial reactive oxygen species (mtROS) to sequentially promote lysosomal Ca^{2+} efflux, TFEB translocation and autophagy induction, suggesting a role for TUFM in mtROS regulation. Collectively, these results demonstrated that Kaem could be a potential therapeutic candidate/small molecule tool for the treatment of metabolic dysregulation and revealed a role for TUFM in autophagy for metabolic regulation with lipid overload (5).

Second, we discovered that one of the active principles of Korean ginseng, Rg3, attenuated cellular senescence in an autophagy-dependent manner. Rg3 enhanced the activation of AMP-activated protein kinase (AMPK) signaling, and ablation of AMPK abolished Rg3-induced autophagy, indicating pivotal role of AMPK in autophagy enhancing activity of Rg3. In addition, inhibition of Calcium/calmodulin dependent protein kinase 2 (CAMKK2) abolished Rg3-induced AMPK activation, suggesting that Ca^{2+} is required for GR to activate AMPK. To investigate the source of Ca^{2+} modulated by GR, Ca^{2+} chelator was applied, revealing a critical role for extracellular Ca^{2+}. Mechanistically, Rg3 bound to and agonized the plasma membrane Ca^{2+} channel, ORAI1, located in, resulting in Ca^{2+} uptake into the cytoplasm, and promoted the cascade of CAMKK2, AMPK, beclin1 (BECN1), and autophagy. Cells treated with ORAI1 inhibitor, or lacking ORAI1 expression completely abrogated the modulatory effect of GR on AMPK activation. These results demonstrated the potential of Rg3 as a new drug candidate/ molecular tool for the development of anti-aging therapy and revealed a novel role of ORAI1 in cellular senescence (6).

Third, metformin (MetF) is widely used worldwide as a first-line therapy for type 2 diabetes. Recently, there has been increasing interest in the pleiotropic effects of MetF, such as its

anti-cancer and anti-aging properties. However, the molecular target of the MetF and the detailed mechanism underlying its cell growth inhibitory effects through autophagy induction remain incompletely elucidated. Using an innovative label-free DARTS/LC-MS/MS method, we discovered that the mitochondrial voltage-dependent anion channel 1 (VDAC1) is a novel binding protein in the autophagy-related cell death activity of high-dose MetF in hepatocellular carcinoma (HCC). Computational alanine scanning mutagenesis revealed that MetF and VDAC1 (D9, E203) interact electrostatically. MetF disrupts the IP3R-GRP75-VDAC1 complex, which plays a key role in stabilizing mitochondria-associated ER membranes (MAMs) by binding to VDAC1. This disruption leads to increased cytosolic calcium levels, which contributes to autophagy induction. MetF also decreased the AMP/ATP ratio and activated the AMPK pathway. Cells genetically knocked down for VDAC1 mimicked the activity of MetF. Taken together, this study provides new insights into the involvement of MetF in ionic interactions with VDAC1 that contribute to its anticancer effects in HCC. These findings help to elucidate the diverse biological and pharmacological effects of MetF, particularly its influence on autophagy, as well as the potential of MetF as a therapeutic agent for diseases in which VDAC1 is overexpressed (7).

Together, intriguing natural small molecules have been discovered as autophagy inducers, and their target proteins have been identified through our chemical genomics/proteomics approaches. This forward approach will ultimately enable the development of further improved therapeutics that more specifically target partner proteins, thereby accelerating therapeutic efficacy. In addition, these studies demonstrated that the label-free small molecule target identification methods, DARTS and CETSA, are powerful platforms for the discovery of novel targets with the tremendous advantage of using unmodified compounds, thereby accelerating the target identification process.

New insights from these small molecules and target proteins related to autophagy have enabled us to uncover new molecular and signaling mechanisms underlying autophagy and to translate them into the development of new therapeutics for autophagy-related diseases, including obesity, aging, cancer and tauopathies.

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ADVANCEMENT IN PEPTIDOPROTEOMICS FOR CANCER RESEARCH

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Peptidoproteomics is an interdisciplinary field that integrates principles from both peptidomics and proteomics. It involves the systematic analysis of peptides and proteins within cells, tissues, or organisms, complementing other “omics” approaches such as genomics and transcriptomics by providing insights into peptide and protein identities, structures, and functions. Peptides and proteins play crucial roles in biological processes, including signaling pathways, post-translational modifications, and complex system interactions. Peptidoproteomics employs a variety of advanced techniques, including mass spectrometry, chromatography, and bioinformatics, to identify, quantify, and characterize peptides and proteins. Among these, mass spectrometry is a pivotal tool in modern peptidoproteomics research. The use of peptide barcodes through MALDI-TOF MS generates unique peptide profiles, which can serve as biomarkers for disease diagnosis, personalized medicine, and the study of complex biological systems. Additionally, LC-MS facilitates the identification and quantification of peptides and proteins, with a particular focus on their temporal and spatial dynamics in biological pathways. Peptidoproteomics has a broad range of applications, including the discovery of diagnostic markers, vaccine development, investigation of peptide protein interactions for drug target identification, and exploration of natural defense molecules with therapeutic potential.

Introduction:

Cancer continues to be a major global cause of mortality. In Thailand, approximately 381 new cancer cases are diagnosed daily, amounting to around 130,000 cases annually (1). Additionally, cancer leads to the deaths of approximately 230 individuals each day in Thailand, resulting in an annual total of about 84,000 deaths, underscoring the need for tailored prevention, early detection, and treatment strategies (2). The need for early diagnosis, personalized and targeted therapies approaches arises in response to current limitations, driving the ongoing quest for more effective and tolerable cancer treatments.

Mass spectrometry is a highly effective tool for detecting protein and peptide expression in biological fluids (3), and it has garnered significant interest for its application in developing biomarker panels (4). Peptides play essential roles in numerous physiological processes, functioning as metabolic products, hormones, and components of proteolytic enzymes (5). Proteolytic degradation has been observed within serum peptidomes, and the analysis of peptidome patterns provides valuable insights into pathophysiological processes, particularly in cancer (6).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a powerful technique for examining peptidomes, particularly low-molecular-weight peptides (≤10 kDa), which represent endogenous peptides found in both extracellular and intracellular environments (7). Furthermore, LC–MS/MS has emerged as a promising approach for the identification and quantification of numerous proteins in biological samples. This proteomic workflow enables the analysis of proteins, the detection of post-translational modifications, the comparison of protein abundance across different sample sets (e.g., healthy individuals versus patients, or across various tumor subtypes and stages), and the exploration of protein interactions and complexes within cellular pathways or networks.

Such peptidoproteomic analyses aim to deepen our understanding of biological processes, elucidate underlying mechanisms, improve disease diagnosis and prognosis, and facilitate precise patient stratification for personalized medicine.

Methodology:

Peptide and protein preparation: Peptides and proteins from serum were isolated following the protocols outlined by Rungkamoltip et al. (8).

Peptidome analysis by Maldi-TOF MS: Each sample was fractionated using a Ziptip C18 column to isolate peptides (Millipore, Burlington, MA, USA). Peptide concentrations were quantified using the Bradford assay (9). Tissues peptides in the eluted solution were mixed with MALDI matrix and applied to a steel target plate for MALDI-TOF MS analysis. Samples were analyzed in linear positive mode over a mass range of 1,000–10,000 Da, with 1,500 laser shots per spot.

Proteome analysis by LC-MS: Protein concentrations were determined using the Lowry assay (10). For each sample, 5 µg of protein was reduced with 10 mM DTT and alkylated with 100 mM iodoacetamide. Following overnight trypsin digestion at 37°C, the resulting peptides were submitted for LC-MS/MS analysis. MaxQuant software was used for quantification, based on MS signal intensities, with protein identification performed using the human UniProt database and Andromeda search engine with a significance threshold of $p < 0.05$ (11). UniProtKB was used for detailed protein sequence and functional information. LC-MS analysis was conducted in triplicate for each sample.

Bioinformatic analysis: Visualization and statistical analysis of the LC-MS data, including partial least squares discriminant analysis (PLS-DA), differential analysis (volcano plots, heatmaps), were conducted using MetaboAnalyst version 6.0, with a significance threshold of $p < 0.05$ (12). A Venn diagram was used to illustrate differences between protein lists from distinct differential analyses (13). Functional categorization and biological process analysis of differentially expressed proteins were performed using Metascape (14). KEGG pathway enrichment analysis was performed using ShinyGO (15).

Result and Discussion:

Peptidome profiling by MALDI-TOF MS: Peptide samples, eluted through reverse-phase chromatography using hydrophobic C18-Ziptips, were analyzed by MALDI-TOF MS, yielding distinct spectral signals. Peptide barcodes were identified for both healthy individuals and cancer patients within the mass range of 1,000–10,000 Da, as shown in Figure 1A. Unique peaks specific to each group were observed, alongside common peaks shared between groups. To further assess the differentiation between peptidome profiles, Partial Least Squares Discriminant Analysis (PLS-DA) was conducted. The PLS-DA results revealed clear distinctions between the peptide patterns of cancer patients and healthy controls, as depicted in Figure 1B. The MALDI-TOF MS analysis demonstrated high accuracy, with a 95% confidence interval. These peptide barcodes show potential as a high-throughput, sensitive diagnostic tool for the rapid screening of cancer. However, as classification accuracy is contingent on the number of reference spectra in the database, future studies should aim to expand the database with additional peptide barcodes from various cancer stages to enhance classification reliability.

Proteome profiling using LC-MS: A total of 680 proteins were identified across serum samples from healthy controls, and patients with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer (Figure 2A). These proteins are involved in a wide range of biological processes, as illustrated by the functional categorization and pathway analyses (Figure 2B, 2C, and 2D). Notably, 45 proteins were exclusively detected in cancer samples and absent in healthy controls, highlighting their potential as candidate biomarkers for the diagnosis and therapeutic development of breast, cervical, cholangiocarcinoma, and thyroid cancers.

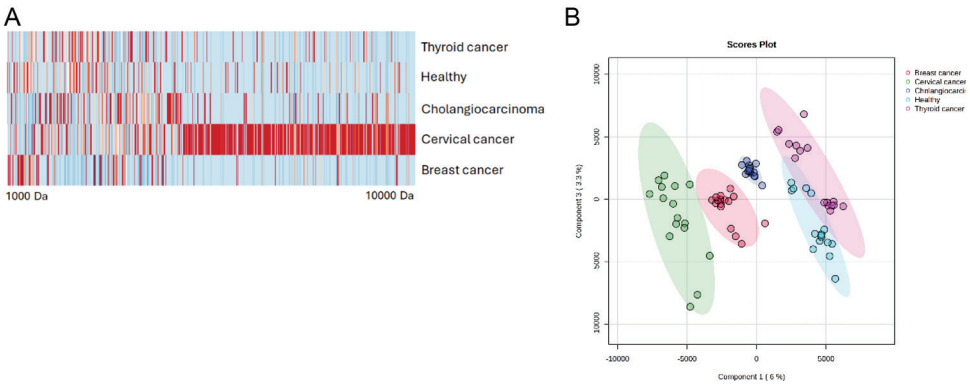

Figure 1. Peptidome profiling of serum using MALDI-TOF MS.

(A) Peptide barcodes derived from serum samples of healthy individuals and patients with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer.

(B) Two-dimensional Partial Least Squares Discriminant Analysis (PLS-DA) scatterplot illustrating the separation of serum samples from healthy individuals and those with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer based on peptidomic data.

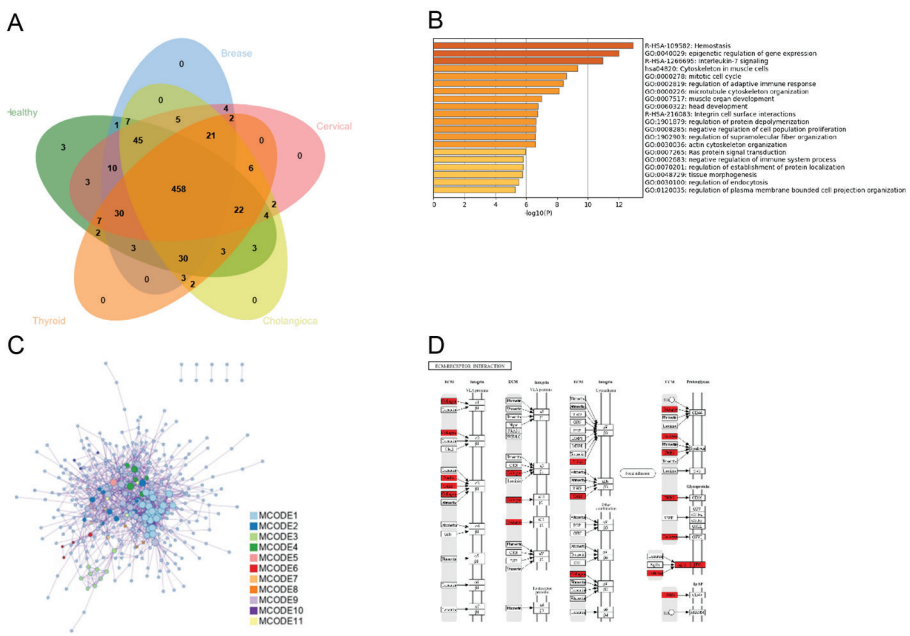

Figure 2. Proteome profiling of serum using LC-MS.

(A) Venn diagram depicting serum proteins significantly differentially expressed between breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer, compared to healthy controls.

(B) Gene Ontology (GO) classification of proteins identified in the serum of healthy individuals and patients with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer.

(C) Protein-protein interaction network of proteins identified in the serum of healthy individuals and those with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer.

(D) KEGG pathway enrichment analysis of proteins identified in the serum of healthy individuals and patients with breast cancer, cervical cancer, cholangiocarcinoma, and thyroid cancer.

Conclusion: MALDI-TOF MS has potential to be used for rapid diagnosis of cancer. With the combination of peptide barcodes (generated by MALDI-TOF MS) and proteome analysis (by LC-MS), potential peptide and protein candidates associated with cancer were identified. Further studies should be performed in a larger population to evaluate these potential candidate biomarkers.

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EXPLORING MICROPROTEINS AND INTERFERON PATHWAYS IN COLORECTAL CANCER: IMPLICATIONS FOR DISEASE PROGRESSION AND FOLFOX CHEMORESISTANCE

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Colorectal cancer (CRC), also known as colon adenocarcinoma, is the most common gastrointestinal cancer. In 2020, it accounted for 10% of all global cancer diagnoses, with approximately 1.93 million new cases, making it the third most diagnosed cancer worldwide. Additionally, it was responsible for around 935,000 deaths, representing 9.4% of all cancer-related fatalities. In Malaysia, CRC ranks among the top three most prevalent cancers. These statistics highlight the significant global burden of CRC, which continues to rise due to aging populations and shifting risk factors. The high prevalence of CRC in industrialized nations points to lifestyle factors and increased life expectancy, underscoring the urgent need for effective diagnostic biomarkers.

CRC prognosis is often poor, primarily due to challenges in early detection and delayed treatment. More than half of patients are diagnosed at an advanced stage, when metastasis renders curative surgery impossible. In such cases, chemotherapy becomes the primary treatment to eliminate cancer cells and prolong survival. A commonly used first-line treatment regimen combines fluorouracil, leucovorin, and oxaliplatin (FOLFOX). However, patient responses to this chemotherapy vary, with some developing chemoresistance or experiencing severe toxicities.

In this presentation, I will discuss two ongoing studies in my lab that employ mass spectrometry-based proteomics to investigate CRC. In the first project, we aim to identify and quantify microproteins—small proteins encoded by short open reading frames (sORFs)—that are differentially expressed at various stages of CRC. These microproteins, consisting of fewer than 100 amino acids, often remain unannotated by conventional genome annotation pipelines due to arbitrary coding sequence length thresholds. Moreover, sORFs typically possess weak sequence features and exhibit poor conservation across species, further complicating their annotation. However, recent advancements in ribosome profiling (Ribo-seq) have shown that sORFs can indeed be translated, though direct evidence of the resulting peptides remains limited. Using a mass spectrometry (MS)-based peptidomics approach, we aim to validate the translation of these sORFs at the peptide level and examine the expression profiles of CRC-associated microproteins.

To achieve this, we developed a sORF-oriented peptidomics pipeline and applied MS-based peptidomics to identify and quantify microproteins in CRC cell lines. We cultured six colorectal adenocarcinoma cell lines representing different Dukes' stages of CRC, including SW1116 (Dukes' Type A), SW480 (Dukes' Type B), SW48 (Dukes' Type C), COLO320DM (Dukes' Type C), and COLO205 (Dukes' Type D), along with the non-cancerous colorectal cell line CCD112CoN. Cells were cultured to 90% confluence, harvested, and lysed. Low molecular weight polypeptides were enriched using 0.25% acetic acid precipitation, followed by a bottom-up proteomics approach utilizing a Bruker timsTOF mass spectrometer. The MS data was analyzed using FragPipe against a custom FASTA database containing protein sequences from SwissProt, OpenProt, and Ribo-seq data

In addition to the six CRC cell lines, we downloaded and reanalyzed raw MS data from the Proteomic Data Commons (PDC000116) using the same pipeline. These data were originally

part of CPTAC's proteomic and phosphoproteomic CRC studies, which included 97 tumors and 100 normal colon specimens. Among these, 96 standard samples were paired with tumor samples from the same individuals, yielding data from 100 patients.

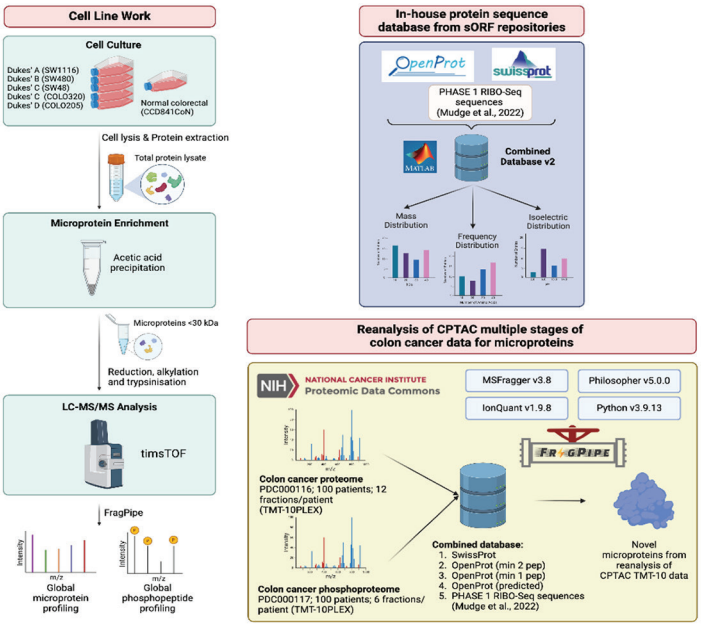
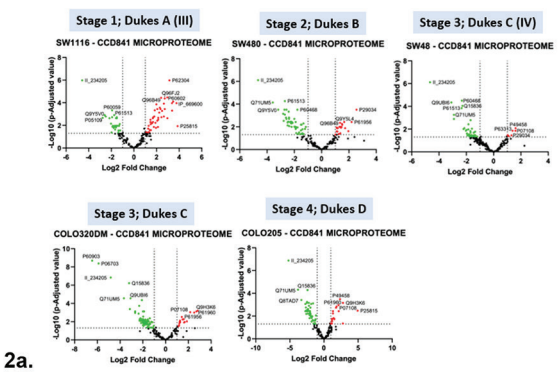


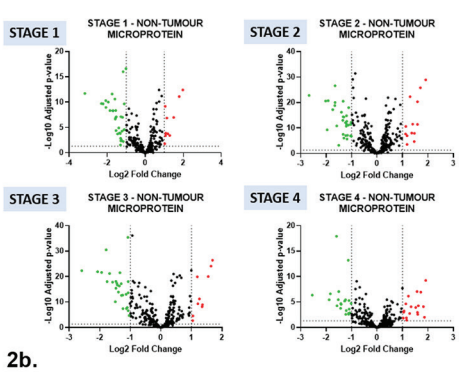
Figure 1. *Overview of the experimental design. Six cultured colorectal cancer (CRC) cell lines from various Dukes' stages and a non-cancerous control cell line were harvested and lysed. Low molecular weight polypeptides were enriched using 0.25% acetic acid. The enriched peptides were then analyzed using a bottom-up proteomics approach on a Bruker timsTOF mass spectrometer. Data were processed using bioinformatics pipeline incorporating a custom-built FASTA containing SwissProt, OpenProt, and Ribo-seq entries, leading to the identification and quantification of CRC-associated microproteins. CPTAC-derived MS data from patient specimens were analyzed using the same pipeline.*

Our analysis identified 207 microproteins from the CRC cell lines and 665 from the CPTAC data, with 167 and 66 differentially expressed, respectively. Notably, the newly annotated microproteins PIGBOS and NoBody, hypothesized to play roles in cancer, were downregulated across all CRC cell lines. Among the annotated microproteins, S100P and UQCR11 were upregulated, while ADIRF, PCP4, and NMES1 were downregulated in specific cell lines and across CPTAC stages.

To explore the biological functions of these novel microproteins, we developed a "guilt-by-association" strategy. By reanalyzing interactome MS data from BioPlex, we identified baits that bind to the same microprotein prey and conducted enrichment analysis. This approach allowed us to assign functions to three novel microproteins: (i) IP_662645, associated with NOD-like receptor signaling; (ii) IP_624439, involved in SCF-dependent proteasomal catabolism; and (iii) IP_671454, linked to actin-binding functions. These findings highlight the critical roles of sORFs and microproteins in tumorigenesis, revealing their differential expression in CRC.



2a.



2b.

Figure 2a. *Volcano plots showing the differentially expressed microproteins of CRC cell lines from various Dukes' stages against that of the normal control cell line.* ***Figure 2b***. *Volcano plots show the differentially expressed microproteins of CRC tumors derived from various TNM stages against the paired adjacent non-cancer controls.*

In the second project, we applied MS-based proteomics to investigate the development of chemoresistance to the FOLFOX regimen in CRC. While FOLFOX remains the standard chemotherapy for metastatic CRC, chemoresistance—where cancer cells adapt and become resistant to treatment—remains a significant challenge. Despite its clinical importance, the mechanisms underlying FOLFOX resistance are poorly understood.

To address this, we characterized the global proteome and phosphoproteome profiles associated with FOLFOX resistance. In the discovery phase, we analyzed proteomic and phosphoproteomic profiles of FOLFOX-responsive and FOLFOX-resistant CRC cells, identifying proteins and phosphorylation sites linked to resistance. We developed FOLFOX-resistant models by repeatedly exposing HCT-116 cells to FOLFOX for ten cycles, followed by proteomic profiling using data-independent acquisition (DIA).

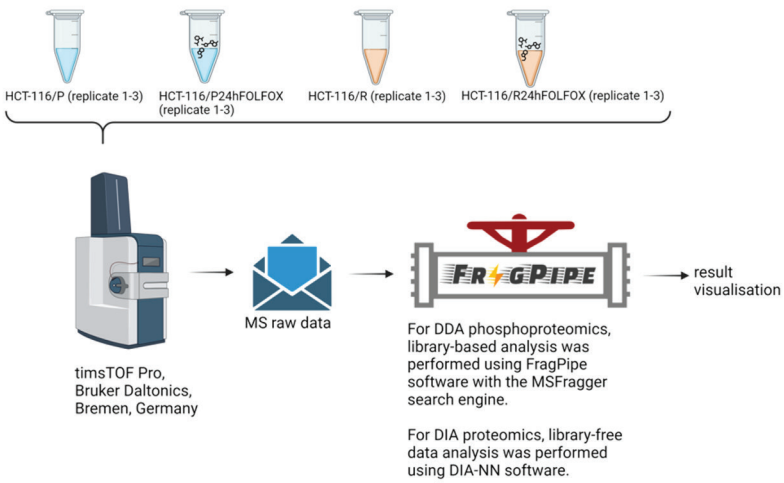


***Figure 3**. HCT-116 cell lines were treated with 25 μM 5-fluorouracil and 3.125 μM oxaliplatin for 10 cycles to induce chemoresistance. Resistant cells were then maintained at a low dose of FOLFOX (25 μM 5-fluorouracil and 0.625 μM oxaliplatin). Before the proteomics experiment, parental and resistant cells were exposed to Folfox for 24 hours.*

Our proteomic analysis revealed several key findings. First, we demonstrated that resistant HCT-116 cells did not activate DNA damage response or cell cycle arrest pathways, which are typically triggered in parental cells. This corroborates the acquisition of chemoresistance. We observed overexpression of TP53, AURKA, CCNA2, and RRM2 in parental HCT-116 cells following 24 hours of FOLFOX treatment, whereas no such overexpression was seen in resistant cell lines post-treatment.

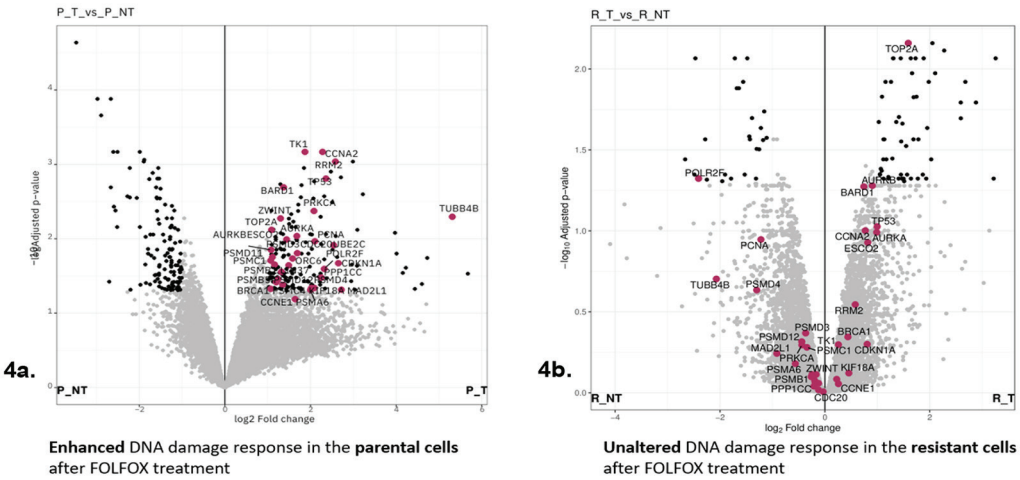


***Figure 4a** depicts a volcano plot of differentially expressed proteins comparing parental HCT-116 cell lines before and after 24 hours of FOLFOX treatment. Many proteins (red dots) associated with DNA damage response and cell cycle arrest were upregulated in the treated parental cells, corroborating the DNA-damaging effects of the FOLFOX regime. However, these effects were not observed in resistant HCT-116 cells post-treatment (**Figure 4b**.).*

Additionally, we found that 51 out of 87 quantified interferon-stimulated genes (ISGs) were significantly upregulated in resistant cells before FOLFOX exposure, with 31 maintaining elevated expression after 24 hours of FOLFOX treatment. This suggests that interferon signaling may play a role in FOLFOX resistance. Key ISGs, including ISG15, IFI35, and STAT1, emerged as potential biomarkers for predicting chemotherapy response in CRC patients. We compared our list of ISGs with relevant literature from the past decade to validate our findings, which revealed a unique ISG signature in our study that correlates with previous transcriptome analyses of DNA-damaging agents used to treat cancer.

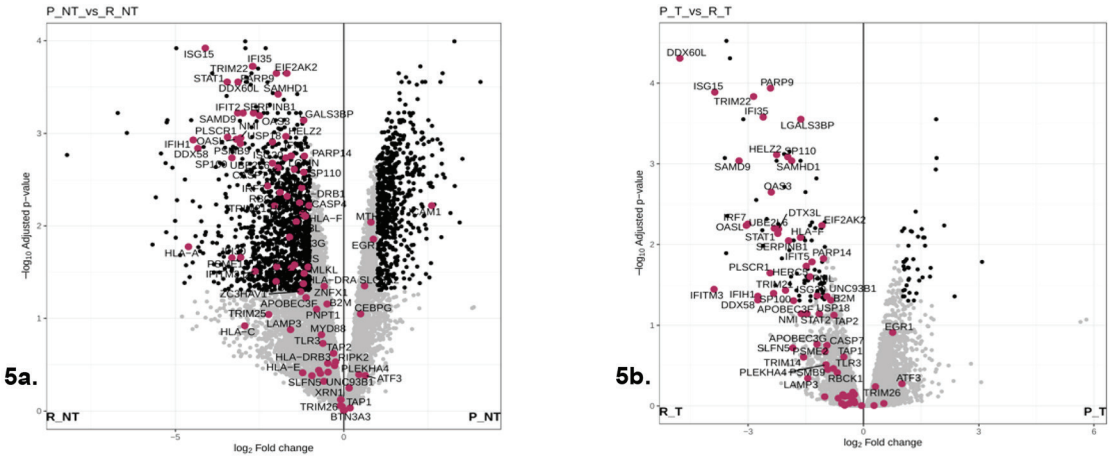


Figure 5a *shows that from all the 87 Interferon Stimulated Genes (ISGs) identified and quantified (red dots), 51 ISGs were upregulated in non-treated resistant HCT-116 cells compared to the no-treated parental cells. This implies that the interferon pathways were activated at the basal state of resistant cells. Upon 24 hours of FOLFOX treatment* ***(Figure 5b****.), the number of upregulated ISGs reduced to 31 in comparison to the treated parental cells.*

These studies emphasize the crucial roles of microproteins and ISGs in CRC, offering new insights into tumor progression and chemotherapy resistance. They pave the way for identifying novel biomarkers and therapeutic targets to improve treatment outcomes for CRC.

GENETIC AND EPIGENETIC CUES SHAPE INITIATION, PROMOTION AND THERAPY RESISTANCE IN CHILDHOOD LEUKAEMIA

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Childhood leukaemia provides a tractable paradigm in which to study the initiation, promotion and evolution of cancer as well as mechanism that underpin therapy resistance. Childhood acute lymphoblastic leukaemia is thought in many cases to initiate *in utero.* The factors that influence the formation of the initiating lesions are not well understood although the nature of these genetic aberrations is well documented and include in approximately a quarter of children the presence of the t12;21 chromosomal translocation that fuses the transcription factors Tel (ETV6) and Aml1 (RUNX1). The Etv6-Runx1 fusion gene produces a pre-leukaemic clone but in and of itself is insufficient to produce frank leukaemic transformation. For this, additional mutations are required and it remains unclear what factors influence their acquisition. To gain insight with these issues we have been exploring the target genes of TEL-AML1 and associated second hits as well as developing new foetal specific models in which to examine the biological impact of TEL-AML1. Our results allow us to develop transcriptional networks that inform how different mutations may collaborate to allow leukemic progression. As part of this analysis we revealed that Etv6-Runx1 functions as a first hit mutation primarily through competition for RUNX1 binding sites and transcriptional repression. In frank leukemia, knockdown of RUNX1 or its co-factor CBFβ results in cell death suggesting sustained requirement for RUNX1 activity which is recapitulated by chemical perturbation using an allosteric CBFβ-inhibitor. Strikingly, we show that RUNX1 addiction extends to other genetic subtypes of paediatric B-ALL and also adult disease. Importantly, inhibition of RUNX1 activity spares normal hematopoiesis. Our results suggest that chemical intervention in the RUNX1 program may provide a therapeutic opportunity in ALL. Finally, modelling of the independent and combinatorial role of mutations associated with ALL gives insight into the mechanisms underlying leukaemogenesis in this disease. Interestingly, these studies implicated processes associated with viral infection and/or inflammation in the promotion of initiated pre-leukaemic clones

TOWARDS AN UNDERSTANDING OF AIR POLLUTION DRIVEN LUNG CANCER PROMOTION

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Environmental exposure to carcinogens significantly contributes to global disease burden. Recent studies suggest that many environmental carcinogens do not directly cause mutations, with normal human tissues often containing a mix of mutant cell clones, some with oncogenic mutations. There is insufficient data on how environmental factors influence tissue stability and promote tumor development without direct mutational causes. The mechanisms behind lung cancer initiation in never smokers remain unclear. We hypothesized that 2.5 μm particulate matter ($PM_{2.5}$), as an environmental carcinogen, could facilitate lung cancer initiation through non-mutagenic pathways by inducing inflammation and allowing existing mutant clones in normal tissue to grow.

We analyzed $PM_{2.5}$ levels and cancer incidence data from WHO, UK Biobank, Public Health England, Taiwan's Chang Gung Memorial Hospital, and Korea's Samsung Medical Centre, involving 495,276 individuals from 2010 to 2018. Our findings show a correlation between $PM_{2.5}$ levels and various cancers, including EGFR mutant lung cancer, which is more prevalent among never smokers. Pollution was found to promote a progenitor-like state in EGFR mutant lung cells, accelerating cancer progression in mouse models. Deep mutational profiling of normal lung tissues revealed oncogenic mutations in 18% of samples for EGFR and 45% for KRAS. In studies of $PM_{2.5}$ exposure, we identified that IL1B is released from lung epithelium and may drive the expansion of EGFR mutant clones, suggesting targeting IL1B could help prevent lung cancer in $PM_{2.5}$-exposed communities. These results highlight the urgent need to address urban air pollution.

Environmental exposures influence health throughout life, with approximately 19% of all cancers linked to factors such as air pollution and second-hand smoke (Prüss-Üstün et al., 2016). The lungs, as barrier organs, are particularly affected, with over 20 agents classified as lung carcinogens (IARC, 2015). Lung cancer in never smokers (LCINS) is notably concerning, being the eighth leading cause of cancer death in the UK (Bhopal et al., 2019), and current screening protocols often exclude these patients.

LCINS exhibits distinct clinical and molecular features, frequently harboring EGFR mutations and showing a lower mutation burden without a clear environmental signature (Cancer Genome Atlas Research Network, 2014; Devarakonda et al., 2021). Environmental factors, such as air pollution, are particularly significant; 99% of people live in areas exceeding WHO air quality guidelines. Ambient air pollution is the fourth leading cause of death globally, responsible for 6.7 million deaths in 2019 (GBD Lancet, 2020).

The mechanisms by which PM pollution drives lung cancer initiation are not well understood. Traditional beliefs hold that carcinogens induce DNA damage, yet recent findings reveal no mutational signatures in mouse tumors after exposure to many environmental carcinogens (Riva et al., 2020). Advanced sequencing has shown mutations in normal tissues, suggesting that while driver mutations may be necessary for tumor initiation, they are not always sufficient (Lee-Six et al., 2019).

We hypothesized that air pollution might trigger inflammatory changes that allow mutated cells to expand and potentially initiate tumors. Our research combined epidemiological data, preclinical mouse models, and $PM_{2.5}$ exposure studies to explore how air pollution contributes to lung tumor development.

In conclusion, our findings indicate that PM exposure can promote clonal expansions. We

observed that $PM_{2.5}$ fosters a progenitor-like state in EGFR mutant cells, enabling tumor initiation only in cells with existing oncogenic mutations. This aligns with evidence that mutant cells can rapidly progress to carcinoma after exposure to promoting agents, even long after mutation acquisition (Balmain, 2020).

Ultimately, these results suggest that cancer risk arises not only from acquiring driver mutations but also from environmental exposures that facilitate the growth of mutant cells. Addressing the mechanistic factors of environmental carcinogenesis is crucial. A comprehensive approach is needed to identify hormonal, environmental, and genetic influences that affect mutant clone expansion, potentially revealing intervention opportunities for at-risk populations. This requires collecting global exposure data, studying at-risk cohorts, and conducting research to understand how environmental factors disrupt normal physiology and promote mutant expansion. Such efforts could inform new screening strategies and targeted cancer prevention methods. Notably, the antibody Canikumumab, which targets IL1B—a factor induced by $PM_{2.5}$ exposure—has shown promise in reducing lung cancer incidence in the CANTOS trial (Ridker et al., 2017).

In the short term, these data reinforce the causal link between pollution and lung cancer, first proposed by Doll and Hill in 1950 (Doll and Hill, 1999), underscoring the urgent need for public health measures to reduce particulate emissions in urban areas.

AXONAL INJURY INITIATES GLIOBLASTOMA

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Glioblastoma is the most common and malignant type of primary brain tumour that is universally fatal. Prognosis remains at less than 18 months despite aggressive multimodal treatment protocols. In addition, glioblastoma patients experience severe cognitive and physical impairments caused by the growing tumour mass and its treatment that profoundly impact their quality of life.

At the time glioblastoma is diagnosed it is typically advanced, highly therapy-resistant and intractable. Major causes of therapy resistance are pervasive cellular and molecular heterogeneity, extensive spread and a highly immune suppressive tumour microenvironment. The majority of glioblastoma research to date has focussed on this late-stage disease, which is readily available from patient biopsy or debulking surgery material. In contrast, much less is known about how glioblastoma initiates and progresses to advanced disease. Deciphering these processes is essential to our understanding of glioblastoma biology and evolution and may identify novel, more effective treatment strategies for delaying its progression or inhibiting recurrence.

Our team has developed a suite of disease-relevant somatic mouse models that enable analysis of tumour cells from acquisition of mutations to terminal disease. Leveraging these models, we explored the early events of glioblastoma development, from SVZ neural stem and progenitor cells, a common cell of origin for this disease. We found a key role for injury programmes, triggered by axonal degeneration and sustained by neuroinflammation. I will describe these findings and present preclinical evidence that targeting the injury microenvironment can delay tumour progression whilst ameliorating cognition, with important implications for cancer interception.

WHAT DOES OUR KNOWLEDGE OF INNATE IMMUNE REGULATION DURING INFECTION TELL US ABOUT INNATE IMMUNE CONTRIBUTION TO CANCER OUTCOME?

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Immunotherapy for cancer has saved numerous lives since its inception. However, some cancers do not respond to immunotherapy and even when treating sensitive cancers, not all patients benefit. Mechanisms of immunotherapy resistance are poorly understood with some studies suggesting type I interferon and inflammatory phenotypes are associated with the best outcomes.

Interferon is produced by cells when they detect pathogens. Pattern recognition receptors recognize pathogen associated molecular patterns to activate transcription factors including IRFs and NF-kB. Gene expression is initiated, and this includes IFNs, which activate defensive gene expression in nearby cells that have not yet see the pathogen. Ideally pathogen replication is suppressed and the host is protected. Typically, pathogens have evolved countermeasures allowing them to evade and shut down IFN activities allowing them to successfully infect their host. The ongoing defensive inflammatory responses tend to cause pathogenesis. Importantly, host molecules can also activate IFN expression in health and non-infectious disease, but how this is initiated in cancer is poorly understood. Recent work has focused on activation of nucleic acid sensing by host nucleic acid, particularly virus-like sequences, which we collectively refer to as transposable elements (TE). IFN Induction by TE expression in the absence of infection has been referred to as viral mimicry.

TE make up around half of our genome. When expressed, they may produce nucleic acids that can trigger nucleic acid sensing pathways and IFN causing sterile inflammation including in cancer. It is unclear when, or whether, sensing of expressed TE contributes to resolution of diseases, including infection and cancer, or whether it drives disease by causing inflammation. Manipulating TE expression to trigger immune sensing on demand, for example, to enhance immunotherapy is of great interest. While *in vitro* and animal model studies have suggested that stimulating TE expression can enhance interferon responses and improve immunotherapy efficacy, the link between TE expression and type I interferon responses in cancer patients remains largely correlative.

Analyzing RNA-seq samples from multiple cancer types in The Cancer Genome Atlas (TCGA), we found that high IFNB1 and interferon stimulated gene (ISG) expression is associated with better survival outcomes in some cancers (particularly Ovarian (OV)) and worse outcome in others (particularly Kidney Renal Clear Cell Carcinoma (KIRC)(Fig. 1).

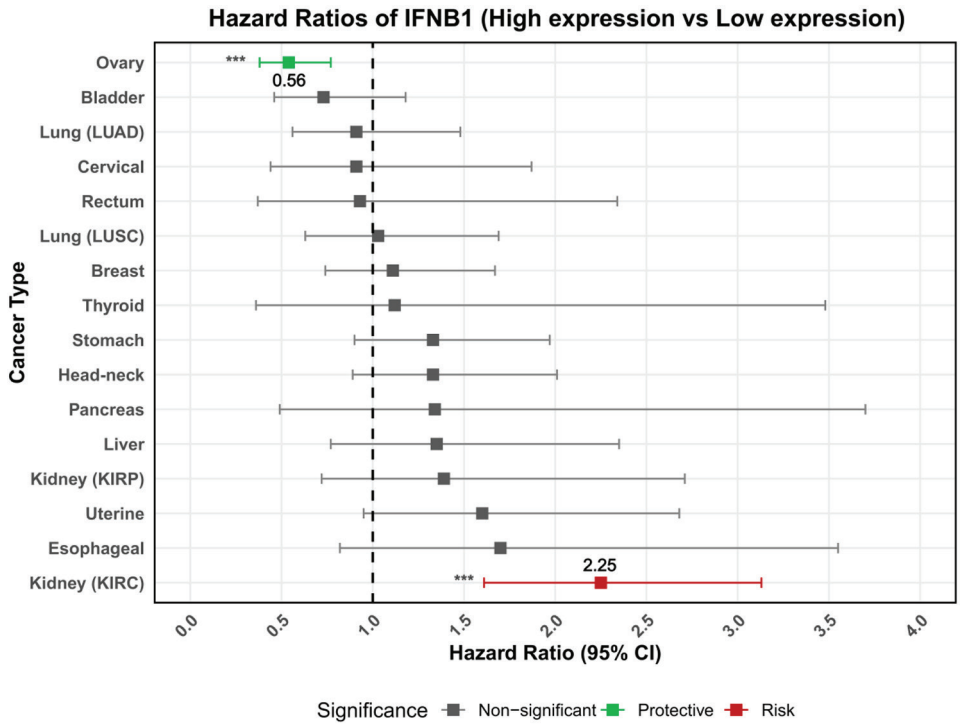

Figure 1. Effects of the expression of IFNB1 (Q1 vs Q4) on overall survival in TCGA cohort.

It is unclear what drives sterile inflammation in cancer. Considering a role for TE in IFN induction in OV we found that many TEs were merely co-expressed with nearby ISGs with significant chromosomal co-localization, suggesting they were a consequence rather than a cause of sensing activation (Fig. 2).

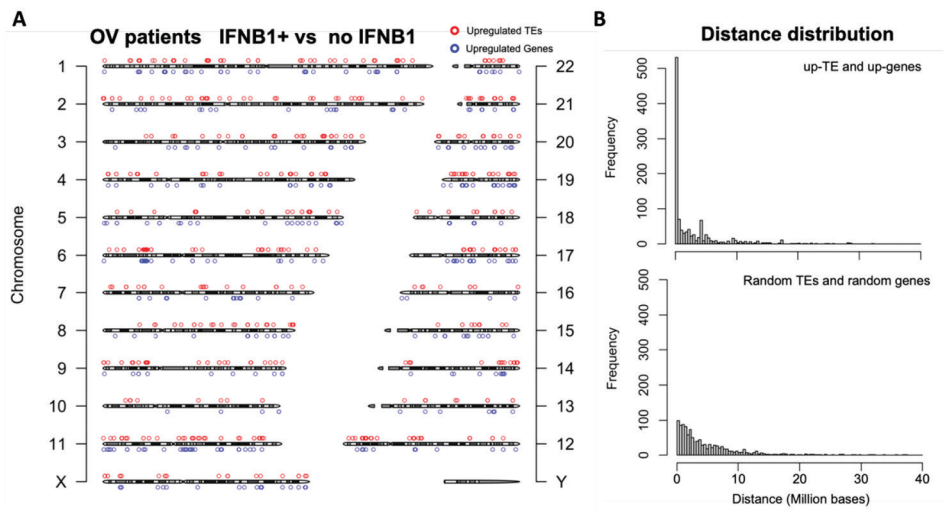

Figure 2 (A) Chromosomal map of upregulated TE loci and genes in IFNB1-positive versus IFNB1-negative patients (TCGA-OV). (B) Distances distribution between upregulated TE loci and nearest upregulated genes. Control: Distance distribution of equal numbers of random TE locus and random genes. The difference is significant ($p<0.0001$).

Indeed, analysis of a series of sequencing data sets from infection, cancer and non-infectious experiments we found widespread TE expression with the number of upregulated TE loci correlating well with the number of upregulated genes (Fig. 3).

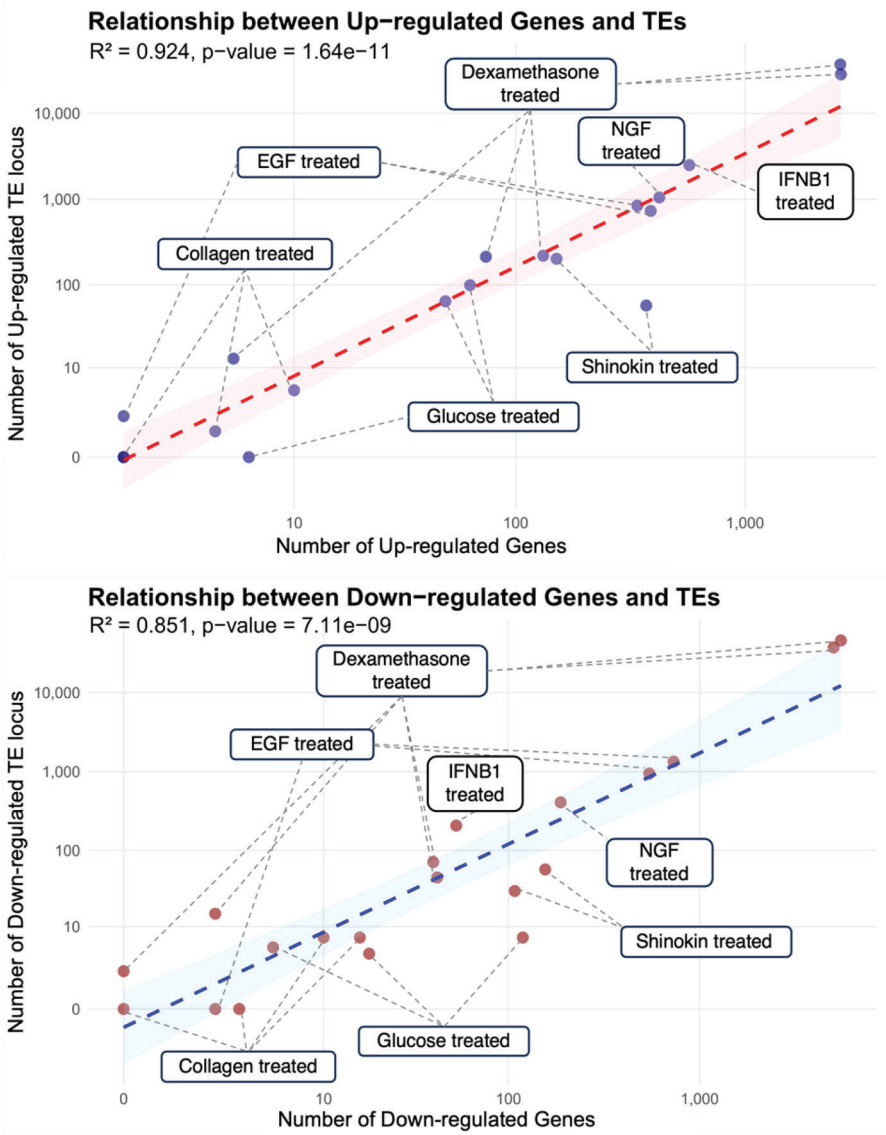

Figure 3. The relationship between the number of up/down-regulated genes and the number of up/down-regulated TE locus in the gene expression dataset of cells treated with different compounds.

Co-expression network analysis suggested that 15%-70% of TE expression is explained by adjacent gene expression. To remove the confounding effect of co-expression we eliminated IFNB1-correlated TEs adjacent to ISGs and other expressed genes. This revealed expressed gene independent TE loci which were strongly correlated with IFNB1 expression with over representation of Alu and LINE1 elements. We conclude that TEs are regularly expressed but the majority are not contributing to innate immune activation. Further work focuses on understanding the nature and regulation of expression of the specific TE loci that do not appear to be driven by co-expression and may drive the sterile inflammation that is associated with improved outcome in OV.

THE INFINITE LOOP: MACHINE LEARNING FOR DISCOVERY, DELIVERY, AND RAPID MANUFACTURING OF POTENTIAL MEDICINES

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The use of deep learning for the delivery of antisense oligonucleotides (ASOs) into cells is a transformative approach that addresses the challenge of poor cellular permeability of ASOs. ASOs, such as phosphorodiamidate morpholino oligomers (PMOs), are promising therapeutic agents but often require high doses for effective intracellular delivery. In response, machine learning models have been developed to design novel cell-penetrating miniproteins, which facilitate the efficient delivery of ASOs into cells. By leveraging robust datasets and advanced input representations, deep learning enables the generation and optimization of sequences with enhanced nuclear delivery capabilities. These models use convolutional neural networks to predict and optimize the activity of these miniproteins, leading to the development of non-toxic and highly effective ASO-delivering proteins that are capable of trafficking cargo across cellular membranes. The use of this technology has demonstrated significant improvements in ASO delivery, offering a 50-fold increase in efficiency, paving the way for more effective gene therapies(Schissel, *NatChem.*, 2021).

Rapid protein and peptide synthesis by flow chemistry offers an innovative approach to manufacturing biologically active molecules with unprecedented speed and precision. The process utilizes automated fast-flow solid-phase peptide synthesis (AFPS) to assemble peptide chains through a continuous flow system, which significantly reduces reaction times and improves overall efficiency. This method, developed in recent years, allows for the production of long peptide chains, including complex proteins, in just a few hours, rivaling traditional biological expression methods. The technology excels at maintaining high fidelity in peptide bond formation, while reducing common side reactions, enabling the synthesis of peptides exceeding 50 amino acids in length. By optimizing reaction conditions and employing high-performance liquid chromatography (HPLC) for real-time monitoring, the AFPS platform delivers high-quality synthetic peptides that can be folded and used in various biophysical and enzymatic applications(Hartrampf, *Science*, 2020).

Affinity selection-mass spectrometry (AS-MS) is a cutting-edge tool used to discover ligands for drug targeting by rapidly identifying high-affinity binders from large peptide libraries. This technique allows researchers to explore chemical space with greater diversity, testing millions of peptides in a single experiment. Through AS-MS, small molecule binders can be identified by capturing ligands that exhibit specific interactions with target proteins. The method employs nanoscale liquid chromatography-tandem mass spectrometry (nLC-MS/MS) to decode peptide sequences from complex mixtures, dramatically increasing the efficiency of ligand discovery. This technology has been successfully applied to discover binders for oncogenic proteins and other therapeutic targets, offering a powerful approach to building a drug-targeting engine that is both precise and scalable (Rossler, *Science*, 2023)(Quartararo, *NatCommun.*, 2020).

Peptide-encoded libraries (PELs) represent a novel platform for the discovery of small molecules with drug-like properties. PELs utilize abiotic peptides as carriers of information, which can be decoded using tandem mass spectrometry. The stability and versatility of these peptide tags allow for a broad range of chemical reactions during the synthesis of small molecules. By encoding synthesis information within peptide sequences, PELs can generate large combinatorial libraries that are subsequently screened for interactions with target proteins. This method has been shown to yield high-affinity binders for various therapeutic targets, including oncogenic proteins, and has the potential to accelerate the discovery of novel small molecules

with optimized properties(Rossler, *Science*, 2023).

By integrating deep learning for delivery, rapid flow-based peptide synthesis, AS-MS for ligand discovery, and PELs for encoding small molecule libraries, we are constructing what can be described as “The Infinite Loop.” This closed-loop system leverages machine learning, automation, and mass spectrometry to iteratively design, synthesize, and screen molecules at an unprecedented scale. Together, these technologies create a continuous, self-improving cycle for drug discovery and development, allowing for the rapid identification and production of potential medicines. This approach not only enhances the efficiency of therapeutic discovery but also expands the possibilities of molecular innovation.

ANTIBODY-DRUG CONJUGATES (ADCS) – A PERFECT SYNERGY?

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Over a hundred years ago, Paul Ehrlich pioneered the use of cytotoxic chemotherapy, and also pointed to the future of antibodies as “magic bullets”. These two therapeutic classes are both widely used in cancer treatment, but they have largely developed independently. Naked antibodies have specificity but often have limited antitumour activity as single agents, particularly against solid tumours. Cytotoxic chemotherapy suffers from lack of tumour specificity and significant systemic toxicity, but can be combined successfully with antibodies due to their favourable safety profile.

The aim of antibody-drug conjugates (ADCs) is to achieve potent and selective target cell killing by taking advantage of the selectivity of a monoclonal antibody, combined with the potent cell-killing activity of a small molecule cytotoxic. Binding of an ADC to its target cell-surface antigen results in internalisation into the cell and subsequent intracellular release of the cytotoxic agent. Several approved ADCs contain cathepsin cleavable peptide linkers stochastically conjugated to cysteine residues on the antibody. Although simple in concept, developing clinically effective ADCs has proven to be highly challenging and it is only relatively recently that they are emerging as a rapidly growing and important class of therapeutic agents against both haematological malignancies and solid tumours.

The three components of an ADC, the antibody, linker and drug, all need careful design and optimisation. In addition, the choice of target antigen and target cancer indication are critical to the ultimate clinical activity and tolerability of an ADC. Until relatively recently, the cytotoxic warheads in the majority of ADCs in clinical development were based on two families of antimitotic agents - auristatins and maytansinoids. Few tumours, however, are inherently sensitive to such anti-microtubule agents. The search for next generation warheads has led to several novel drug classes including topoisomerase I inhibitors and DNA interacting agents. An example of the latter is the pyrrolobenzodiazepine (PBD) dimer class of DNA cross-linking agent. In addition, rational design has enabled improved linker and conjugation technologies in next generation ADCs.

The PBD dimers were rationally designed to induce DNA interstrand cross-links in the minor groove of DNA [1]. These sequence selective cross-links persist in cells due to minimal distortion to the DNA structure and are highly cytotoxic. The only PBD dimer to enter the clinic as a standalone agent, SJG-136 (SG2000), showed potent cytotoxic activity against human tumour cell lines in vitro in the low nanomolar range. Rational structural modification of the PBD pharmacophore subsequently produced agents with enhanced cross-linking activity and picomolar, and in some cases sub-picomolar, activity. Examples of these latter compounds were then developed into drug-linker payloads talirine and tesirine (Figure 1) in novel ADCs, where the potency of the PBD dimer warheads enabled activity to be achieved with a drug-antibody ratio (DAR) of around 2 [2].

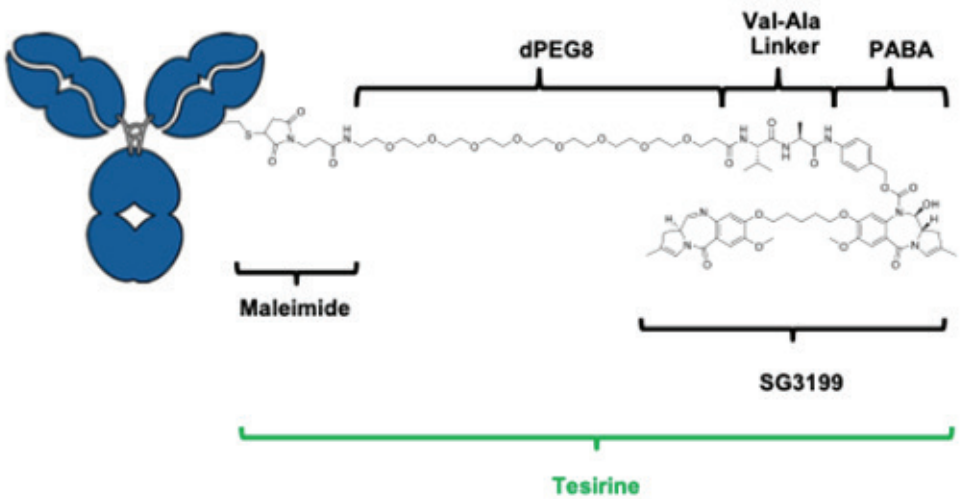

Figure 1. Structure of the drug-linker payload tesirine. The maleimide group allows conjugation to cysteine residues on an antibody. The valine-alanine is cleaved by cathepsin and upon cleavage the PABA group self-immolates to release the drug. SG3199 is a highly cytotoxic PBD dimer DNA cross-linking drug.

Several PBD dimer-containing ADCs have entered clinical development against both haematological malignancies and solid tumours including camidanlumab tesirine [3] and loncatuximab tesirine [4] targeting antigens CD25 and CD19, respectively. Pre-clinical studies demonstrated potent and selective *in vitro* activity and impressive antitumour activity against human tumour xenograft models with single intravenous administration of ADC at doses as low as 0.5 mg/kg. A phase I study of camidanlumab tesirine in relapsed or refractory lymphoma (dose range 3 to 150 μg/kg) gave an overall response rate of 58% (29% complete response) [5]. In Hodgkin lymphoma patients the response rate was 71% (42% CR), and at an expanded dose level of 45 μg/kg, the response rate was 86% (49% CR). Similarly, loncastuximab tesirine in phase 1 at 15 to 200 μg/kg gave a response rate of 45.6% in relapsed or refractory B-cell non-Hodgkin lymphoma [6], which was confirmed in 145 patients in a Phase 2 study in r/r diffuse large B-cell lymphoma with a response rate of 48.3% (24.8% CR) [7]. This latter agent subsequently gained accelerated approved in the USA and Europe as Zynlonta™.

The activity of an ADC depends both on the potency of the drug and the DAR. In contrast to loncastuximab tesirine (Zynlonta™) which delivers a highly potent PBD dimer with a DAR of 2, trastuzumab deruxtecan (EnhertuTM) delivers a less potent topoisomerase I inhibitor drug, but with a relatively high DAR of 8. The high DAR approach can be applied to other drug classes, including PBD dimers, and may increase tolerability with ADCs that target antigens that have some expression on critical normal tissues.

ADCs are now an important therapeutic modality in oncology showing superior clinical profiles compared to standard chemotherapy across multiple indications. Improvements in linker and conjugation technology combined with strategic warhead choice have the potential to widen therapeutic index by reducing non-specific and off target effects and improving targeted delivery. The range of antigens that can be targeted is also expanding both within oncology (e.g. against targets within the tumour microenvironment) and beyond (e.g as antibody-antibiotic conjugates (AACs)).

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DEVELOPMENTS OF MOLECULAR PROBES FOR DETECTION OF BIOLOGICAL TARGETS

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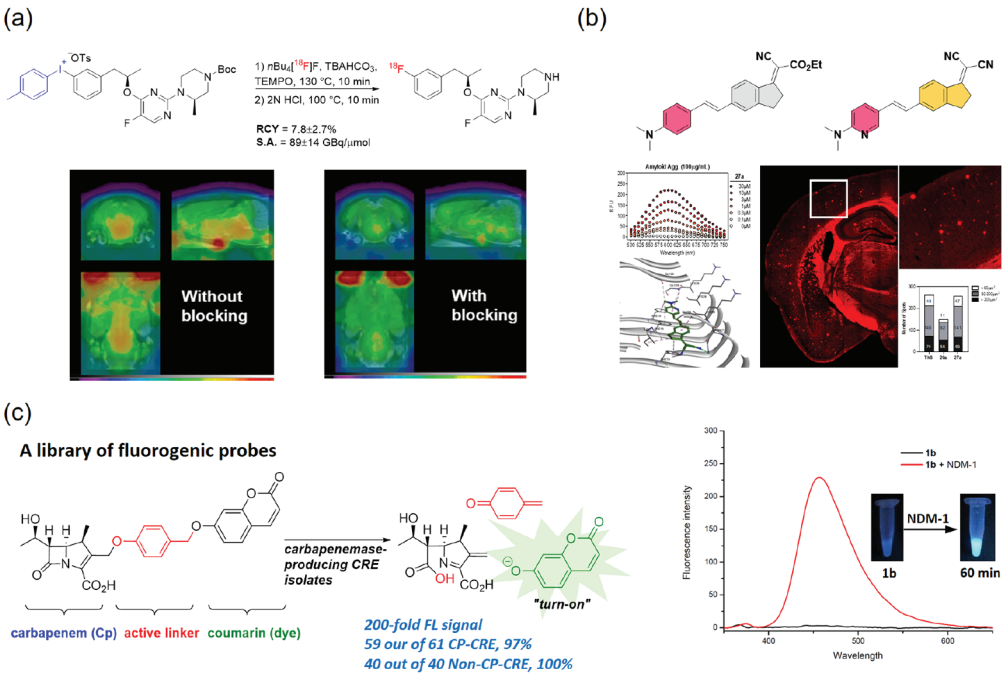
Chemical probes are small molecules that are used to study and manipulate a biological system such as a cell or an organism by reversibly binding to and alternating to the function of a biological target within that system. Probes ideally have a high affinity, high efficacy and binding selectivity for a specific molecular target, mostly protein. Due to their target specificity as well as physicochemical properties, they have been applied to a variety of molecular imaging techniques such as MRI (magnetic resonance imaging), optical imaging, and PET (positron emission tomography) for investigating particular targets inside organism in a noninvasive manner. These chemical probes for molecular imaging opens up new possibilities for medical application, including early detection and treatment of disease and basic pharmaceutical development. Our research is currently focused on the development of new chemical probes to invasively visualize biological targets in the nervous system, particularly related to neurological diseases.

Serotonin (5-HT) is a major excitatory neurotransmitter that plays an important role in normal physiological conditions. They are mediated by multiple receptor subtypes that have been classified into seven subfamilies (5-HT1-7). Among them, the 5-HT_{2c} is widely distributed in the human brain, in particular, displaying a high density in striatal, cortical, and limbic regions. It has been reported that dysfunction of the 5-HT_{2c} contributed to a variety of brain-related disorders such as schizophrenia, Parkinson's disease, and anxiety. Up to date, it is difficult to study a direct mechanism of action between the 5-HT_{2c} and such brain diseases due to lack of proper chemical probes. Accordingly, development of an in vivo method for measuring its function and density is important to identify the role of the 5-HT_{2c} receptor in the brain. Thus, PET imagining might be one of the potential tools for this purpose. A number of selective 5-HT_{2c} ligands have been developed, but only a few of them have been evaluated as PET radiotracers. Herein, we describe synthesis and biological evaluation of a pyrimidine derivative as a selective 5-HT_{2c} PET radiotracer. We have successfully developed a synthetic route toward [18F]-labeled pyrimidine derivative through late-stage fluorination reaction using an aryliodonium tosylate as a key intermediate. The in vivo evaluation of this selective PET radioligand in normal rats indicated that our PET radiotracer exhibits a high level of specific binding to 5-HT_{2c} receptors in the rat brain.

Second, the development of fluorescent imaging probes for the detection of Alzheimer's disease (AD)-associated protein aggregates will be presented. Alzheimer's disease (AD) is a neurodegenerative disease characterized by cognitive decline and memory impairment. With the rapid increase of the population of AD patients, the disease has become a significant economic and social burden throughout the world. Currently, clinically approved treatments for Alzheimer's disease only delay the progression of the disease or alleviate symptoms. Therefore, the early diagnosis of AD patients is important to prevent the disease from becoming severe. Amyloid-beta (Aβ) peptide deposition and hyperphosphorylated tau protein are considered critical pathological hallmarks in AD. Chemical probes to visualize these biomarkers have been developed to test the progression of the disease, but it is still needed to discover novel imaging probes for the identification of a precise disease stage. In this work, indane derivatives with a donor-π-acceptor (D-π-A) structure were designed and synthesized. The probes were evaluated for their ability to bind to β-amyloid (Aβ) protein aggregates, which are a key pathological hallmark of AD. Further analysis revealed that the most active compound could effectively stain Aβ plaques in brain tissue samples from AD transgenic mice.

Finally, development of novel fluorogenic carbapenemase substrates for the specific detection of the carbapenemase-producing carbapenem-resistant enterobateriaceae (CP-CRE) is described. Current detection methods of β-lactamase producing bacteria can be categorized into phenotypic and genotypic methods. The genotypic methods based on molecular diagnostics are accurate but they can detect only the genes of which sequence are known. Alternatively, many phenotypic methods have been reported, whereas they are still time-consuming and lack of sensitivity and specificity. Most recently, activity-based detection methods have been developed to overcome these disadvantages. In particular, fluorescence-based assays are attractive owing to relatively low cost, high sensitivity and operational simplicity. Our probes consist of beta-lactam substructure of carbapenem, a cleavable linker and a fluorescent dye, which exhibit fluorescent signal enhancement upon hydrolysis by carbapenemase. The result from clinical assessment of our probes indicated that they would provide practical screening tools for rapid and accurate detection of the CP-CRE.



Scheme 1. Molecular probes for detection of biological targets. (a) 5-HT_{2c}–selective PET radiotracer (b) Fluorescent probes for Alzheimer's disease (AD)-associated protein aggregates (c) fluorogenic β-lactamase substrates

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FROM STRUCTURAL BIOLOGY TO ORGANIC SYNTHESIS: A COLLABORATIVE SEARCH FOR NEW ANTI-MYCOBACTERIAL DRUGS

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Tuberculosis, caused by *Mycobacterium tuberculosis*, remains a serious threat to human health, especially in developing countries, with 6.4 million cases in 2019.[1] The danger is increasing due to the development of resistance to the few existing drugs. This is exacerbated by the paucity of new drugs[2] with Johnson & Johnson's bedaquiline (Sirturo) being the first FDA approved drug for the disease in 40 years.[3] In addition, other mycobacteria also present threats to human health. *M. abscessus*, for instance, is an increasing cause of skin infections of hospital patients and treatment requires prolonged antiobiotic treatment.[4]

One approach to the discovery of new drugs is based upon solid knowledge of biochemical mechanisms and protein structures. One enzyme that is essential for life is ATP synthase (Fig. 1). While this enzyme is largely conserved over many species (including *Homo sapiens*), ATP synthase in mycobacteria possesses sufficient differences due to the environment in which the organism survives to allow it to be a valid drug target. Our collaborators in the School of Biological Sciences have an extensive structural and functional understanding of ATP synthase in mycobacteria. This has enables them to identify regions where there are differences in protein compared to other organisms (including us). Specifically, they identified such regions known as ε, δ and γ. With this knowledge, virtual screening was conducted using the ZINC database to generate lead compounds.[5] This approach generated a wide range of compounds which could then be screened.

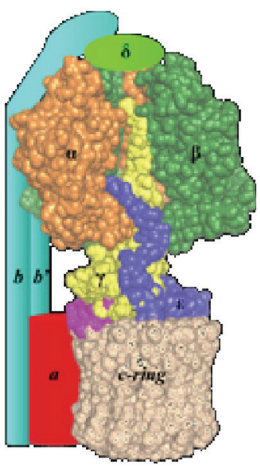

Figure 1. ATP synthase

Compounds that showed activity could then be assessed for chemical synthesis, as well as the design and preparation of analogs, and further biochemical studies. Naturally, many of these lead compounds, despite being enzyme inhibitors, did not progess further. A particular challenge with mycobacteria is the structure of the cell membrane, described as a "formidable permeability barrier", distinctly different to that of other organisms and presenting a significant challenge.[6]

One such compound from virtual screening is EpMF2 **1** which targets the ε-subunit of FTP synthase. This compound had an enzyme inhibitory IC_{50} value of 0.43 μM when tested against *M. smegmatis*. While we were able to prepare analogs (Fig. 2) that had slightly improved enzyme inhibitory activity, such as compounds **2** and **3**, we faced the problem that none of the compounds showed activity against the organism itself, most likely due to failure to penetrate the membrane. Our novel strategy to overcome the problem of membrane penetration will be presented.

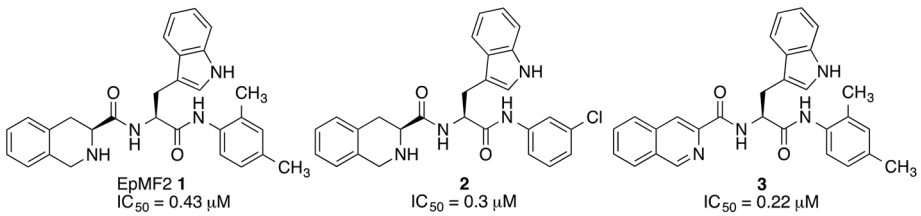

Figure 2. EpMF2 and analogs

Virtual screening also provided the molecule DeMF1 **4** which is predicted to bind to the δ-subunit.[7] While this compound did inhibit ATP synthesis, we considered that it was inappropriate as a drug molecule due to both the ketone and ester functional groups. As the dihedral angle between the ketone and the α-C-O bond is calculated to be only 20°, we selected the oxazole ring[8] as a replacement for the acetoxy ketone moiety (Fig. 3). A series of trisubstituted oxazoles, such as compound **5**, were synthesized by modification of a reported CH activation protocol.[9] A number of these showed ATP synthesis inhibitory activity, but failed to have an affect on the organism. Thus, oxazole **5** inhibited ATP synthase with a respectable IC_{50} of 0.14 μM, but had no effect on the growth of the whole organism. It was notable that the active compounds in the oxazole series lacked the cyclopentyl moiety. This appears to be due to a slightly different binding mode, with the oxazole being deeper in the pocket. In contrast, two compounds including pyridine **6**, had no affect on ATP synthesis but did inhibit growth of the organism, implying both an ability to penetrate the membrane and a different mechanism of action.

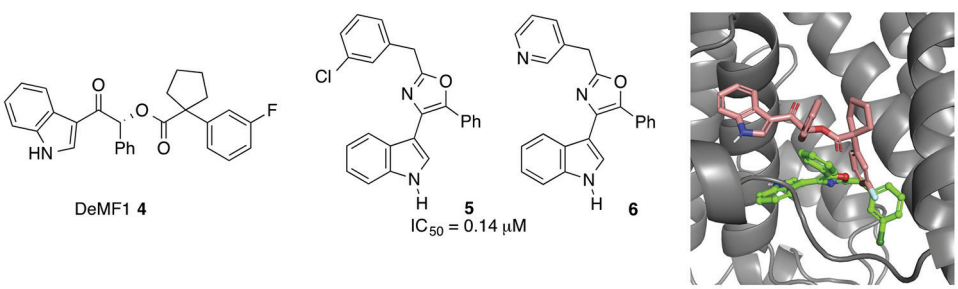

Figure 3. left: DeMF1 and oxazole analogs; right: oxazole (green) vs. DeMF1 (pink) binding.

A third molecule revealed by virtual screening was EGCG **7**, a compound well known in the natural products literature and present in many plants, including Camellia sinensis or tea.[10] It is also present in the beverage green tea, but not in black tea due to its sensitivity to oxidation. Tested in *M. smegmatis*, EGCG inhibited ATP synthase with an IC_{50} of 0.16 μM.[11] Using the method of LI and Chan,[12] or starting from commercially available catechin, we synthesized a wide range of EGCG analogs with both *cis* and *trans* stereochemistry. Amongst the analogs, we found that one of those in the trans series, compound **8,** showed activity only slightly lower than EGCG (Fig. 4). When the binding of these two compounds to the epsilon sub unit was measured by NMR titration, the results were in complete contrast. While compound **8** did not bind, EGCG did bind, but non-specifically. This calls into question whether EGCG exerts its activity by simple non-covalent binding, and also raises the question of the target protein of compound **8**. While there have been numerous literature reports about the biological activity of EGCG **7,**[13] our hypothesis about its activity brings into question whether it can ever be considered drugable.

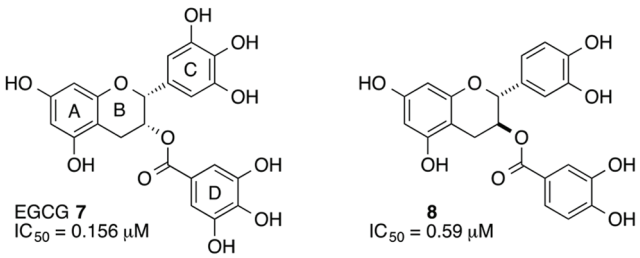

Figure 4. EGCG and a catechin analog

The γ-sub-unit is a small loop not present in other organisms. Virtual screening for compounds that bind to this sub unit led to GaMF1 **9** (Fig. 5).[14] This compound was a micromolar inhibitory of ATP synthase and its mode of binding was confirmed by NMR experiments. Most importantly, it inhibited the growth of the organism meaning that it had the ability to penetrate the cell membrane. Indeed, beyond inhibition, we have also shown it to be bacteriocidal. The compound shows similar activity against M. abscessus, including clinical isolates,15 but was inactive against *E. coli* and *S. aureus*, which lack the γ-loop. This is an important result, as the drug pipeline for *M.abscessus* is even more poorly populated than that for TB. We were able to develop a simple and modular synthesis of this compound that enabled us to synthesise a wide range of analogs, including a family in which the pyrimidine moiety is replaced with a triazene. Nevertheless, GaMF1 **9** remained the most active with the exception of a small set of benzimidazoles in which some rotation has been restricted.

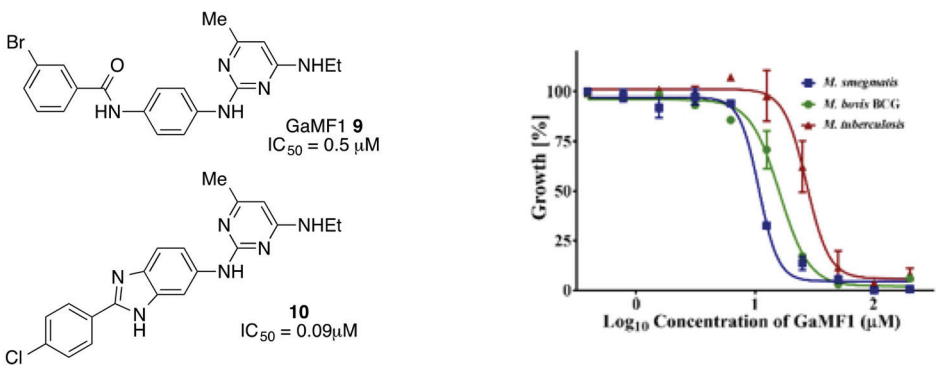

Figure 5. GaMF1 and an analog; growth inhibition by GaMF1

Discovery of new anti-mycobacterial compounds remains a task that is both challenging and essential. A combination of thorough biochemical understanding, in silico screening methods and synthetic organic chemistry has enabled us to develop several distinctly different lead compounds. While many fail to affect the organism, most likely due to the problem of membrane penetration, GaMF1 **9** and its benzimidazole **10** analog are effective against *M. tuberculosis* and other mycobacteria. These compounds have now been licensed and are undergoing further development.

Acknowledgement

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ROUNDTABLE DISCUSSION

Roundtable Discussion - Regulatory Innovation

Moderators: Ram Sasisekharan (U.S.A.) and **Surachoke Tangwiwat** (Thailand)

- U.S. FDA's Efforts to Advance Gene Therapy Development [Virtual Presentation]
  Peter Marks *(U.S.A.)*
- Perspectives from the Thai FDA
  Worasuda Yoongthong *(Thailand)*
- Case Studies of Regulatory Innovations
  Ram Sasisekharan *(U.S.A.)*

Roundtable Discussion - AMR Policy Impacting South and Southeast Asia

Moderators: Helen Boucher (U.S.A.) and **Julie L. Gerberding** (U.S.A.)

Panelists:
- **Helen Boucher** (U.S.A.)
- **George F. Gao** (P.R. China)
- **Julie L. Gerberding** (U.S.A.)
- **Ramanan Laxminarayan** (U.S.A.)
- **David Paterson** (Singapore)

U.S. FDA'S EFFORTS TO ADVANCE GENE THERAPY DEVELOPMENT

Peter Marks

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Although gene therapy has been in development for many years, it has only become a commercially available therapeutic option during the past decade. Many challenges had to be overcome to develop the first cell-based and directly administered gene therapies, and many challenges remain. These include the ability to cost-effectively manufacture high quality gene therapy, efficiently conducting clinical trials evaluating the safety and effectiveness of products, and the challenges negotiating a complex global regulatory environment. In 2017, the U.S. FDA approved the first chimeric antigen receptor T cell (CAR-T) product and approved the first directly administered gene therapy, a locally administered adeno-associated viral (AAV) product (Table 1).

Table 1. U.S. FDA Approved Gene Therapies and Year Approved (in parentheses)

Modified T Cells	Modified Stem Cells	Directly Administered
Kymriah (2017)	Zynteglo (2022)	Luxturna (2017)
Yescarta (2017)	Skysona (2023)	Zolgensma (2019)
Tecartus (2020)	Lyfgenia (2023)	Hemgenix (2022)
Breyanzi (2021)	Casgevy (2023, 2024)*	Adstiladrin (2022)
Abecema (2021)	Lenmeldy (2024)	Vyjuvec (2023)
Carvykti (2022)		Elevidys (2023)
Tecelra (2024)		Roctavian (2023)
		Beqvez (2024)

*Approved for beta-thalassemia in 2023 and sickle cell disease in 2024

Despite the availability of approved gene therapy products for several rare diseases, there are potentially thousands of rare diseases that might be addressed by gene therapy products. Hundreds of products are currently in sponsored clinical trials; however, the development of a significant number of products has been discontinued because of challenges encountered: the time it takes to conduct such trials and their cost are not insignificant. That said, promising new technologies, such as CRISPR genome editing, offer the potential to transform the currently landscape of gene therapy, making it more efficient and affordable to manufacture and deliver to patients.

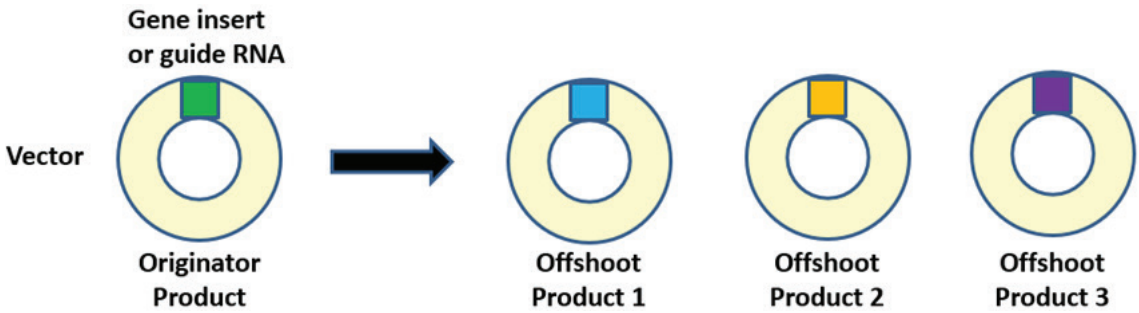
U.S FDA is undertaking a variety of efforts to help expedite the development of gene therapy locally and globally. By addressing challenges in manufacturing, clinical development, and regulatory approval, it hopes to advance the development and availability of an increased number of products that can benefit people in medical need around the globe.

To start, many existing directly administered gene therapies use AAV vectors that are challenging from several different perspectives. They generally cannot be repeatedly administered systemically because of cross-reactive antibodies that develop to the large doses of virus administered. They are also very challenging to manufacture at scale from both the perspectives of complexity and cost because appropriately packaged AAV must be produced and subsequently purified from

cultured cells. Because U.S. FDA identified that differences in manufacture at various institutions and benchtop production are leading to product variability and to increased costs, we are doing what we can to standardize and encourage automation of the AAV production process.

U.S FDA also realizes that regulatory streamlining is possible with gene therapy vectors like AAV. An approach that treats these products as platform technologies may be taken. For example, The AAV backbone can potentially be reused from one product to the next with same manufacturing process, swapping between different gene inserts (Figure 1). Such an approach could potentially allow the same toxicology and manufacturing information to be reused for different products, thereby reducing cost.

Figure 1. A Platform Therapy Approach to Gene Therapy



Although use of the platform approach for AAV is attractive, the platform approach is potentially even more relevant for genome editing using CRISPR technology. In particular, as CRISPR technology advances, constructs are being developed that allow something akin to read/write editing of the genome by using CRISPR-PRIME editors in which the CRISPR construct is linked to a reverse transcriptase. The reason the platform approach is so compelling for these products in that products targeting very different diseases might be quite similar. For example, hemophilia B and familial hypercholesterolemia might both be corrected with a nanoparticle encapsulated CRISPR-PRIME editor construct delivered to the liver; the two large macromolecular assemblies might only differ by a few hundred base pairs of a guide RNA. U.S. FDA is therefore actively preparing for the novel regulatory framework that will be necessary to accommodate this class of products.

Relevant to both AAV and CRISPR-based products, this includes expediting the clinical development process using accelerated approval. Gene therapy approaches are often well suited to application of biomarkers as surrogate endpoints, because measurement of the gene therapy product or a metabolite upstream or downstream in a pathway can reasonably likely predict clinical effectiveness, which can subsequently be confirmed in clinical studies conducted over a longer period of time.

U.S FDA also notes that we need to address the current limitations to global access to gene therapy. Obviously, reducing cost and simplifying delivery will be critical to this effort. However, in addition, it has been recognized that unnecessary differences in the how gene therapy products are regulated in various jurisdictions lead to a disincentive for multinational development and deployment. To address this FDA is taking two key actions: 1) it is working with the World Health Organization to facilitate global regulatory convergence, education, and regulatory capacity building in cell and gene therapy, and 2) it has launched a pilot program

with key global regulatory colleagues to work to resolve regulatory differences and concurrently review regulatory submissions. If the pilot proves successful, it will be expanded further.

Finally, during the COVID-19 pandemic in the United States work was undertaken to develop safe and effective vaccines at a record pace (11 months for two mRNA vaccines). A key learning from the steps implemented to accomplish this task was that constant communication on an as needed basis via email or teleconference, rather than formally scheduled meetings, could significantly expedite development for important medical products. The challenge of this approach was the extra staff effort required. Because such effort is potentially justified to address serious unmet medical needs, U.S. FDA is piloting a program (the START pilot) to provide a select number of development programs with this opportunity for ongoing intensive interaction as clinical trials proceed. If measurement of key indicators of development time proves the program to have merit, it may be expanded more broadly.

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SPECIAL PLATFORM SESSION ON ONE HEALTH

sO-1

WORLD HEALTH ORGANIZATION CALLS FOR ENHANCED MULTISECTORAL ACTION TO IMPROVE CHEMICAL SAFETY AND PROTECT PUBLIC HEALTH, INCLUDING THROUGH A ONE HEALTH APPROACH

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The World Health Organization (WHO) recognizes the pace of climate change and environmental degradation as a major threat to human health and has prioritized these concerns in its Fourteen General Programme of Work (GPW14) for 2025-2028.[1] GPW14 also recognized that more effective work is needed across multiple sectors to deliver better health outcomes from hazardous chemicals and air, water and soil pollution and contamination.

The WHO Executive Board and World Health Assembly in 2025 will consider a report by the Director-General on the updates needed to the WHO Chemicals Road Map to enhance health sector engagement in the new Global Framework on Chemicals[2] adopted in 2023 and the importance of health protection strategies for vulnerable populations and specific chemicals of concern, such as lead. World Health Assembly resolution 76.17 (2023) further recognized the importance of a One Health approach to address chemicals, waste, and pollution. [3] A One Health approach recognizes the interconnectedness of human, animal, and environmental health, and promotes multisectoral collaboration and action.[4]

WHO estimated that exposure to only a select number of chemicals caused 2 million deaths in 2019.[5] This burden of disease is underestimated, since it can be estimated for only a few types of chemical exposures due to lack of the prerequisite data for other chemicals. WHO identifies ten chemicals of major public health concern- air pollutants, arsenic, asbestos, benzene, cadmium, dioxins and dioxin-like substances, inadequate or excess fluoride, lead, mercury, and highly hazardous pesticides.

From a One Health perspective, arsenic contamination of groundwater, mercury contamination of seafood, dioxin contamination of soils, and widespread use of highly hazardous pesticides has increased cancer risk in humans, reduced productivity and affected the health of livestock, which consume contaminated crops and water, and entered the human food chain, water supply

[1] World Health Organization Fourteenth General Programme of Work, 2025-2028: https://www.who.int/about/general-programme-of-work/fourteenth

[2] Global Framework on Chemicals. 2023. https://www.unep.org/global-framework-chemicals

[3] World Health Assembly Resolution 76.17. 2023. The impact of chemicals, waste and pollution on human health https://apps.who.int/gb/ebwha/pdf_files/WHA76/A76_R17-en.pdf

[4] A health perspective on the role of the environment in One Health. Copenhagen: WHO Regional Office for Europe; 2022. https://www.who.int/europe/publications/i/item/WHO-EURO-2022-5290-45054-64214

[5] The public health impact of chemicals: knowns and unknowns - data addendum for 2019 https://www.who.int/publications/i/item/WHO-HEP-ECH-EHD-21.01

and ecosystem.[6,7,8,9] Also from a One Health perspective, the exacerbation of air and water pollution by climate change has led to respiratory and cardiovascular health issues, particularly in urban areas. Climate change has also altered the distribution of persistent organic pollutants, increasing exposure risk to humans, animals, and the environment. Chemical pollutants have also exacerbated antimicrobial resistance (AMR) by promoting the spread of AMR genes in the environment, affecting human and animal health. These interlinkages between human, animal, and environmental health call for coordinated multisectoral action at national, regional and global levels to minimize or prevent the adverse effects on human, animal and environmental health caused by hazardous chemicals, waste and pollution.

The WHO Chemicals Road Map (2017) is a framework to enhance chemical safety through multi-sectoral cooperation, focusing on risk reduction, knowledge and evidence, institutional capacity, and leadership and coordination.[10] Countries can use the Road Map's Workbook[11] to identify priority actions, leveraging the health sector's role to foster collaboration among stakeholders. By implementing tailored national plans, countries can address specific chemical safety challenges, promote sustainable development, and protect public health.

Figure 1. WHO Chemicals Road Map Action Areas

Figure 1 Legend – The WHO Chemicals Road Map identifies concrete actions where the health sector has either a lead or important supporting role in the sound management of chemicals

The WHO Chemicals Road Map notes several key actions for the WHO Secretariat, such as enhancing risk assessment methodologies, increasing biomonitoring and surveillance, and estimating the burden of disease from chemicals. The Secretariat is also responsible for coordinating international efforts and providing technical support to Member States, such as through the WHO Global Chemicals and Health Network[12] of national health ministry focal points, established in 2018, to facilitate health sector implementation of the Road Map.

[6] World Health Organization. Arsenic Fact Sheet. 2022. https://www.who.int/news-room/fact-sheets/detail/arsenic

[7] World Health Organization. Dioxins Fact Sheet. 2023. https://www.who.int/news-room/fact-sheets/detail/dioxins-and-their-effects-on-human-health

[8] World Health Organization. Mercury Fact Sheet. 2024. https://www.who.int/news-room/fact-sheets/detail/mercury-and-health

[9] World Health Organization. Exposure to highly hazardous pesticides: a major public health concern. 2019. https://www.who.int/publications/i/item/WHO-CED-PHE-EPE-19.4.6

[10] WHO Chemicals Road Map. 2017. https://www.who.int/publications/i/item/WHO-FWC-PHE-EPE-17.03

[11] WHO Chemicals Road Map Workbook. 2018. https://www.who.int/publications/i/item/978924151363

[12] WHO Global Chemicals and Health Network. https://www.who.int/publications/m/item/global-chemicals-and-health-network-flyer

The Secretariat also coordinates the WHO Chemical Risk Assessment Network of 100+ institutions [13] , the INTOX network of poison centers[14] , and an extensive network of WHO collaborating centers on chemical safety and environmental health issues, such as the WHO Collaborating Centre for Capacity Building and Research in Environmental Health Science and Toxicology at the Chulabhorn Research Institute.

Chemical safety offers numerous examples of multi-sectoral One Health approaches due to their ubiquitous presence and impact on human, animal, and environmental health, such as the WHO-United Nations Environment Programme (UNEP) compendium of over 500 interventions involving chemicals, waste, and pollution to improve health by creating healthier environments. [15] WHO and UNEP are also updating the WHO-UNEP 2012 State-of-the-Science report on endocrine disrupting chemicals, which will be presented at the United Nations Environment Assembly (UNEA7) in December 2025. Another WHO initiative with UNEP works to phase out the use of mercury in medical devices and skin lightening products, including developing waste management strategies to protect human, animal, and environmental health.[16]

WHO also works with the Food and Agriculture Organization of the United Nations (FAO), the United Nations Development Programme (UNDP), the International Labour Organization (ILO) and UNEP to establish a Global Alliance on Highly Hazardous Pesticides, which aims to phase out their use in agriculture and promote safer, more sustainable alternatives[17]. Another example is WHO's initiative to evaluate highly hazardous and environmentally persistent Per- and Poly-FluoroAlkyl Substances (PFAS), which is being coordinated across WHO's chemical safety, food safety, and drinking water quality units.

WHO is also one of 10 intergovernmental organizations participating in the Inter-Organization Programme on the Sound Management of Chemicals (IOMC), with WHO serving as the Secretariat for IOMC and also for development and implementation of the IOMC Toolbox for decision making in chemicals management.[18] In addition to UNEP, FAO, UNDP, and ILO, the Organization for Economic Cooperation and Development (OECD), United Nations Institute for Training and Research (UNITAR), the World Bank, the United Nations Industrial Development Organization (UNIDO) and the Basel, Rotterdam, and Stockholm Convention (BRS) Secretariat also participate in IOMC along with WHO to help countries manage chemicals, waste and pollution to protect human and environmental health. [19]

In summary, the WHO calls for enhanced multisectoral action to improve chemical safety and protect public health, including through a One Health approach. Through multi-sectoral coordination and collaboration, focusing on risk reduction, knowledge and evidence, institutional capacity, and leadership and coordination, WHO can support countries to implement tailored national plans, address specific chemical safety challenges, promote sustainable development,[20] and protect public health.

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[13] WHO Chemical Risk Assessment Network. https://www.who.int/groups/chemical-risk-assessment-network

[14] WHO INTOX network of poison centres. https://www.who.int/groups/intox-network-of-poisons-centres

[15] Compendium of WHO and other UN guidance in health and environment, 2024 update. https://www.who.int/publications/i/item/9789240095380

[16] https://www.who.int/news/item/15-05-2024-nations-unite-to-eliminate-mercury-containing-medical-devices

[17] As endorsed by resolution V/11 of the 5th International Conference on Chemicals Management (ICCM5) in 2023 in parallel with agreement of the Bonn Declaration and the Global Framework on Chemicals. https://www.chemicalsframework.org/page/resolution-v11-highly-hazardous-pesticides

[18] IOMC Toolbox for decision making in chemicals management. https://www.iomctoolbox.org/

[19] International Organization on the Management of Chemicals. https://partnership.who.int/iomc/participating-organizations

sO-2

DEVELOPMENT OF GUIDANCE ON PROBLEM FORMULATION FOR IMPLICATIONS FOR ONE HEALTH APPROACHES

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Problem formulation (PF) is a systematic approach that identifies all factors critical to a risk assessment and considers the purpose of the assessment, scope and depth of the necessary analysis, analytical approach, available resources and outcomes and overall risk management (protection) goal (Solomon et al., 2016). It entails consideration of hazard characterization and exposure assessment as a basis to plan the risk assessment process. PF includes, for example, consideration of appropriate methods and endpoints for hazard assessment tailored to the nature of the decision to be made. In problem formulation, then, the complexity of the envisaged hazard characterization is tailored to the context of decision-making; approaches are necessarily flexible and iterative, permitting efficient identification and generation of the specific information essential to further assessment or risk management. At each stage, uncertainties (data gaps) are identified to address next steps. Refinement of key knowledge during the risk assessment informs reformulation of the problem such that the risk assessment can proceed with the degree of certainty and precision necessary to enable a decision to be made. The scope of the assessment sets the boundaries, with the goal of making it ‘‘fit for purpose’’. In the planning and scoping stage, the terms of reference (ToR) which comprise the focus of the risk assessment and the key questions to be addressed are outlined with objective to provide relevant, science-based evidence to answer the concerns of decision makers and other stakeholders, in an appropriate time-frame and within the available resources. In this sense, the PF is the refinement and translation of questions from decision makers and various stakeholders into scientifically testable hypotheses. A key activity in the planning and scoping phase is also clear identification of the goals for protecting human health and/or the environment. These ‘‘protection goals’’ are usually defined in the various statutes and regulations that relate to the protection of human health and the environment and, although these differ somewhat between jurisdictions, the main objective is the protection of individuals, populations and ecosystems against a specific risk or against any harm. Assessment endpoints are actual measures against which protection can be evaluated. They are predicated on the (implicit) protection goals but are quantitative so that they can be easily combined and/or compared in a mathematical sense. Though definitions of problem formulation and the scope of assessments varies, the objective of PF is to identify critical aspects and issues early in the assessment to ensure efficiency and effectiveness to meet risk management objectives. It is particularly important in One Health approaches where there is a need for efficient integrative management to unifying approaches to sustainably balance and optimize the health of people, animals and ecosystems (FAO, OIE, WHO and UNEP, 2017). Though the importance and benefits of problem formulation are widely recognized, currently, there is limited existing Guidance on its specific content, format or associated process for conduct (see, e.g., see Chapter 2 in Risk Sciences International, 2022). To address this gap, the International Programme on Chemical Safety (IPCS) of the World Health Organization (WHO) is developing guidance on problem formulation in chemical risk assessment. Its content is likely to inform considerably One Health approaches. The Guidance is envisaged to include indication of the nature of information relevant to transparent reporting of problem formulation. This reflects experience internationally in the important role of the development of templates to describe appropriate considerations based on collective experience (see, for example, Meek and Lipscomb, 2012). These templates “cue” assessors/ programs concerning aspects that should be considered and need to be reported. As such,

they contribute not only to increase understanding of the components/factors which need to be formally addressed in PF but promote transparency to facilitate greater consistency over the longer term, in the application of tailored "fit for purpose" approaches, considerations and tools to defensibly streamline integrative approaches to risk assessment and management of people, animals and ecosystems. Delineation of the relevant aspects to be addressed also indicates the nature of collective expertise required to address multifactorial, multidisciplinary, integrative approaches in One Health. Health and environmental assessments are a function of not only content, but the process for their preparation (e.g., the extent of peer engagement. This has not previously been documented in relation to PF. Transparent problem formulation/scoping and planning provides opportunity for early engagement with risk managers, stakeholders and the public to promote efficiency and accountability in adequately considering critical aspects of the assessment and documenting/communicating the path forward. Problem formulation in concert with scoping and planning represents, then, an artful combination of technical and management aspects to ensure efficient, transparent and informed assessment that meets the needs of stakeholders and the public, while conserving resources and protecting public and ecosystem health. The objectives, as outlined here, necessarily dictate the need for a consultative and iterative process, taking into account relevant documentation to focus on critical issues and questions. Components of envisaged guidance, then, are likely to include templates to outline the content of formal problem formulation – i.e., documented "framing" of the proposed approach to assessment, in response to a series of questions on focus (appropriate tools)/content/process, taking into account associated resources (indicated) and scoping of key reviews and new data (Meek, 2018). In this manner, in addition to increasing transparency as a basis to facilitate communication, formal problem formulation focuses the assessment to conserve resources. Development of a formal problem formulation also facilitates early input and/or review by oversight or peer review groups as a basis to solicit input, serves as supporting documentation for the assessment (rationale for focus) and provides relevant text for non-critical aspects of the assessment. In relation to efficiency of assessment, the template is designed to ensure early and maximum utilization of available well documented, peer reviewed assessments and more recent information in critical areas identified in rapid screening. This entails:

- identifying issues as early as possible, as a basis to focus resources and documentation and
- tailoring the content of assessments to improve transparency in critical areas while minimizing documentation of less important aspects. Normally, this relates to robust characterization of important aspects of dose-response, and associated, focused critical supporting information on hazard that directly supports protection goals and associated exposure estimation.

A preliminary scoping of key data is undertaken in problem formulation to "focus" effort. It normally involves assimilating relevant data in specified format drawing as much as possible on existing assessments, as a basis to solicit early input on likely issues, including:

- relevant recent reviews of exposure, hazard and dose-response analysis involving peer engagement (e.g., those conducted by regulatory Agencies) as a basis to scope key data on pathways of exposure, effects (i.e., toxicity profiling) and related considerations (e.g., mode action, pharmacokinetics)
- conduct of a literature search to identify references in critical areas published/prepared since the issue of the most recent relevant authoritative review and
- addressing a series of questions or bullets designed to solicit thought, input and documentation on appropriate focus/content at the outset of an assessment. Responses are based on a scoping exercise taking into account, principally existing reviews

This facilitates streamlining of the content of the assessment. Information that doesn't materially impact the relevant outcome is minimized while ensuring adequate transparency of documentation on critical aspects. For areas that are not likely to be critical (i.e., those aspects

which are well documented in existing assessments, not directly relevant to characterization to meet protection goals or for which there is limited controversy), a very short overview or assimilated summary is sufficient. The Problem Formulation Report constitutes supporting documentation for the assessment and contributes to the rationale in the Introduction for focus on critical aspects relevant to meeting the specified objectives. It also provides documentation of the process for preparation of the assessment (e.g., nature of peer engagement). Accompanying process to introduce formal "problem formulation" might include the following steps:

1. completion of a formal problem formulation template based on scoping of assessments/ reviews and recent data.
2. review at a meeting of internal senior staff to agree focus, content and resourcing for the assessment
3. sign-off on agreed focus, proposed schedule for completion and suggested peer engagement.
4. circulation of the agreed problem formulation to others (potentially impacted) for input (including identification of relevant planned, completed or in progress assessments and relevant data)
5. circulation of the agreed problem formulation to stakeholders with a specific request to flag additional potential "issues" in their areas of expertise with subsequent revision of focus, if necessary and
6. additional iteration of the steps above, as appropriate (e.g., should there be a proposed significant change in focus/envisaged content)
7. The state of development of the WHO Guidance will be presented in the context of its important implications for One Health approaches.

sO-3

HARNESSING NANO-AND BIO-TECHNOLOGY PLATFORMS FOR HUMAN AND PLANT HEALTH

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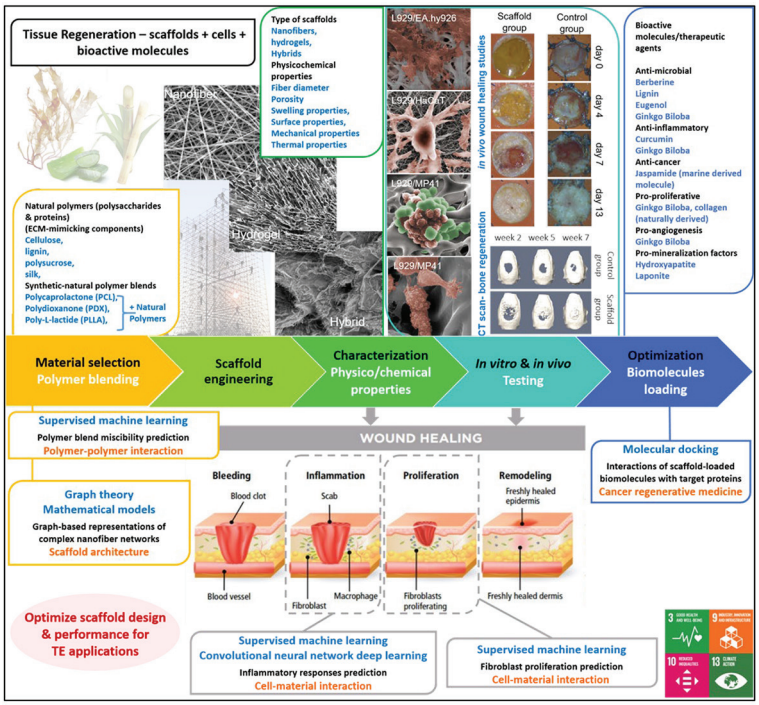
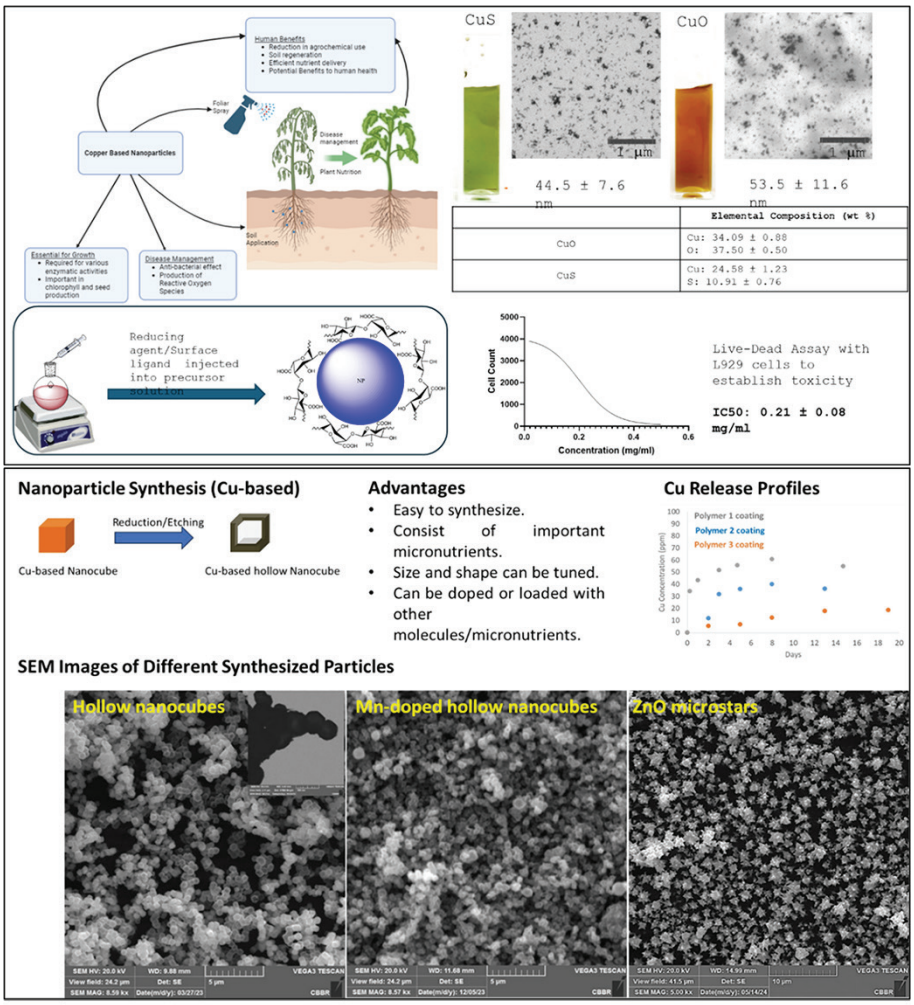
Over the decades, numerous successful techniques have been developed for the engineering of nanostructures in the form of nanofibers, nanoparticles with various morphologies and hybrid systems. Materials engineering and design are at the center of this success. We first developed nanofibrous scaffolds with multiple functionality for tissue engineering applications addressing infected wounds and post-operative cancer resection wounds. [1-5] Bio/materials' physico-chemical-biological properties and the structural designs of the scaffolds were investigated and correlated through machine learning models and tested *in vitro/in vivo*. (Scheme 1) Using our previous knowledge in nanoparticle synthesis, this nanotechnology platform was used to develop nanoparticles with various morphologies/shapes such as star, cubic, ruffles to promote plant growth and defence mechanisms mimicking nanodrug delivery systems in health which were designed to enhance delivery efficacy and reduce the toxicity of drugs/vaccines to human cells (Scheme 2). 3D Bioprinting, hydrogel technology and mathematical modeling are now being applied to improve the architecture of our systems for both plant and human health which remains highly intertwined. Nanobiotechnology, a combination of nanotechnology and biotechnology, has given rise to systems such as the mRNA vaccines. The medical, industrial, agricultural and environmental fields are benefiting from the convergence of these two platforms. However developing an interchangeable biotechnology-nanotechnology platform is a challenging endeavor. The development pathways and work accomplished on the above systems will be presented.



Scheme 1. Bio/materials engineering for humans



Scheme 2. Bio/materials engineering for plants

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sO-4

TRACKING COMMUNICABLE DISEASES USING WASTEWATER IN AN ENVIRONMENTAL ONE HEALTH FRAMEWORK

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Wastewater-based epidemiology (WBE) and microbial source tracking (MST) have emerged as powerful tools for detecting and monitoring communicable diseases within an environmental One Health framework. MST identifies specific sources of fecal contamination in water bodies by detecting gut microorganisms from humans and animals, or through host-specific DNA markers. By linking microbial contamination to its sources, MST facilitates effective water quality management and supports microbial risk assessments as recommended by the World Health Organization (WHO). MST methods vary from bacterial and viral cultivation to molecular assays, with diverse requirements for instrumentation, expertise, and costs. WBE gained global recognition during the COVID-19 pandemic, when it was used to monitor SARS-CoV-2 spread and provide early warnings of outbreaks. Beyond COVID-19, WBE continues to be an economical and effective surveillance method for tracking a variety of pathogens in low- and middle-income countries, where it complements clinical surveillance at the population level. However, national WBE systems require cross-sector cooperation, legislative support, and sustainable funding. Sentinel cities play a critical role in providing early warnings of new disease dynamics and emerging pathogen variants. This presentation focuses on the application of WBE and MST to track communicable diseases and antimicrobial resistance genes (ARGs) in human wastewater and environmental water. Through case studies, we demonstrate how these approaches provide early detection of disease outbreaks, identify contamination sources, and support public health interventions. Integrating WBE and MST within an environmental One Health perspective enhances disease surveillance, helping to mitigate the spread of communicable diseases and improve global health monitoring.

SPECIAL PLATFORM SESSION ON CANCERS

sO-5

THE LANDSCAPE OF ETIOLOGICAL PATTERNS OF HEPATOCELLULAR CARCINOMA AND INTRAHEPATIC CHOLANGIOCARCINOMA IN THAILAND

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Thailand is among countries with the highest global incidence and mortality rates of hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA). While viral hepatitis and liver fluke infections have been associated with HCC and iCCA, respectively, other environmental risk factors, overall risk factor commonality and combinatorial roles, and effects on survival have not been systematically examined. We conducted a TIGER-LC consortium-based population study covering all high-incidence areas of both malignancies across Thailand: 837 HCC, 1474 iCCA, and 1112 controls (2011–2019) were comprehensively queried on lifelong environmental exposures, lifestyle, and medical history. Multivariate logistic regression and Cox proportional hazards analyses were used to evaluate risk factors and associated survival patterns. Our models identified shared risk factors between HCC and iCCA, such as viral hepatitis infection, liver fluke infection, and diabetes, including *novel and shared* associations of agricultural pesticide exposure (OR range of 1.50; 95% CI: 1.06–2.11 to 2.91; 95% CI: 1.82–4.63) along with vulnerable sources of drinking water. Most patients had multiple risk factors, magnifying their risk considerably. Patients with lower risk levels had better survival in both HCC (HR 0.78; 95% CI: 0.64–0.96) and iCCA (HR 0.84; 95% CI: 0.70–0.99). Risk factor co-exposures and their common associations with HCC and iCCA in Thailand emphasize the importance for future prevention and control measures, especially in its large agricultural sector. The observed mortality patterns suggest ways to stratify patients for anticipated survivorship and develop plans to support medical care of longer-term survivors, including behavioral changes to reduce exposures.

sO-6

INTEGRATED GENOMIC APPLICATIONS DISCERN MOLECULAR SUBGROUPS AND TREATMENT RESPONSE IN LIVER CANCER

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Primary liver cancers (PLC), encompassing hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA), are leading causes of cancer mortality worldwide with rising incidence in the U.S. for several decades, particularly among underserved populations. Tumor heterogeneity and presentation with advanced disease is linked to poor prognosis and treatment failure. More recently, immunotherapy-based treatments, such as immune checkpoint inhibitors (ICIs), have revolutionized cancer treatment in PLC, however only a fraction of patients achieve an objective response rate, and median survival has remained suboptimal. Therefore, there is an urgent need to identify homogeneous molecular subgroups and related predictive biomarkers of treatment response and outcome to select patients who are more likely to respond. In a U.S.-based multi-site clinical study (NCI-CLARITY) with retrospective and prospective arms, we have profiled the bulk and single-cell transcriptome as well as genomic alterations among PLC patients, prior to and following ICI treatment. In the retrospective arm, we have established a formalin fixed, paraffin embedded (FFPE)-based workflow to perform comprehensive omics profiling among tissue biopsies from 230 PLC tumors and adjacent non-tumor tissues. Four stable survival-related molecular subgroups were identified, defined by orthogonal axes of aggressive tumor biology and immune infiltration, the latter associated with ICI response. Although the underlying molecular status of tumors prior to ICI treatment was largely maintained following treatment, some molecular responses tracked with patient outcome. In single-cell analyses, the degree of transcriptomic diversity in tumors were associated with worse overall survival and VEGF expression. Longitudinal tracking of intratumoral cell states and their hierarchical relationship revealed osteopontin, encoded by the gene SPP1, as a candidate regulator of tumor evolution in response to treatment. Furthermore, we defined a lineage and ecological score, termed CASCADE, that measures the joint dynamics of tumor cells and their microenvironments in response to therapeutic intervention, classifying four main states in the lineage-ecological space, which are associated with clinical outcomes. Moreover, to improve health equity in PLC, the transcriptome is being explored to identify molecular fingerprints among race/ethnic groups related to treatment response and outcome. In sum, patients with heterogenous PLC may be stratified by molecular status related to tumor biology and microenvironmental features. These molecular indicators of biological and outcome patterns suggest approaches to stratify patients to improve anticipated survivorship and treatment response.

sO-7

IONIZING RADIATION-ASSOCIATED LIVER CANCERS IN THE MAYAK WORKER COHORT

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Occupational exposure to ionizing radiation, especially plutonium, is associated with increased risks for the development of liver cancers, but the molecular mechanisms and epidemiological characteristics are not well known. My team assessed the pathological characteristics of the liver cancers that occurred in a Russian cohort of nuclear workers of the Mayak Production Association, and studied the relationships between dosimetry, gender, histology, and gene sequences. The subjects included two groups of workers whose biological specimens had been collected during autopsies: 32 with liver cancers (cases), and 38 workers free of cancer (worker controls). An independent pathologist reviewed all tissues and to confirm the diagnoses of angiosarcoma of the liver (ASL), hepatocellular carcinoma (HCC), or cholangiocarcinoma (CCA). Radiation dose levels and gender and age distributions were compared among the groups. Female workers predominated among those who developed ASL (9 of 13, 69%), whereas male predominance characterized both HCC (9 of 9; 100%) and CCA (8 of 9, 89%). There was also a male predominance in the group of workers without liver cancer (30 of 38, 79%). Those who developed ASL had the largest cumulative external doses (> 2 Gy in some cases) and the largest absorbed doses to the liver. DNA extracted from tumor blocks was subjected to whole exome sequencing, and comparisons of aberrant sequences in tumor versus adjacent non-tumor tissue revealed common genes and genetic pathways across the three tumor types (actin cytoskeleton signaling and DNA double strand breakage repair genes). Specific pathways included: angiogenesis and AKT/MTOR signaling in ASL; chromatin remodeling in CCA; and Wnt/β-catenin genes in HCC. These observations offer possible clues to the unusual pattern of liver malignancies, particularly angiosarcoma, in relation to aspects of plutonium exposure.

sO-8

URINARY BIOMARKERS FOR EARLY LUNG CANCER DETECTION IN NON-SMOKERS: A STEP TOWARD PERSONALIZED SCREENING

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Nonsmokers represent around 10% to 13% of all lung cancer cases in the United States. The causes of lung cancer in nonsmokers include secondhand smoke, asbestos exposure, environmental pollutants, and radon. However, these risk factors are not currently included in the criteria for early lung cancer screening using low-dose CT (LDCT). To address this gap, we analyzed collected urine samples from two independent groups: an exploratory cohort of 846 participants and a validation cohort of 505 participants. They analyzed levels of two cancer-related urinary biomarkers, creatine riboside (CR) and N-acetylneuraminic acid (NANA), using liquid chromatography-mass spectrometry. The aim was to see if these biomarkers could help distinguish nonsmoker lung cancer cases from age- and sex-matched controls, as well as from tobacco smoker cases and controls. This could potentially refine the criteria for LDCT screening eligibility. The study found that urinary levels of CR and NANA were significantly elevated in both nonsmokers and smokers with lung cancer compared to control groups in both cohorts. When analyzing the data using receiver operating characteristic (ROC) curves, the combination of CR and NANA showed strong predictive accuracy for detecting lung cancer in nonsmokers. In the exploratory cohort, the area under the curve (AUC) was 0.94, indicating high accuracy, while the validation cohort had an AUC of 0.80. Additionally, Kaplan-Meier survival analysis revealed that higher levels of these biomarkers were linked to a greater risk of cancer-specific death in both nonsmokers and smokers with lung cancer. These findings suggest that measuring CR and NANA in urine samples could be a valuable tool for identifying nonsmokers at higher risk for lung cancer, making them candidates for LDCT screening. This approach could lead to more personalized screening criteria and underscores the need for further studies to validate the use of these biomarkers in clinical practice.

FREE COMMUNICATION

O-1

THE IMPACT OF GLYPHOSATE ON EMERGENCE OF ANTIMICROBIAL RESISTANCE

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Antimicrobial resistance (AMR) is a global threat to the human health and recognized as a silence pandemic. In 2019, 4.95 million deaths globally were associated with AMR, whereas 1.27 million deaths directly caused by bacterial AMR. Sustainable solutions are urgently needed. In addition to the development of novel antibiotics, understanding the driving forces behind AMR will provide an insight into the sustainable solutions for AMR crisis. Not only the antibiotics, but also the agrochemicals, like glyphosate, could be possible AMR drivers. However, more scientific evidence for glyphosate-induced AMR is still needed. This study revealed that the agrochemical glyphosate, a widely use herbicide, is one of the AMR drivers. Using the human pathogen model, *S. maltophilia* K279a, supplementation with glyphosate at sublethal concentrations significantly accelerated the development of strains that reduced susceptibility to several antibiotics belonging to the quinolone and aminoglycoside groups. Expression analysis using reverse transcription PCR showed that these resistance phenotypes are related to the upregulation of several efflux pump genes, such as *smeABC, smeVWX, mfsA, tcrA,* and *qnr*. These results aligned with the proteomic profiles of glyphosate treated cells, in which efflux pump proteins were significantly increased, compared to the unexposed cells. Altogether, the results indicated that not only the antibiotics but also the herbicide glyphosate could be a driving force for AMR. Thus, reducing a release of these AMR drivers will curb the AMR development, resulting in lower emergence of AMR.

O-2

BIOCIDE-ASSOCIATED RESISTANCE MECHANISMS IN *PSEUDOMONAS AERUGINOSA*: IMPACTS ON ANTIBIOTICS AND HOST IMMUNITY

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Pseudomonas aeruginosa, an opportunistic pathogen, exhibits significant inter- and intra-strain variability due to its highly plastic genome, which contributes to its metabolic versatility and phenotypic flexibility. This bacterium shows notable resilience to both biocides and antibiotics, partly due to its capacity to develop biocide-induced resistance mechanisms. Key factors driving this resistance include the upregulation of efflux pumps and enhanced biofilm formation, which aid in the bacterium's survival in hostile environments. Exposure to biocides not only promotes these resistance mechanisms but also diminishes antibiotic efficacy, complicating treatment strategies. Our research demonstrated that resistance to paraquat, mediated through the polyamine degradation pathway, results in the induced expression of an efflux pump and antibiotic resistance in *P. aeruginosa*. Additionally, oxidant-induced expression of iron-sulfur cluster biogenesis and repair genes under the IscR regulon alters susceptibility to various antibiotics. Biocide-induced resistance can also interfere with host immune responses, enabling *P. aeruginosa* to evade immune recognition and clearance. For instance, resistance to bleach mediated by RcsA and resistance to chlorhexidine mediated by Ppk could enhance bacterial survival in both animal hosts and mammalian immune cells. These data highlight the complex interplay between biocide-induced resistance mechanisms and their effects on host immunity and antibiotic susceptibility. Understanding these interactions is essential for developing targeted infection management strategies and effective antimicrobial stewardship practices. We are also exploring advanced adjuvants in vaccine development, potential drug targets, and biosensors for detecting specific biocide residues in the environment. Ultimately, misuse or overuse of biocides can drive adaptive resistance mechanisms in bacteria, making infections harder to treat. Responsible biocide uses and heightened social awareness of antimicrobial resistance issues are critical to preventing reduced antibiotic effectiveness and addressing complex infection control challenges.

O-3

NANO BOOST: ENHANCING ANTIBACTERIAL EFFICACY OF CEFTRIAXONE WITH MAGNESIUM OXIDE NANOPARTICLES TO TACKLE ANTIMICROBIAL RESISTANCE

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Antimicrobial resistance is a leading issue for one health perspective. Researchers are working to cope with this issue through different approaches like discovering new antibiotics, Peptide like substances, computer aided antibiotics, nano biotics etc. The antibacterial effect of metallic nanoparticles (NPs) has been observed but they are cytotoxic at higher doses. In this study, we are aimed to determine the boosting of antibacterial effect of resistant antibiotic (Ceftriaxone) using nanoparticles [Magnesium oxide nanoparticles (MgO NPs)] and thus obtaining a good antibacterial effect of NPs at lower doses. *Salmonella typhi* and MRSA stain were selected as test organism as it is not susceptible to Ceftriaxone. The MgO NPs was synthesized as prescribed by Narendran et al. (2019). Conjugation of MgO with Chitosan (CS) to prepare CS-MgO NPs. Then, CS-MgO NPs was conjugated with ceftriaxone to obtain Ceftriaxone-CS-MgO. *In vitro* drug release kinetics was performed through membrane dialysis technique. The conjugation ability of ceftriaxone was assessed and characterization of Ceftriaxone-CS-MgO NPs was done through the use of visible spectroscopy, transmission electron microscopy and X-Ray diffraction. The antibacterial efficacy of Ceftriaxone, MgO NPs and Ceftriaxone-CS-MgO NPs against *S. typhi* and MRSA strains was evaluated using disc diffusion method. The Minimum inhibitory concentration (MIC) of ceftriaxone, MgO NPs and Ceftriaxone-CS-MgO NPs was assessed on 96 well plates. The size of MgO NPs, CS-MgO NPs and Ceftriaxone-CS-MgO NPs ranges between 6 to 18nm, 37nm and 40nm respectively and all had spherical shape. The ceftriaxone-CS-MgO NPs have enhanced antibacterial activity than MgO NPs and Ceftriaxone alone. The highest reduction in the MIC of ceftriaxone-CS-MgO NPs was noted against tested strains ranging from 22% to 96%. Ceftriaxone-CS-MgO NPs have no cytotoxic effects on normal cells at low doses. The study successfully enhanced the antibacterial effect of Ceftriaxone through conjugation with MgO NPs. This research holds promise to address antimicrobial resistance by producing high antibacterial outcomes at low doses of antibiotics and explore potential of ceftriaxone-CS-MgO NPs to combat multi drug resistant bacterial infections.

O-4

EVALUATING AMR SURVEILLANCE METHODOLOGIES IN CHOLISTANI AND SAHIWAL CATTLE: A COMPARATIVE STUDY OF SAMPLING STRATEGIES AND TESTING TECHNIQUES IN PAKISTAN

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Withdrawn

O-5

EMERGENCE OF MULTI-DRUG RESISTANT BACTERIA DUE TO IRRATIONAL ANTIBIOTIC USE IN LIVESTOCK

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The irrational use of antibiotics in poultry, pig, and cow farms by farmers lacking proper knowledge poses a significant risk in Sri Lanka, where these antibiotics are readily available in farm shops without prescription. This improper use, such as dissolving antibiotics in large quantities of drinking water, can lead to sub-lethal concentrations in the environment, contributing to the development of antibacterial resistance. Under the One Health concept, this study investigates the emergence of resistance in Escherichia coli and Staphylococcus aureus after exposure to sub-lethal concentrations (MIC/5 and MIC/10) of several antibiotics commonly sold in farm shops, including enrofloxacin, neomycin-oxytetracycline (Neo-OTC), sulphamethoxazole-trimethoprim (Trim-Sulfa), tylosin, tilmicosin, and sulphabenzpyrazine-2,4-diamino-5-veratrylpyrimidine (SBVP). Minimum inhibitory concentrations (MICs) for E. coli and S. aureus were determined using the broth dilution method followed by the Alamar Blue assay. The bacteria were cultured in LB broth with sub-lethal concentrations of each antibiotic for 7 days at 37°C, with controls maintained without antibiotics. After centrifugation and washing with sterile saline, bacterial pellets were cultured, and colonies were isolated on Mueller-Hinton agar. Disk diffusion tests assessed the susceptibility of both exposed and control strains to erythromycin, ciprofloxacin, doxycycline, gentamicin, ceftriaxone, and amoxicillin. The inhibition zone diameters were compared using one-way ANOVA followed by Dunnett's multiple comparison test, with $p < 0.05$ considered significant. Results showed that enrofloxacin resulted in significant resistance in *S. aureus* against doxycycline and ciprofloxacin, with a marked reduction in inhibition zone diameter for ciprofloxacin. In *E. coli*, enrofloxacin led to resistance against gentamicin, doxycycline, and ciprofloxacin, potentially due to oxidative stress. The Neo-OTC combination conferred resistance in *S. aureus* to all antibiotics except amoxicillin and ceftriaxone, and in *E. coli*, resistance was noted for all except erythromycin and amoxicillin. The Trim-Sulfa affected *S. aureus* for erythromycin, gentamicin, and doxycycline, while *E. coli* showed reduced susceptibility to doxycycline and ciprofloxacin. Tylosin conferred resistance in *S. aureus* to all antibiotics except ciprofloxacin, with *E. coli* showing reduced susceptibility to erythromycin, doxycycline, and amoxicillin. The combination of SBVP similarly impacted S. aureus, with resistance to all antibiotics except doxycycline, and in *E. coli*, to doxycycline and ciprofloxacin. Tilmicosin reduced susceptibility in *S. aureus* to erythromycin, gentamicin, and ciprofloxacin, and in *E. coli* to erythromycin and ciprofloxacin. This study reveals that exposure to sub-lethal concentrations of antibiotics can foster not only resistance to specific drug classes but also multi-drug resistance, likely due to the stress induced by these concentrations. To prevent the emergence of multi-drug-resistant bacteria, stricter regulation of antibiotic use in farming is essential globally. Future research should focus on additional bacterial strains, particularly those associated with zoonotic diseases.

O-6

THE GUT MICROBIOTA IN CHILDREN FROM AN E-WASTE RECYCLING AREA WITH 6PPDQ AND OTHER POLLUTANT EXPOSURE

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The composition and metabolites of the gut microbiota can be altered by environmental pollutants. However, the effect of co-exposure to multiple pollutants on the human gut microbiota has not been sufficiently studied. In this study, gut microorganisms and their metabolites were compared between 33 children from Guiyu, an e-waste recycling area, and 34 children from Haojiang, an area without e-waste recycling activity. The exposure level was assessed by estimating the daily intake (EDI) of 6PPD-quinone (6PPDQ), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and metal(loid)s in kindergarten dust. Significant correlations were found between the EDIs of 6PPDQ, BDE28, PCB52, Ni, Cu, and the composition of gut microbiota and specific metabolites. The Bayesian kernel machine regression model showed negative correlations between the EDIs of five pollutants (6PPDQ, BDE28, PCB52, Ni, and Cu) and the composition of gut microbiota. The EDIs of these five pollutants were positively correlated with the levels of the metabolite 2,4-diaminobutyric acid, while negatively correlated with the levels of d-erythro-sphingosine and d-threitol. Our study suggests that exposure to 6PPDQ, BDE28, PCB52, Ni, and Cu in kindergarten dust is associated with alterations in the composition and metabolites of the gut microbiota. These alterations may be associated with children's health.

O-7

HEAVY METAL(LOID) EXPOSURE INCREASES THE RISK OF CHILDHOOD ANEMIA: EVIDENCE FROM A TYPICAL E-WASTE RECYCLING AREA IN CHINA

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Childhood anemia poses a significant global health concern. Exposure to heavy metal or metalloid is one of the main causes of anemia in children, but evidence of the comprehensive effects of multiple heavy metal(loid) exposure on childhood anemia-sensitive indicator erythrocyte-related parameters is still insufficient. This study aimed to explore the association between high levels of heavy metal(loid) exposure and child erythrocyte-related parameters, alongside investigated the potential modifying effects of children's gender, age, and body mass index (BMI). In this study, a total of 588 children aged 3 to 7 from were recruited from e-waste recycling area, Guiyu, China. Inductively coupled plasma mass spectrometry (ICP-MS) was used to measure child blood heavy metal(loid) levels. Erythrocyte-related parameters including hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), and red blood cell distribution width (RDW) were measured by an automated hematology analyzer. K-means clustering was used to analyze and explore the differences in erythrocyte-related parameters between subgroups according to the concentration of sensitive elements. Interaction analysis was conducted by child gender, age, and BMI to explore the potential modifying effects of stratified factors. The results showed that children in the e-waste recycling area had higher levels of heavy metal(loid) in blood. Erythrocyte-related parameters were negatively correlated with the Cu and Cu/Zn ratios and positively correlated with Cr, Ni, Zn, and Se by Spearman correlation analysis. Only blood Cu level was negatively correlated with HGB [β=−2.74, (95% CI: −4.49, −0.995)], MCH [β=−0.505, (95% CI: −0.785, −0.226)], MCV [β=−1.024, (95% CI: −1.767, −0.281)], and MCHC [β=−2.137, (95% CI: −3.54, −0.734)] by multiple linear regression analysis. Cluster analysis found that children with high levels of blood Cu exposure had lower erythrocyte-related parameters compared to the low concentration group, indicating the sensitivity of Cu element to erythrocyte-related parameters and implying a potential risk of anemia. Interaction analysis indicated that metal(loid)s exposure effects were modified by child sex, age, and body mass index (BMI). The Bayesian Kernel Machine Regression (BKMR) model analysis indicated a negative correlation between the combined exposure to Cu, Zn, Pb, Cr and MCH or MCV. The single-factor analysis showed a considerable statistical difference only with Cu on MCV, MCH, and HGB. Furthermore, the interaction analysis highlighted the interdependent effects of Cu and Zn, Pb and Zn, and Cr and Zn on MCH and MCV levels, indicating high levels of exposure to heavy metal(loid)s increase the risk of anemia in children. Additionally, the oxidation and/or antioxidation reactions may play a significant role in the development of metal(loid)-induced anemia risk. It is crucial to investigate the effects of co-exposure to multiple heavy metal(loid) elements on anemia, especially the interrelationships and mechanisms among them.

O-8

INFLUENCE OF E-WASTE EXPOSURE ON DNA DAMAGE AND DNA METHYLATION IN PEOPLE LIVING NEAR RECYCLING SITES

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The association between long-term exposure to e-waste and poor health is well established, but how e-waste exposure affects DNA methylation is understudied. In this study, we measured the DNA damage levels and the alternation of DNA methylation in peripheral blood mononuclear cells (PBMCs) collected from a population exposed to e-waste. The concentration of 28 PCB congeners in the blood samples of e-waste recycling workers was elevated than those of the reference group. DNA damage levels were significantly higher than that of samples from the reference group by detecting the SCGE, CA, and CBMN assays. Eventually, we found that the methylation level of 1233 gene loci was changed in the exposure group. Bioinformatic analysis of differential genes revealed that the hypermethylated genes were enriched in cell component movement and regulation of cell function, and hypomethylated genes were involved in the cellular metabolic process. Among the 30 genes we tested, 14 genes showed a negative correlation between methylation level and expression level. Therefore, e-waste exposure potentially increased the levels of DNA damage and alters DNA methylation, which would likely impact human health.

O-9

THE HEALTH STATUS ASSESSMENT OF PERSONNEL HANDLING ELECTRONIC WASTE IN DEVELOPING COUNTRY: A SITUATION ANALYSIS

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Electronic wastes are referred to electrical and electronic equipment and its components that have been thrown out which can't be re used. A cross-sectional study was carried out among workers handling electronic wastes. Informed consent was obtained in English and the local language from each individual before collecting the Sample. A total of 215 subjects participated in the study. The aim of the study was to investigate health status of these workers and assessment of body burden of e-waste contaminants. The heavy metal concentration, hematological (CBC), biochemical, genotoxicity assessment and urinary 8-OHdG levels were carried out. Hematological parameters were found to be normal, except for microcytic hypochromic and microcytic normochromic anemia in about 8% and 11.5% of workers respectively. The assessment of cytogenetic damage using comet assay showed a high frequency of DNA damage index among study participants. Significant ($p<0.05$) increases in the levels of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and abnormal levels of liver enzyme (SGOT and SGPT) were observed among study participants. Additionally, chronic health complaints i.e. asthma (1.4%), hypertension (6.9%) and diabetes mellitus (3.5%) were observed among e-waste workers. The workers complained backache (26.45%), neck pain (15.30%) due to their sitting posture during work. The blood metal concentrations of cadmium, chromium manganese, nickel and lead were significantly ($p<0.05$) higher than the levels detected in office staff those not handling e waste processing work. Other health complaints reported include Tiredness, backache, diarrhoea, vomiting, neck pain, joint pain, headache, eye irritation, and blurred vision were also reported among study subjects. The workers and the management were apprised about the importance of using personal protection equipment during the work to prevent exposures of pollutants.

O-10

BLOOD HEAVY METALS, DNA DAMAGE, AND INTELLIGENCE QUOTIENT AMONG CHILDREN FROM AN INFORMAL E-WASTE PROCESSING VILLAGE IN VIETNAM

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This study was conducted to compare the levels of heavy metals in blood, DNA damage, and intelligence quotient (IQ) in children living in an informal e-waste processing village (the exposed village) with those from a reference village in Northern Vietnam. The findings revealed that blood levels of nickel and arsenic in the children at the exposed village were significantly higher than those at the reference village at $p < 0.05$; however, no significant differences were observed for lead, cadmium, and chromium in children's blood between the two villages. The overall levels of five heavy metals in exposed children's blood were significantly higher than those in non-exposed children at $p < 0.01$. The DNA damage of the children at the exposed village was significantly greater compared to that of the children at the reference village at $p < 0.01$. Otherwise, there were no significant differences in children's IQ among the two villages. The overall blood metal level of the children at both villages was positively correlated with DNA damage given as Tail Length (Spearman $r = 0.249$, $n = 80$, $p<0.05$), indicating that a higher level of DNA damage could be induced by higher heavy metal exposure among children. A negative correlation was observed between IQ and DNA damage in children from both villages. Factors such as parental engaging e-waste processing activities, distance to the nearest e-waste processing area, processing e-waste at home, engaging e-waste processing while pregnant, and breastfeeding while handling e-waste were the significant contributors to higher health effects on children. It was found that heavy metal pollution from e-waste processing activities could pose a potential risk to children's health.

O-11

ENVIRONMENTAL HEALTH RISKS OF POST-BAN PARAQUAT RESIDUES IN PHITSANULOK'S NAN RIVER AND VICINITY AREAS

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Paraquat, a herbicide extensively used for weed management, was banned in Thailand in 2020. Despite this ban, residual contamination in water sources may continue to pose significant public health and ecological risks due to paraquat's toxicity and adverse environmental effects. This study investigates paraquat residues in water from the Nan River and associated canals in agricultural areas of Phitsanulok, Thailand, and evaluates its ecotoxicological impact using the Hazard Quotient (HQ). Twelve water sampling stations were established: six along the Nan River in areas with intensive agriculture and six from irrigation canals utilized for rice cultivation. The water quality parameters, including pH, turbidity, and total dissolved solids, were measured. Paraquat concentrations were determined using a standard color comparison kit and quantified by spectrophotometry at 395 nm. HQ values were derived from the ratio of Estimated Environmental Concentration (EEC) to Predicted No-Effect Concentration (PNEC). The results revealed that 58% of water samples contained paraquat residues. Concentrations in the Nan River ranged from non-detectable to 3.80 ± 0.026 mg/L, significantly higher than those in the canals, where levels ranged from non-detectable to 0.47 ± 0.010 mg/L. The average paraquat concentration in the river (2.24 ± 1.605 mg/L) was ≈ 6 times greater than in the canals (0.40 ± 0.070 mg/L). HQ values exceeded 1 at both sites, indicating notable ecotoxicological risks. While pH levels were within Thailand's water quality standards (7.504 and 7.479), turbidity was higher in the river, whereas total dissolved solids (TDS) were higher in the canals. The persistence of paraquat residues, even after the ban, underscores a critical environmental health challenge. The elevated paraquat levels in the Nan River indicate the urgent need for ongoing monitoring and effective remediation strategies to protect public health and aquatic ecosystems.

O-12

ENHANCING ANTI-GLIOMA ACTIVITY WITH COMBINED EPIGENETIC AGENTS AND MAGE-D4 PEPTIDE-SPECIFIC T CELLS

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This study evaluates the efficacy of combining epigenetic drugs—decitabine (DAC), valproic acid (VPA), and trichostatin A (TSA) with immunotherapy targeting glioma. We analyzed MAGE-D4 expression and prognosis in glioma using online databases. The impact of epigenetic drugs on MAGE-D4 and HLA-A2 expression in glioma was assessed using qRT-PCR, Western blot, and flow cytometry. The methylation status of the MAGE-D4 promoter was determined by pyrosequencing. An HLA-A2-restricted MAGE-D4 peptide was predicted, synthesized, and its affinity for HLA was measured through affinity and peptide/HLA complex stability assays. The functionality of MAGE-D4 peptide-specific T cells was evaluated using CCK8, CFSE, ELISA, and ELISPOT assays. The cytotoxic effects of these T cells combined with epigenetic drugs against glioma were analyzed in vitro using flow cytometry, ELISA, and cytotoxicity assays. Additionally, the inhibitory effect of MAGE-D4 peptide-specific T cells on gliomas was tested in a glioma-loaded mouse model. MAGE-D4 was found to be highly expressed in glioma and associated with poor prognosis. Epigenetic drugs successfully induced MAGE-D4 and HLA-A2 expression in glioma cells. MAGE-D4-associated peptides effectively stimulated dendritic cells to induce T-cell proliferation, IL-2 secretion, and IFN-γ production. MAGE-D4 peptide-specific T cells showed the highest cytotoxicity when treated with TSA alone or in combination with DAC, though this effect was significantly reduced after HLA blocking. *In vivo*, MAGE-D4-specific T cells inhibited glioma growth in TSA-treated mice. AGE-D4 is highly expressed in glioma and correlates with prognosis of glioma. The identified MAGE-D4 peptide effectively stimulates MAGE-D4-specific T cells, which, when combined with epigenetic drugs, can significantly enhance the inhibition of glioma growth.

O-13

ADIPOSE TISSUE LEVELS OF PBDES IN RELATION TO CHEMOTHERAPY TOXICITY, CHEMORESISTANCE AND PROGNOSIS IN BREAST CANCER PATIENTS

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Exposure to polybrominated diphenyl ethers (PBDEs) has been linked to an increased risk for breast cancer. However, the implication of PBDEs in chemotherapy response, prognosis and chemotherapy toxicity in breast cancer patients are poorly understood. This study aimed to identify the cancer recurrence, survival and chemotherapy-associated hematotoxicity/hepatotoxicity associated with PBDE adipose levels in patients with breast cancer. A total of 183 breast cancer patients from a hospital located in the eastern area of southern China were enrolled in the current study, of which 126 received chemotherapy. Utilizing gas chromatography-mass spectrometry, BDE-153, BDE-209 and BDE-183 were determined as the most prevalent congeners in this population. Multivariable logistic regression analysis showed BDE-99 and 190 levels were positively associated with clinical stage and N-stage respectively (OR = 2.61 [1.26–5.40], OR = 2.78 [1.04–7.46]). BDE-28, BDE-183, BDE-190, BDE-209, and total PBDEs were positively associated with chemotherapy toxicity (OR = 91.4-280.1%, all $P < 0.05$). Additionally, BDE-100, BDE-154, BDE-153, BDE-138, BDE-183, BDE-190, BDE-209, and total PBDEs were positively associated with the chemoresistance risk (all $P < 0.05$). Restricted cubic splines analysis indicated a general L-shaped trend for increased risk of chemotherapy toxicity with the elevated BDE-28, BDE-153, BDE-154, BDE-183, BDE-190, and total PBDE levels ($P < 0.05$ for non-linear). Meanwhile, BDE-209 exhibited a positive linear correlation (non-linear $P = 0.7848$). A non-linear relationship between chemoresistance risk and BDE-100, BDE-153, BDE-154, BDE-183, BDE-190, and total PBDEs was also observed (all P for non-linear < 0.05). Nevertheless, quantile g-computation and Bayesian kernel machine regression (BKMR) analysis found no significant association between PBDE mixtures and chemotherapy toxicity or resistance, albeit with a positive link. Notably, using Kaplan-Meier and Cox regression, BDE-47, BDE-99, and BDE-183 were identified as independent prognostic factors for shorter PFS, respectively (adjusted HR = 3.14 [1.26–7.82]; 2.25 [1.03–4.94]; 2.60 [1.08–6.25]). In conclusion, the loads of certain PBDE congeners in breast cancer patients may contribute to chemotherapy toxicity, recurrence and poor prognosis.

O-14

GENETIC UNDERPINNINGS OF HYPOGLYCEMIA-INDUCED EPILEPSY: INSIGHTS FROM GYS2 GENE MUTATIONS

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Epilepsy is a complex neurological disorder characterized by recurrent seizures. Recent advances in genetic research have implicated various genes in its pathogenesis. Glycogen synthase 2 (GYS2) is primarily expressed in the liver and plays a crucial role in glycogen synthesis. Biallelic variants in GYS2 have recently been associated with epilepsy in humans. We performed whole-exome sequencing (WES) on individuals with clinically suspected epilepsy. Bioinformatic analysis was conducted to identify pathogenic variants, focusing on genes known to be associated with epilepsy. Functional studies were performed to evaluate the impact of identified variants on protein function and cellular processes. WES identified biallelic variants in the GYS2 gene in multiple individuals with epilepsy. The identified variants included missense mutations, deletions, and splicing defects. Functional analysis demonstrated that these variants led to reduced glycogen synthase activity, impaired glycogen storage, and increased susceptibility to seizures in cellular and animal models. The discovery of GYS2 biallelic variants in individuals with epilepsy expands our understanding of the genetic basis of this disorder. Our findings suggest that impaired glycogen synthesis may contribute to the pathophysiology of epilepsy. These results have important implications for genetic counseling and potential therapeutic interventions targeting glycogen metabolism. This study highlights the significance of GYS2 in epilepsy and underscores the importance of genetic testing in the diagnostic workup of patients with epilepsy. Further research is needed to explore targeted therapies that can modulate glycogen synthesis pathways in epilepsy patients.

O-15

AMINOACYL-TRNA SYNTHETASE DEFICIENCIES: CLINICAL PRESENTATIONS, BIOCHEMICAL ANALYSES, AND FUNCTIONAL STUDIES

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Aminoacyl-tRNA synthetase (ARS) deficiencies represent a diverse group of inborn errors of metabolism caused by defects in ARS enzymes, which catalyze the attachment of amino acids to their corresponding tRNA. These enzymes typically exist in two isoforms: cytosolic (xARS1) and mitochondrial (xARS2), with the exceptions of GARS1, QARS1, and KARS1, which can function in both locations. Patients with mitochondrial ARS deficiencies often present with neurological or cardiac symptoms, resembling other primary mitochondrial disorders, while those with cytosolic ARS defects exhibit a wider range of clinical manifestations. In this presentation, we discuss two cases: one patient experienced developmental regression accompanied by abnormal brain imaging following an illness and another patient presenting with developmental delay and recurrent liver failure. Exome sequencing of the first proband identified compound heterozygous variants in *DARS2*, the gene encoding mitochondrial aspartyl-tRNA synthetase, which is associated with leukoencephalopathy with brainstem and spinal cord involvement and elevated lactate levels (LBSL). Genetic analysis of the second patient revealed compound heterozygous variants in *IARS1,* which encodes cytosolic isoleucyl-tRNA synthetase and is linked to growth retardation, cognitive impairment, hypotonia, and hepatopathy (GRIDHH). Although biochemical analyses of clinical specimens yielded inconclusive results, signs of mitochondrial dysfunction were observed in both patients. Functional studies using a baker's yeast model supported the pathogenicity of the identified variants. This study underscores the heterogeneity of ARS deficiencies and the diagnostic challenges they pose, while also validating the use of yeast models for functional investigations.

O-16

DIFFERENTIAL REQUIREMENT FOR IL-2 AND IL-23 IN THE DIFFERENTIATION AND EFFECTOR FUNCTIONS OF T CELL AGAINST CANCER

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A well-documented Achilles heel of current cancer immunotherapy approaches is T cell exhaustion within solid tumor tissues. The pro-inflammatory cytokine interleukin-23 (IL-23) has been utilised to augment chimeric antigen receptor (CAR)-T cells survival and tumor immunity. However, in-depth interrogation of molecular events downstream of IL-23/IL23R signaling is hampered by a paucity of suitable cell models. The current study investigates the differential contribution of IL-2 and IL-23 to the maintenance and differentiation of the IL-23 responsive Kit225 T-cell line. We observed that IL-23 enhanced cellular fitness and survival but was insufficient to drive proliferation. IL-23 rapidly induced phosphorylation of STAT1, 3 and 4, and mRNA expression of IL17A, the archetypal effector cytokine of Th17 cells, but not their lineage markers *RORC* and *NCR1*. These observations suggest that IL-23 endowed Th17/ILC3-like effector function but did not promote their differentiation. In contrast, spontaneous differentiation of Kit225 cells towards a Th17/ILC3-like phenotype was induced by prolonged IL-2 withdrawal. This was marked by strongly elevated basal *IL17A* and *IL17F* expression and the secretion of IL-17. Together, our data present Kit225 cells as a valuable model for studying the interplay between cytokines and their contribution to T cell survival, proliferation, and differentiation.

O-17

CLINICAL FEASIBILITY OF MALDETEC-CTC FOR DETECTION OF CIRCULATING TUMOR CELLS IN OSTEOSARCOMA USING WHOLE-CELL MALDI-TOF MASS FINGERPRINT

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Osteosarcoma is a bone malignancy with a high tendency for metastasis, which is the leading cause of death. For improving survival rates, the methods for earlier detection of metastasis are needed. Circulating tumor cells (CTCs) representing in bloodstream reflect tumor alteration in real time and metastases. However, most studies are limited by technical challenges associated with isolation and identification of CTCs due to their rarity in the bloodstream and white blood cell contamination after CTC-enrichment process. Osteosarcoma CTCs with a mesenchymal cell type could not be detected using the EpCAM-based standard method. Here, we aim to develop MALDETECT-CTC, a novel platform by coupling negative-selection CTC enrichment and MALDI-TOF-MS for detecting CTCs in osteosarcoma. We generate a mass spectral profile (MSP) database from normal cells, carcinoma and sarcoma cell lines, patient-derived osteosarcoma cell lines (PDCs), and osteosarcoma blood samples using MALDI-Biotyper. The sensitivity and specificity of the analytical platform were analyzed in 11 healthy donors and 12 osteosarcoma patients. MALDI-Biotyper was used to detect the CTCs in osteosarcoma blood samples based on the logarithmic score calculated from the MSP of individual samples. MSPs enabled the differentiation of osteosarcoma cell lines from other cancer types and normal blood cells. Most of the MSPs of CTC-enriched osteosarcoma samples resembled their PDC counterparts with high MALDI-log prediction scores. The diagnostic power of MALDETEC-CTC achieved high specificity (100%) but inferior sensitivity (50%). The sensitivity of CTCs for metastatic diagnosis was 100%, and the specificity was 60%. Additionally, CTC-positive group had a higher tendency of metastasis after initial diagnosis than patients with CTC-negative. Our combination technique successfully demonstrated the clinical value and feasibility to detect metastatic disease by identifying circulating osteosarcoma cells. We demonstrate the proof-of-concept that this workflow might be used to detect circulating cancer cells in cancer patients with a rapid and easy operation.

O-18

CANDIDA ALBICANS DNA-PROTEIN CROSSLINK REPAIR AND ITS ROLES IN STRESS RESPONSE

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Candida albicans is an important opportunistic pathogen that is becoming a problem worldwide. Due to the increase in the number of population with immunocompromising conditions and increase in antifungal drug resistance, it is critical to better understand *C.albicans* pathogenesis in order to devise novel antifungal strategies. To survive in the host, *C. albicans* have to tolerate oxidative stress, a major microbicidal mechanism of phagocytes that can cause several types of molecular damages, including DNA-protein crosslinks (DPCs), a deleterious DNA lesions that can lead to genomic instability. Our works characterized the roles of DPC repair proteases, Wss1 and Ddi1, in *C. albicans* when exposed to oxidative stress inducers. This presentation will summarize our findings and propose that DPC repair are crucial for *C.albicans* survival under oxidative stress and in phagocytes, and hence its pathogenicity.

O-19

METABOLIC DYSFUNCTION IN MICE WITH ADIPOCYTE SPECIFIC ABLATION OF THE ADENOSINE A2A RECEPTOR

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It has been well established that adenosine plays a key role in the control of inflammation through a number of GPR coupled receptors and recently shown that it can regulate thermogenesis. Here we investigated the specific requirements of the adenosine A2A receptor (A2AR) in mature adipocytes for thermogenic functionality and metabolic homeostasis. Here we provide novel evidence demonstrating that mice specifically lacking the A2AR in mature adipocytes have reduced expression of thermogenic genes in adipose tissue and decreased thermogenesis. Furthermore, we show that fat specific A2AR knock out (A2AR-FKO) mice exposed to a high fat diet (HFD) have decreased insulin sensitivity and elevated inflammation in visceral adipose tissue. Analysis of livers obtained from A2AR-FKO mice revealed increased hepato-steatosis and -steatitis. Overall, our studies demonstrate that loss of A2AR specifically in fat cells alters brown adipose tissue functionality and increases metabolic dysfunction in response to high caloric intake, providing novel insights into the mechanisms that regulate adipose tissue functionality and uncovering a potential new tissue specific therapeutic target to combat obesity and metabolic dysfunction. We generated fat tissue specific adenosine A2A receptor knock-out mice to assess the influence of signaling through this receptor on brown and beige fat functionality, obesity, insulin sensitivity, inflammation and liver function. Fat specific A2AR knock-out and wild type littermate mice were compared for potential differences in cold tolerance and energy metabolism. In addition, we measured glucose metabolism, Adipose Tissue (AT) inflammation and liver phenotypes in mice of the two genotypes after exposure to a diet rich in fat. Our results provide novel evidence indicating that loss of the adenosine A2A receptor specifically in adipocytes is associated with cold intolerance and decreased oxygen consumption. Furthermore, mice with fat specific ablation of the A2AR receptor exposed to a diet rich in fat showed increased propensity to obesity, decreased insulin sensitivity, elevated adipose tissue inflammation and hepato-steatosis and -steatitis. Overall, our data provide novel evidence that A2AR in mature adipocytes safeguards metabolic homeostasis, suggesting the possibility of targeting this receptor selectively in fat for the treatment of metabolic disease.

O-20

PI3K/AKT MEDIATE COLLAGEN TYPE 1- INDUCED OSTEOGENESIS OF DENTAL PULP STEM CELLS VIA FOCAL ADHESION MECHANISM

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Dental pulp stem cells are widely available sources of stem cells which have been extensively studied for its ability to differentiate into osteoblasts and endothelial cells, to support bone repair and regeneration. Collagen type 1 (Col-1) is a well-known extracellular matrix component, which plays a vital role in regulating the signalling pathway for osteoinduction of bone progenitor cells. However, the exact mechanism pertaining to the active role of Col-1 during osteogenesis of stem cells remains unclear. This study aims to identify the key signalling pathway and proteins interaction associated with Col-1-induced osteogenesis of DPSC. The localisation of OCN protein was assessed by immunocytochemistry analysis, followed by Western blot analysis on OCN, AKT, p-AKT, Smad2/3, p-Smad2/3, ERK1/2, and p-ERK1/2 pathways. Further identification of protein profiling was then performed using gel-free digestion and LC-MS/MS, followed by protein-protein interaction analysis using STRING online tools to assist in determination of link between various pathways. The different method of osteoinduction either using Col-1 or osteogenic differentiation medium exhibit distinct alteration on biological process, cellular component, and molecular functions, along with the presence of abundant osteogenic-related proteins within the Col-1 scaffold group. The proteomics data also indicated that the key signalling pathway involved in Col-1-induced DPSC is the PI3K/AKT pathway, which has a significant impact and potential crosstalk with TGF-β/Smad and MAPK/ERK mainly via focal adhesion protein complexes. The gathered evidences suggested that PI3K/AKT signalling pathway is more dominant than the TGF-β/Smad and MAPK/ERK pathways during the Col-1 induced osteogenic differentiation of DPSC, by stimulation of focal adhesion protein complex. Together, the findings provides deeper insight on cellular biology of differentiated stem cells for potential manipulation in bone tissue repair and regeneration.

O-21

SYNERGISTIC EFFECTS OF AMPK ACTIVATION AND TBK1 INHIBITION TO IMPROVE METABOLIC HEALTH AND INSULIN SENSITIVITY IN OBESE MICE

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Obesity and type 2 diabetes have emerged as global health crises. The activity of AMP-activated protein kinase (AMPK), a key regulator of energy homeostasis, is diminished in both obese mice and patients. This reduction is partly attributed to the inhibitory role of TANK-binding kinase 1 (TBK1) during metabolic stress, where TBK1 suppresses AMPK through a feedback loop, limiting its catabolic effects. We hypothesized that combining an AMPK activator with TBK1 inhibition would enhance AMPK activity and promote greater energy expenditure compared to either intervention alone. In this study, mice fed a 60% high-fat diet (HFD) for 12 weeks were treated with either 25 mg/kg amlexanox (AMX), 100 mg/kg AICAR, or a combination of both for 21 days. Our results show that the combination of AMX and AICAR significantly reduced body weight and improved insulin sensitivity in obese mice. Furthermore, the combination therapy enhanced AMPK activity and more effectively suppressed pro-inflammatory and lipogenesis-related gene expression compared to either AMX or AICAR alone. These findings suggest that targeting both AMPK activation and TBK1 inhibition could provide a more effective approach to increasing energy expenditure and improving metabolic health in obesity.

O-22

UNRAVELING THE INTRACELLULAR ACTION OF ANTIMICROBIAL PEPTIDE A11 THROUGH PROTEOMIC ANALYSIS IN *ACINETOBACTER BAUMANNII*

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Antimicrobial peptides (AMPs) are promising therapeutic agents for treating infections caused by drug-resistant bacteria due to their potent antimicrobial activity and low tendency to induce bacterial resistance. While AMPs are traditionally thought to target bacterial membranes, increasing evidence suggests they employ multiple mechanisms to achieve bacterial death. This study investigates the antibacterial mechanism of A11, a potential AMP, against *Acinetobacter baumannii*, a leading cause of hospital-acquired infections (HAIs) and a serious global health threat. We found that A11 induces significant membrane depolarization, as shown by flow cytometry and electron microscopy, but causes minimal membrane lysis, indicating a predominantly non-lytic killing mechanism. Confocal microscopy revealed rapid intracellular penetration of A11 peptide, further supporting this hypothesis. Quantitative proteomics analysis demonstrated that A11 disrupts critical cellular functions in *A. baumannii*, including energy metabolism, protein homeostasis, fatty acid synthesis essential for membrane integrity, and biofilm formation. Additionally, A11 impairs stress responses, DNA repair, and signal transduction processes necessary for bacterial survival. A11 also exhibited robust activity against multidrug-resistant (MDR) and extensively drug-resistant (XDR) clinical isolates of *A. baumannii*, with synergy observed when combined with levofloxacin and minocycline. Importantly, A11 showed a low propensity for inducing bacterial resistance. These findings underscore the therapeutic potential of A11 as a novel antimicrobial agent against drug-resistant *A. baumannii* and highlight the need for further investigation.

O-23

GLYCOLIPID BIOSURFACTANT AND ITS POTENTIAL APPLICATIONS IN BIOMEDICINES

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Withdrawn

O-24

GENOME PERSISTENCE & SURVIVAL OF CLOSTRIDIUM PERFRINGENS FROM ICHTHYOFAUNA OF DAL LAKE HIMALAYA - A POSSIBLE FUTURE PUBLIC HEALTH HAZARD

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Clostridium perfringens is one of the most prolific toxin-producing bacteria, an indicator of fecal contamination in aquatic habitats. This bacterium is not known to cause any disease in fish but its importance in fish is primarily due to the health hazards to humans, as *C. perfringens* has been found to be the causative agent of many outbreaks of food poisoning associated with the consumption of fish and its products. Aquatic resources of Kashmir are in a serious state of eutrophication-particularly cultural, more pronounced in Dal Lake, which has witnessed an increase in food borne pathogens. We aimed to investigate the presence of different toxinotypes of *C. perfringens* in commonly consumed fishes of Kashmir. Collection of samples. Isolation and identification by culture & staining. DNA extraction and PCR using species-specific 16S rRNA gene amplification, The toxinotyping of *C. perfringens* isolates by multiplex-PCR targeting six toxin genes (*cpa, cpb, ɛtx, ıtx, cpb2* and *cpe*). Phylogenetic analysis and antibiotic sensitivity tests. All the 45 isolates from water and 37 isolates from fish (21 from scale-carp and 16 from snow-trout) were found to carry *cpa* gene alone as a major toxin gene and thus were designated as toxinotype A. None of the isolates carried *cpb, ɛtx, ıtx, cpb2* or *cpe* genes indicating the absence of *C. perfringens* toxinotypes B, C, D or E in the water and fish samples of the Dal Lake. The prevalence of *C. perfringens* was slightly higher in scale-carps (35%) compared to snow-trouts (26.6%). Phylogenetic analysis of the cpa toxin genes of *C. perfringens* revealed 95% to 98% homology with corresponding GenBank published sequences and close relationship with corresponding AA sequences of *C. perfringens* strains reported from India, Egypt and China. Presence of *C. perfringens* toxinotype A in fish for the first time in the Dal Lake of the Kashmir valley and implied that fish may be a possible source of *C. perfringens* type A intoxication to humans through food chain posing a serious public health concern. The antibiogram pattern of the *C. perfringens* type A isolates revealed a higher antimicrobial resistance to amikacin, ceftriaxone, metronidazole, norfloxacin and tetracycline.

O-25

TOXICOLOGICAL IMPACTS OF CLOFIBRIC ACID, DICLOFENAC AND IBUPROFEN ON FISH: AN ENVIRONMENTAL HEALTH ISSUE

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The hotspot pollutant, pharmaceutical drugs (PDs) have extensively been produced and prescribed to cure and control various types of human and animal diseases, and against many health issues. The large-scale production, widespread use, disposal of used and expired medicines, discharge of domestic waste waters, excretion via water and sewage treatment systems are the main source of continuous input of PDs in the aquatic ecosystem. In recent times, the presence of these active ingredients and their metabolites has widely been detected in various segments of the environment (sewage effluents, ground water, surface water, drinking water and estuaries) at concentrations ranging from μg/L to ng/L. Such lower-level occurrence of PDs may pose a serious threat to the aquatic systems and organisms. Most of the available literature envisage their level of occurrence and fate. Nevertheless, their ecotoxicological impacts, and behavioural and physiological responses of aquatic animals exposed to different types of PDs are meager. In addition, little toxicological research has sought to integrate the behavioural effects of PDs with physiological processes. Consequently, the current study was aimed to investigate the toxicological impacts of most commonly used PDs namely Clofibric acid (CA), Diclofenac (DCF) and Ibuprofen (IB) on the freshwater fish, *Cirrhinus mrigala* (one of the Indian major carps) treated with the environmental concentrations (1, 10 and 100 μg/L) under short (96 hrs) and long-term (35 days) exposures. In this study, the behavioral responses and physiological indicators such as hormonal (T_3, T_4 and TSH), enzymological (GOT, GPT, LDH and Na^+/K^+- ATPase), ionoregulatory (Na^+, K^+ and Cl^-), biochemical (glucose, protein and cholesterol) and hematological (RBCs, WBCs and Hb) were investigated. The results obtained from this work showed that significant ($P < 0.05$) responses were observed in all parameters studied in fish exposed to CA, DCF and IB including 1 μg/L, and recommends the alterations of these parameters can be used as potential biomarkers; and useful in monitoring and understanding of pharmaceutical toxicity in aquatic environment.

O-26

EXPLORING AND CHARACTERIZING THE BIOSYNTHETIC MACHINERIES FOR SYNTHESIZING FUNGAL NATURAL PRODUCTS

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Natural products have been pivotal in advancing chemistry and drug discovery, offering diverse scaffolds and semisynthetic derivatives that give rise to new chemical entities with various biological activities and pharmacological potential. The recent surge in genome sequencing has accelerated the identification of genes and enzymes involved in secondary metabolite biosynthesis. Biocatalysts in green chemistry present significant benefits, such as reducing the need for hazardous reagents and minimizing energy consumption under mild conditions. However, designing biocatalysts for specific reaction pathways or tailoring chemical backbones remains a formidable challenge. To expand the repertoire of biosynthetic enzymes, we report the identification and characterization of biosynthetic gene clusters responsible for the synthesis of terpenoids in Ascomycota and Basidiomycota fungi. These findings not only offer new insights into natural product biosynthesis but also create opportunities for enzymatic derivatization in synthetic biology.

O-27

IN VITRO SCREENING FOR ANTI-VIRAL AND VIRUCIDAL EFFECTS AGAINST HIV-1 RT AND SARS-COV-2 BY PHENYLAMINO-PHENOXY-QUINOLINE

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The primary focus of this study was on the design, synthesis, and preliminary evaluation of the potential biological activity of novel quinoline compound derivatives as antiviral agents targeting the reverse transcriptase (RT) enzyme of HIV-1. The novel quinoline derivative discovered in this study was created utilizing a molecular hybridization technique consisting of pharmacophore templates from current HIV-1 RT inhibitors such as nevirapine, efavirenz, and rilpivirine. The designs suggested the synthesis of quinoline derivatives to incorporate the phenyl-amino and phenyl-oxy as substituents in the core structure of quinoline. The investigation indicated that the presence of substituents at positions 2 and 4 of the quinoline core structure is beneficial. Moreover, the investigation revealed that the incorporation of nitrogen atoms into the synthesized compounds, particularly in the side chain, serves as an enhancement to their biological efficacy in inhibiting the reverse transcriptase (RT) enzyme of HIV-1. These compounds demonstrate effective inhibitory activity against HIV-1 RT, and molecular docking studies exhibit strong interactions of these compounds with crucial key amino acids of HIV-1 RT, including LYS101, HIS235, and TYR318, which are required for their inhibitory effects. The compounds are highly effective, with IC_{50} values equivalent to conventional medicines for HIV-1 RT. Furthermore, it was shown that these synthesized compounds have specific toxicity to cancer cells while not being potentially dangerous to normal cells. Additionally, the quinoline derivatives have been investigated and assessed for their ability to inhibit the SARS-CoV-2 virus, specifically focusing on the SARS-CoV-2 main protease (M^{pro}), which is crucial for viral reproduction. The methods employed in this study included molecular docking, molecular dynamics simulations, cytotoxicity testing, and antiviral activity assays. The findings of this study demonstrate that quinoline derivatives and several current medications (ritonavir, entecavir, nimatrelvir, and paxlovid) exhibit efficient interactions with the active site of M^{pro} via hydrogen bonding, hydrophobic interactions, and pi-sulfur interactions. This study emphasizes the potential of quinoline derivatives as multifunctional antiviral agents capable of targeting both HIV-1 reverse transcriptase and SARS-CoV-2. Their ability to interact with crucial viral proteins and their low cytotoxicity to normal cells warrant the potential for further developments as antiviral and anticancer medications.

O-28

GENOME PERSISTENCE & SURVIVAL OF CLOSTRIDIUM PERFRINGENS FROM ICHTHYOFAUNA OF DAL LAKE HIMALAYA - A POSSIBLE FUTURE PUBLIC HEALTH HAZARD

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The uncontrolled growth of cells characterizes cancer. It is a primary cause of death worldwide, and considerably impacts mortality rates in low- and middle-income countries. Predictions show that cancer deaths will increase from 4 to 8 million by 2002 to 2030 due to population growth and demographic changes. Recently, there has been a significant rise in the number of patients undergoing chemotherapy. Given the toxicity of cytotoxic agents to humans, including patients and healthcare professionals, it is essential to develop reliable analytical methods for examining these compounds. Electrochemical sensors are evidenced to be excellent tools for the detection of antineoplastic agents. The current study presents a remarkable, simple, and straightforward protocol for producing polyvinylpyrrolidone functionalized strontium oxide nanoparticles (PVP-SrO NPs). The synthesized PVP-SrO nanoparticles were applied as a sensor to detect vinblastin sulfate (VNB) (an anticancer drug). The synthesized PVP-SrO NPs were characterized using different characterization techniques, such as UV-visible spectroscopy, FTIR, XRD, SEM, and EDS to confirm their functionality, phase purity, surface morphology, and elemental composition. The electrochemical behavior of the fabricated PVP-SrO sensor was investigated by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV). Remarkably, the electrochemical sensor exhibited a wide linear dynamic concentration range for the vinblastine sulfate, ranging from 0.05 µM to 60 µM. In addition, it exhibits a low detection limit (0.005 µM), and a limit of quantification (0.017 µM). The proposed sensor possesses excellent sensitivity and selectivity. In addition, the capabilities of the PVP-SrO sensor can be successfully used for monitoring vinblastine sulfate (VNB) in blood serum samples with a satisfactory level of recovery.

O-29

UNRAVELING HEPARAN SULFATE–SARS-COV-2 SPIKE PROTEIN INTERACTION: STRATEGY TO DEVELOP NOVEL VIRAL INHIBITORS

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection begins with the recognition of cellular heparan sulfate (HS) by the receptor-binding domain of the spike protein (S1-RBD). This event facilitates the adhesion of viral particles to the cell surface and promotes the subsequent binding of S1-RBD to the angiotensin-converting enzyme 2 (ACE2) receptor, triggering the fusion of viral and host cell membranes. Heparin and other HS mimetics have been shown to experimentally inhibit the attachment and invasion of SARS-CoV-2 in Vero cells. This study aims to elucidate the structural basis of the interaction between HS and S1-RBD using ligand-based NMR techniques and computational methods, with a focus on how this interaction is influenced by the genetic evolution of the virus. Synthetic HS oligosaccharides were used as molecular probes to investigate the binding of HS to selected S1-RBD variants, including those from the Wuhan and Omicron strains. Saturation transfer difference (STD) NMR and transferred nuclear Overhauser enhancement (trNOE) experiments were employed to map the binding epitope and define the conformation of the ligands in the protein-bound state. Additionally, docking and molecular dynamics (MD) simulations revealed the contacts and intermolecular forces that drive the ligand-protein interactions, providing detailed 3D models of the complexes. Key amino acids – R346, N354, R355, K356, R357, R466, K444 – were identified as principal binding sites for HS, leaving flexible loops of S1-RBD available to engage ACE2. Our data indicate that HS oligosaccharides interact with S1-RBD in multiple modes, characterized by low specificity and various orientations of the ligands on the protein surface. The evidence of multiple binding modes, which enhances the initial attachment of viral particles to the cell surface, supports a highly dynamic interaction between HS and S1-RBD. This low-specificity interaction is thought to facilitate the diffusion of the virus toward the ACE2 receptor, enhancing its invasion efficiency. Our results suggest that highly specific inhibitors may not be optimal against the spike protein. Instead, HS-based oligosaccharides with high affinity, including multivalent compounds, may be required to inhibit viral entry. This research provides valuable insights into the early mechanisms of SARS-CoV-2 infection, guiding the development of novel therapeutic strategies.

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E. ENVIRONMENTAL HEALTH ISSUES

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A.
DISEASES OF PUBLIC HEALTH CONCERNS (GENETIC DISEASES, CANCER)

PA-01

APOPTOSIS INDUCTION AND MOLECULAR DOCKING OF 3,4-DIHYDRO-LACTUCIN FROM *MICROBISPORA ROSEA* AL22 WITH ANTICANCER PROPERTIES

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Tetrazolium-based cell proliferation assays using MDA-MB-231 and HeLa cells revealed that 3,4-dihydro-lactucin (3,4-DHL), a compound isolated from *Microbispora rosea* AL22, possesses anticancer properties. Apoptotic cell death was observed in 3,4-DHL-treated cells. Lactucopicrin, a related compound, reportedly exerts anticancer activity against different cancer types. However, data on the anticancer mechanism of lactucins are limited. This study aimed to investigate apoptosis induction in MDA-MB-231 cells treated with 3,4-DHL. Morphological changes, mitochondrial membrane potential (MMP), and apoptosis induction in MDA-MB-231 cells treated with 3,4-DHL were investigated. Furthermore, molecular docking and absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis of anti-apoptotic proteins were performed to determine the effector mechanism of 3,4-DHL. 3,4-DHL induced cytotoxicity at a half-maximal inhibitory concentration of 37.62 µg/ml, along with various morphological alterations in apoptotic and viable cells. Furthermore, 3,4-DHL-treated cells showed loss of MMP, intensity of annexin V–fluorescein isothiocyanate staining, and caspase 3 and 8 activities. Molecular-docking studies demonstrated that 3,4-DHL should bind to the active site of various anti-apoptotic proteins, forming stable complexes. Our findings revealed that 3,4-DHL has great potential to be used as an apoptosis-inducing agent in cancer therapy. However, further in vivo confirmation is required in evaluation of 3,4-DHL as an anticancer agent in cancer chemotherapy.

PA-02

KINASE LIBRARY SCREENING IDENTIFIES IGF-1R AS AN ONCOGENIC VULNERABILITY IN CHOLANGIOCARCINOMA STEM-LIKE CELLS

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Intrahepatic cholangiocarcinoma (iCCA) is a highly aggressive cancer of the peripheral bile ducts and is recognized by the abundance of cancer stem-like cells (CSCs) within the tumor mass. While CSC markers in iCCA are well-defined, the molecular vulnerabilities of this subpopulation remain elusive. The 96-well, three dimensional (3D) tumorsphere culture was adapted from a well-established CSC model, validated for CSC markers through gene expression analysis. Kinase library screening was then conducted to reveal potential oncogenic vulnerable pathways. RNA interference was utilized to stably silence the candidate gene in three iCCA cell lines and its impact on iCCA cell proliferation and tumorsphere formation efficiency (TFE) was evaluated. Kinase inhibitor library screening identified the top 50 kinase inhibitors crucial for tumorsphere viability, with 11 inhibitors targeting the IGF-1R/PI3K/AKT axis. Further dose-dependent analysis of the top 'hit' inhibitors confirmed IGF-1R as the candidate molecule. Upon stably silencing of IGF-1R, all three iCCA cell lines exhibited decreased AKT activation, impeded proliferation and reduced TFE, indicating a decline in CSC subpopulations. IGF-1R plays a critical role in maintaining iCCA-stem like cell populations. General significance: Our data highlight the potential utility of IGF-1R as a prognostic marker of iCCA and a therapeutic target for eliminating its CSC subpopulation.

PA-03

INHIBITION OF BREAST CANCER CELL MIGRATION AND INVASION BY A NOVEL NEUTRALIZING MONOCLONAL ANTIBODY TARGETING ADAM9

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Breast cancer is a leading cause of mortality in women. ADAM9, a transmembrane protein, is overexpressed in breast carcinoma that promotes cancer progression. Therefore, targeting ADAM9 with immunotherapy may inhibit breast cancer cell migration and invasion. This study evaluated a novel monoclonal antibody (mAb), which binds to the ADAM9's cysteine-rich domain and has previously been shown to inhibit oral cancer cell invasion, for its effects on breast cancer cell migration and invasion. ADAM9 expression in SK-Br3 and MCF-7 breast cancer cell lines was detected by immunoblotting, immunofluorescence, and flow cytometry. The effects of mAb on cell viability, migration, and invasion were assessed by the alamarBlue™, wound healing, and Transwell® invasion assays, respectively. The mAb successfully detected ADAM9 expression in both cell lines via immunoblotting, consistent with the results of using a commercial anti-ADAM9 mAb. Immunofluorescence revealed ADAM9 localization in the cytoplasm and on the membrane, compared to no ADAM9 staining by the isotype control. Flow cytometry did not detect ADAM9 expression. The mAb showed no significant toxicity up to 72 hours but significantly inhibited cell migration and invasion, particularly in MCF-7 cells at 10 μg/ml ($p < 0.05$), compared to the isotype control. This novel mAb effectively inhibits breast cancer cell migration and invasion in vitro, suggesting its potential use in breast cancer immunotherapy.

PA-04

GENE ALTERATIONS DETECTION IN THAI PATIENTS WITH SOLID TUMORS

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Next-generation sequencing (NGS) is a transformative technology for DNA and RNA sequencing, enabling rapid detection of genetic variants and mutations across extensive genomic regions, or even entire genomes. This study focused on the clinical application of NGS, combined with real-time PCR, to detect gene alterations in 20 solid tumor cases at Chulabhorn Hospital between February 2022 and August 2023. The cases included five cancer types: lung (16 cases), breast (1 case), sarcoma (1 case), duodenum (1 case), and one unknown type. Among the lung cancer patients, 11 were male, 5 were female, and 6 of the males had a smoking history. The most common gene alterations observed were amplifications, heterozygous deletions, and missense mutations. Specifically, *EGFR* (22%), *TP53* (22%), and *PIK3CA* (9%) were the most frequently altered genes in lung cancer cases. This study highlights the effectiveness of NGS and real-time PCR in identifying critical gene alterations across various cancer types, particularly in lung cancer, where these genetic changes are essential for guiding treatment decisions. The use of multigene panels proves to be a robust method for revealing genomic changes in solid tumors, aiding in the development of personalized cancer therapies.

PA-05

PREVALENCE OF *JAK2*^{V617F} MUTATION IN POLYCYTHEMIA VERA AND ESSENTIAL THROMBOCYTHEMIA PATIENTS AT CHULABHORN HOSPITAL

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The JAK2 protein plays an important role in controlling the production of blood cells. Mutations of JAK2 cause the cell to rapidly divide, producing too many of one cell type. *JAK2*^{V617F} mutation is present in most patients with polycythemia vera (PV), essential thrombocytosis (ET) and primary myelofibrosis (PMF). This study, we analyzed the samples by allele-specific polymerase chain reaction (PCR) to determine the *JAK2*^{V617F} mutational status in 617 patients with PV (n=392) and ET (n=225). The main clinical and laboratory data at the time of initial diagnosis were collected for analysis. The results showed the increase of WBC, platelet count (Plt), and hematocrit (Hct) levels associated with *JAK2*^{V617F} mutation. The patients with PV had higher hemoglobin (Hb), WBC and Hct than ET patients but in contrast to Plt. Moreover, mutation allele burden was correlated to disease subtypes and patient's age. Patients with PV had higher allele burden more than ET. The investigation of the relationship between age and *JAK2*^{V617F} allele burden found that the patients older than 50 years old had high *JAK2*^{V617F} allele burden. However, this research studied only in Chulabhorn hospital's population. Further studies in the other population of patients might provide more beneficial information.

PA-06

CORRELATION OF HER-2 TESTING METHODS BY IMMUNOHISTOCHEMISTRY AND FLUORESCENCE *IN SITU* HYBRIDIZATION IN CHULABHORN HOSPITAL

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PHuman epidermal growth factor receptor2 (HER2) is an important prognostic and predictive marker of response for therapeutic practice for breast cancer patients. Approximately 15-20 % of breast cancer has HER2 gene amplification. HER2 amplification is a critical prognosis factor and response to HER2-targeted therapy. Screening for HER2 status can be performed by immunohistochemistry (IHC) methods. The HER2 gene amplification can be detected by the Fluorescence in situ Hybridization (FISH) technique in terms of HER2/CEP17 ratio and HER2/nucleus according to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2018 version. In this study, we would like to analyze the correlation between HER2-IHC status and FISH-HER2 expression. We enrolled female patients diagnosed with breast cancer at Chulabhorn Hospital between 1 January 2022 and 31 December 2023. The patients have histologically confirmed invasive ductal carcinoma and have HER2-IHC stains performed, which were interpreted per 2018 CAP/ASCO Update recommendations. The HER2-IHC results of equivocal (score 2+) and positive (score 3+) were later confirmed with FISH for HER2 testing. Patients with negative HER2-IHC results (score 0 and 1+) were not required for FISH for HER2 testing and were excluded. 263 patients were categorized into two groups according to HER2-IHC staining results. 192 patients were classified in the equivocal (score 2+) and 71 in the positive (score 3+). All patients had HER2 status confirmation by HER2-FISH testing. Of 192 patients in the equivocal (score 2+) HER2-IHC staining group, 138 exhibited negative FISH results, and 54 confirmed FISH-positive results. 71 patients revealed positive (3+) HER2 FISH testing group. Our study revealed statistical significance in the relationship between positive (3+) IHC and FISH for HER2 testing with a correlation rate of 100% (r_s=0.639, $p<0.001$) and 28% in equivocal (2+) IHC groups. In our study, 100% (71/71) of HER2 IHC 3+ with reflex ISH-positive were categorized as classical HER-2 amplification or dual-probe ISH group 1. Based on the 2018 ASCO-CAP recommendations, our study confirmed that the ISH testing for HER2 IHC-positive 3+ patients is unnecessary, making the patient receive potential and reducing cost from treatment and side effects. For HER2 equivocal result (2+ stained), the probability of HER2 overexpression was 28 percent.

PA-07

THE ASSESSMENT OF CONCORDANT OF HER2 STATUS USING FLUORESCENCE IN SITU HYBRIDIZATION (FISH) TECHNIQUE WITHIN THE ASCO/CAP GUIDELINE BETWEEN 2013 AND 2018

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Breast cancer is the most common cause of cancer death in women worldwide. The human epidermal growth factor receptor 2 (HER2) gene plays a crucial role in breast cancer. Most breast cancer patients found overexpression of the HER2 protein or HER2 amplification in 15–30% of invasive breast cancers. Fluorescence in situ hybridization (FISH) is regarded as a standard method for determining the abnormality of HER2 overexpression in breast cancer. Assessment of HER2 status is an essential step for prognosis, managing the treatment, and choosing chemotherapy for breast cancer efficacy. Moreover, recently, the HER2 overexpression patient was found to respond to HER2-targeted therapy (Anti-HER2 therapy). In 2018, the American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP) was an improved version for testing HER2 in breast cancer in 2013. They try to decrease equivocal HER2 cases because equivocal case results are challenging clinical treatment. After all, anti-HER2 therapy in equivocal patients is always unpredictable. Furthermore, in 2023, there will be ASCO-CAP HER2 testing guideline updates and no changes in the 2018 ASCO-CAP recommendations. This Update affirms 2018 ASCO-CAP guideline recommendations. This research assesses the concordant HER2 status using the FISH technique within the ASCO/CAP guideline between 2013 and 2018. The result found the HER2 status using the FISH technique within the ASCO/CAP guideline between 2013 and 2018 was concordant. Furthermore, 7 of 75 samples (5.25%) changed from equivocal. This study can conclude that our laboratory can use the ASCO/CAP 2018 guideline for FISH analysis, which will allow the patient to receive potential treatment and reduce costs, unnecessary time, and side effects.

PA-08

DETECTION OF *LEGIONELLA PNEUMOPHILA* AND ITS AMOEBA HOST IN WASTEWATER TREATMENT PLANTS USING DROPLET DIGITAL POLYMERASE CHAIN REACTION

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Legionella pneumophila is the major causative agent of Legionnaires' disease (legionellosis, severe, acute pneumonia). It presents a significant health risk, especially for the elderly and people with a weakened immune system or underlying morbidities. Inhalation or aspiration of contaminated water or soil aerosols (air-borne microparticles) are the major route of infection. Legionella species grow optimally inside free-living amoebae (FLA) to concentrations that increase risks to those who are exposed. Wastewater treatment plants (WWTPs) have been suggested to be hotspots for the emergence and dissemination of pathogens. Wastewater samples were collected from sixteen WWTPs in Bangkok, Thailand. Droplet digital polymerase chain reaction (ddPCR) using species-specific primers has been developed and was applied to detect *L. pneumophila* and FLA (*Acanthamoeba* spp. and *Vermamoeba vermiformis*) from the collected samples. Monitoring the co-occurrence of L. pneumophila and its amoeba host is of interest for water management and to avoid enrichment of Legionella.

PA-09

DEVELOPMENT OF A DROPLET DIGITAL PCR (DDPCR) ASSAY FOR DETECTION OF *LEGIONELLA* SPECIES

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Legionnaires' disease, primarily caused by *Legionella pneumophila* (LP), is a significant public health concern due to its potential for severe outbreaks. *Legionella* bacteria can proliferate in various environmental sources, including water systems and air conditioning systems, increasing the risk of infection. Rapid and accurate detection of Legionella species is crucial for timely disease management and prevention. Traditional culture-based methods often suffer from long processing times and limited sensitivity, which can delay outbreak identification and response. In this study, we developed a droplet digital PCR (ddPCR) assay for the sensitive and specific detection of *Legionella* species. The results demonstrated that the ddPCR method reliably detects low concentrations of *Legionella* with high accuracy. This assay offers potential advantages over conventional methods in terms of efficiency and robustness. Its high specificity and accuracy are particularly valuable for environmental sampling, which could facilitate earlier detection and improve outbreak management. This method has the potential to enhance public health surveillance and intervention strategies for *Legionella* species.

PA-10

FREQUENCY OF CALR MUTATION IN THAI MYELOPROLIFERATIVE NEOPLASM PATIENTS

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Myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) is a myeloid progenitor disorder driven by various gene mutations. Calreticulin gene (CALR) mutation was known as MPNs driver. To determine the frequency of CALR mutation variants in Thai MPNs patients, collected samples of blood/bone marrow of MPNs patients were analyzed by PCR and agarose gel electrophoresis at Chulabhorn Hospital between year 2020 to 2024. The prevalence of insertion and deletion variants in CALR exon 9 mutation were both 10% (8/82). However, CALR mutation was not found in PV patients. 3 of 48 (6.25%) ET patients were deletion subtype and 7 of 48 (14.58%) were insertion CALR mutations. In addition, 5 of 21 (23.81%) and 1 of 21 (4.76%) were identified as deletion and insertion CALR mutations in PMF patients, respectively. This data difference to the previous report presented that CALR mutations were found 46.9% in ET patients and 20.9% in PMF patients. The WBC count in CALR mutation positive ET patients is higher than CALR mutation negative ET patients. Its clinical importance needs to be further characterized

PA-11

CAPSAICIN EXHIBITS ANTI-METASTATIC ACTIVITY IN HUMAN NASOPHARYNGEAL CARCINOMA CELLS THROUGH THE MODULATION OF MTOR SIGNALING

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Nasopharyngeal carcinoma (NPC), an epithelial malignancy of the nasopharynx, is prevalent in Southeast Asia and Southern China. The prognosis of NPC is poor and local recurrence and metastasis often occur. Capsaicin (tran-8-methyl-N-vanillyl-6-nonenamide), a pungent ingredient of hot chili peppers, shows anti-cancer activities such as anti-proliferation and anti-metastasis. Currently, the role of capsaicin in cell metastasis of NPC is not well understood. In this study, we tested whether capsaicin has anti-metastatic property in NPC cell lines. Three NPC cell lines representing different histopathological classifications including well-differentiated (CNE1), poorly-differentiated (HONE1) and undifferentiated (NPC-TW06) cell lines, were treated with various concentrations of capsaicin compound. Cell viability, colony forming ability and cell migration and invasion were assessed by MTT, colony formation, wound healing and Boyden chamber assays, respectively. The alterations of signal transduction pathway involved in cell metastasis were measured by quantitative real-time polymerase chain reaction and Western blotting. Capsaicin suppressed cell proliferation in dose-dependent manner. It inhibited cell metastasis as shown by wound healing and cell migration assays and decreased the expressions of matrix metalloproteinases enzymes. In addition, the phosphorylation of mTOR was downregulated in capsaicin treated cells. Surprisingly, the phosphorylation of Akt, an upstream regulator of mTOR, was upregulated. Combination of capsaicin and rapamycin (mTOR inhibitor) treatments led to increase of anti-growth and anti-metastatic activities. Consequently, capsaicin has potential to be used as an optional therapeutic drug for improving the survival of NPC patients.

PA-12

TARGETING MITOTIC PROTEINS INDUCES G2/M ARREST AND APOPTOSIS IN CHOLANGIOCARCINOMA CELLS

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Cholangiocarcinoma (CCA) is a lethal cancer originating from the epithelial cells within the bile duct and ranks as the second most prevalent form of liver cancer in Thailand. Polo-like kinase 1 (PLK1), a protein serine/threonine kinase, regulates several steps in cell mitosis and is upregulated in several types of cancer, including CCA. Aurora kinase A (AURKA), another serine/threonine kinase, is also essential in mitotic regulation. This study aimed to investigate the effects of PLK1 and AURKA inhibitions in CCA cells. Four CCA cell lines developed from Thai patients, HuCCA1, KKU055, KKU100, and KKU213A, were treated with two PLK1 inhibitors, BI2536 and BI6727, and were transfected with small interfering RNA. The impact on cell proliferation, cell cycle distribution, and cell apoptosis was then analyzed. Results showed that BI2536 and BI6727 inhibited cell proliferation and caused G2/M-phase arrest in CCA cells. Additionally, the number of total apoptotic cells increased following PLK1 inhibition. The expression levels of mitotic proteins, aurora kinase A, phosphorylated PLK1 (T210), and cyclin B1, were augmented in PLK1-inhibited CCA cells. Inhibition of PLK1 also led to increased DNA damage, as determined by the upregulated levels of γH2AX and increased cleavage of poly (ADP-ribose) polymerase, an apoptotic marker. Furthermore, we explored the effects of targeting AURKA using two small-molecule inhibitors, alisertib and VX680, in CCA cell lines. Both inhibitors effectively suppressed CCA cell growth, with varying IC50 values across the cell lines and induced G2/M phase arrest and apoptosis. In conclusion, targeting PLK1 and AURKA provided promising results and presented potential as a candidate for targeted therapy in CCA.

PA-13

DEVELOPMENT OF A DROPLET DIGITAL PCR (DDPCR) ASSAY FOR DETECTION OF LEGIONELLA SPECIES

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The Thailand Initiative in Genomics and Expression Research for Liver Cancer (TIGER-LC) has created a biorepository and database for Thai hepatocellular carcinoma and intrahepatic cholangiocarcinoma (iCCA) patients. Prior research identified shared prognostic molecular subtypes among Thai and Asian liver cancer patients. To further develop a multi-omics platform and explore subtype-specific epigenetic landscapes, methylome profiling was conducted on 51 Thai iCCA cases using the Infinium MethylationEPIC Array. A probe-based analysis identified 2,547 common differentially methylated probes (DMPs) between tumor and normal tissue, as well as between C1 and C2 tumor subtypes. These DMPs distinguished tumor from normal tissue and differentiated C1 from C2 subtypes. Of these, 17% were hypermethylated and 83% were hypomethylated in the C1 subtype. Hypermethylated probes were mainly located in CpG islands and transcription regulatory regions, potentially affecting gene expression. Pathway analysis showed 207 genes linked to hypermethylation involved in transcription regulation, while 1,067 genes linked to hypomethylation were associated with diseases like cancer and type 2 diabetes. These findings expand the multi-omics data platform for Thai iCCA cases, revealing subtype-specific DNA methylation patterns. Ongoing research aims to integrate methylome data with gene expression, somatic mutations, and clinical outcomes to offer deeper insights into the molecular mechanisms and prognostic implications of iCCA.

PA-14

URINARY BIOMARKERS FOR INTRAHEPATIC CHOLANGIOCARCINOMA USING METABOLOMICS

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Intrahepatic cholangiocarcinoma (ICC) is a highly aggressive liver cancer with poor prognosis and limited early detection options. The lack of specific biomarkers complicates early diagnosis. This study explores urinary biomarkers as a non-invasive diagnostic method, aiming to distinguish ICC patients from healthy controls using a global untargeted metabolomics approach. Urine samples from 118 ICC patients and 62 healthy controls were collected under the TIGER-LC consortium, matched for age, sex, BMI, and region. Using the Metabolon HD4 platform with UPLC-MS/MS, 1134 metabolites were analyzed. Two-way ANCOVA and fold change analysis identified 141 significant metabolites, which were then assessed in combinations of three with the Random Forest algorithm to classify ICC patients from healthy controls. Among the 141 significant metabolites, the highest AUC for a single metabolite distinguishing ICC patient from healthy controls was 77%. Evaluating combinations of three metabolite using the Random Forest algorithm, 26 combinations achieved an AUC over 90%, with the highest at 92.5%. The pathways associated with these high-AUC metabolites combinations were mainly xenobiotics, followed by amino acid and lipid pathways. These findings suggest that these metabolite combinations could serve as effective biomarkers for ICC, paving the way for non-invasive diagnostic tools.

PA-15

MANGANESE INDUCED INFLAMMATION IN ORAL CANCER CELLS

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Manganese (Mn) is an essential micronutrient responsible for normal development, metabolism and against free radicals. However, previous studies had been reported that Mn modulated migration and invasion of cancer by increasing the inflammatory responses through various cytokines. Oral squamous cell carcinoma (OSCC) is a common head and neck cancer, which globally contributes to 377,713 cases in 2020, with a majority occurring in Asia and will reach by approximately 40% in 2040, with a high recurrence rate. Nevertheless, the underlying mechanism of manganese on OSCC is not well understood. In the present study enzyme-linked immunosorbent assay (ELISA) and Western immunoblotting were employed to investigate the molecular mechanism of OSCC exposure to Mn by using SCC25 cells. Exposure to manganese with the concentrations between 10 to 1000 μM for 72 hours significantly decreased the viable cells, meanwhile the concentrations below 10 μM did not significantly change of viable cells and were selected for further experiments. We also found that interleukin-6 (IL-6) secretion was significantly increased by Mn exposure in concentration-dependent manners. Moreover, protein expression analysis showed that SCC25 cells exposure to Mn increases the phosphorylation of JAK2/STAT3. Altogether, these findings revealed that Mn can increase inflammatory cytokines causing inflammation in oral cancer.

PA-16

EVALUATION OF THE ANTICANCER POTENTIALS OF SUGAR-RICH FRACTION AND CRUDE AQUEOUS EXTRACT OF *MORINGA OLEIFERA* SEEDS IN ESTROGEN RECEPTOR-POSITIVE AND TRIPLE-NEGATIVE BREAST CANCER CELLS

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In this study, the cytotoxic potentials of Moringa oleifera seeds polysaccharide extracts: sugar-rich fraction (MSRF) and crude aqueous extract (MCE) were investigated. Previously studied MCE revealed anticancer properties but had low sugar content. In this study MCE extract and obtained polysaccharide fraction with enhanced sugar content (MSRF) were evaluated for the anticancer potential against estrogen receptor-positive (MCF-7) and triple-negative (MDA-MB-231) breast cancer cells. The cytotoxicity of the extracts was examined via MTT and clonogenic assays. Cell morphology was examined following treatment with 0, 0.5 IC50 and IC50 concentration of the extracts with a light microscope. Cell cycle progression and apoptosis were studied by flow-cytometry, while lipid accumulation was assessed by Nile-red staining. MSRF shows a 3.78-fold increase in sugar content compared to our previous crude polysaccharide extract, however MCE demonstrated higher cytotoxicity with lower IC50 values (28.00 ± 0.69 for MCF-7 and 33.76 ± 4.37 µg/ml for MDA-MB-231) over time. Both extracts significantly reduced clonogenic ability and disrupted cell morphology. They also showed prominent pro-apoptotic properties especially at the early apoptotic stage, and disrupted cell cycle progression, decreasing the number of cells in the G1 phase, they also caused an increase in MDA cells at the S phase. Furthermore, the extracts caused a significant reduction in lipid accumulation. In conclusion, both MCE and MSRF displayed significant anticancer potentials, with MCE shows greater cytotoxicity, making it a strong candidate for further investigation.

PA-17

LOCAL INVASION SUPPRESSOR IN INTRADUCTAL PAPILLARY NEOPLASM OF THE BILE DUCT TUMORS IDENTIFIED BY CLONAL EVOLUTION ANALYSIS

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Intraductal papillary neoplasm of the bile duct (IPNB) is a specific type of bile duct tumor. The histopathology is characterized by intraluminal papillary tumors with pseudostratified epithelium and crowded nuclei with hyperchromaticity. In IPNB, the cancer cell originates inside the bile duct space, and invades outside the bile duct into the nearby liver tissues, resulting in local invasion (LI-IPNB). IPNB is particularly interesting because it can be an appropriate disease model to investigate mutations in local invasion, the earliest step of cancer metastasis. This study aims to identify potential suppressor genes of LI-IPNB by comparing the somatic mutations of the IPNB and LI-IPNB tissues in individual patients. Ion Ampliseq targeted sequencing was performed for the identification of candidate genes with 11 pairs of macrodissected locally invasive IPNB and IPNB tissues without local invasion from the same patients. The clonal evolution analysis using Pyclone and ClonEvol software revealed an increase in accumulated variant allele frequency (VAF) of the protein-truncating variant RNF213 (c.6967C>T; p.Gln2323X; chr17: 78,319,102 [hg19], exon29), as the most common protein-truncating variant event in LI-IPNB samples (4/11 patients). AlphaFold2, an artificial intelligence (AI) system, was utilized to predict the human RNF213 wide-type and RNF213 mutant protein structures to confirm the loss-of-function mutant RNF213 Q2323X leading to protein-truncation by loss of AAA+ ATPase domain and Zinc finger (RIN-type and RZ-type) domains in an E3 ubiquitin ligase. In summary, we showed that loss-of-function of RNF213 may be a common and early genetic alteration in LI-IPNB tumors.

PA-18

METFORMIN MODULATES PANCREATIC STELLATE CELL MIGRATION AND CANCER STEMNESS IN 3D PANCREATIC DUCTAL ADENOCARCINOMA MODELS, SUGGESTING POTENTIAL FOR ENHANCED THERAPEUTIC EFFICACY

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Pancreatic ductal adenocarcinoma (PDAC) is characterized by a dense stroma that enhances its invasive properties. While metformin has been suggested as a potential adjuvant treatment to improve survival rates in PDAC patients, its mechanisms of action have been primarily studied in two-dimensional cell models. Additionally, pancreatic stellate cells (PSCs) present within the PDAC tumor microenvironment are a significant obstacle to effective drug therapy. In this study, we developed two types of co-culture models: i) a direct co-culture model, where patient-derived PDAC organoids were combined with PSCs in Matrigel™, ii) an indirect co-culture model using a trans-well system, with PDAC organoids at the bottom well and PSCs in the insert well. At a concentration of 10 μM, metformin significantly reduced PSC migration by downregulating matrix metalloproteinase-2 (MMP-2). In the 3D direct co-culture model, metformin also diminished the expression of cancer stemness-related genes. The observed reduction in PSC migration was linked to decreased MMP-2 levels, and knocking down MMP-2 in PSCs replicated this reduced migratory behavior. Furthermore, metformin's anti-migration effects were confirmed in a 3D indirect co-culture model with patient-derived PDAC organoids and primary PSCs. Metformin suppressed PSC migration by lowering MMP-2 levels and also reduced factors associated with cancer stemness. Additionally, oral administration of metformin (30 mg/kg) significantly inhibited the growth of PDAC organoid xenografts in immunocompromised mice. These findings suggest that metformin holds promise as an effective therapeutic option for PDAC. This research was supported by a National Research Foundation of Korea grant funded by the Korean government (NRF-2019-Global Ph.D. Fellowship Program, grant number NRF-2019H1A2A1076751 to S.H).

PA-19

EGFR MUTATIONS IN 592 THAI NSCLC PATIENTS

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The epidermal growth factor receptor (EGFR) is a crucial transmembrane tyrosine kinase receptor involved in cell division and apoptosis. Mutations in *EGFR* gene leading to EGFR protein overexpression are associated with various cancers and have become targets for emerging tyrosine kinase inhibitors (TKIs). These therapies are most effective in cancers with abnormal EGFR signaling. Non-small cell lung cancer (NSCLC) patients who carried somatic mutations in *EGFR* gene often present a good response to TKIs treatment, leading to a notable 60% response rate compared to conventional chemotherapy. Clinical guidelines suggest routine *EGFR* mutation testing in NSCLC patients to identify the patients who may benefit from TKIs treatment. Both tumor tissue and peripheral blood can be used for mutation detection. The plasma-based tests for circulating tumor DNA (ct DNA) are offering a non-invasive method that is an alternative way when tissue biopsy is impractical. In this study, we used real-time PCR to detect pathogenic variants (PVs) in *EGFR* gene. The 592 Thai patients with NSCLC comprising 449 solid tumor samples and 149 circulating tumor samples were analyzed. The real-time PCR were used to identify PVs in 261 patients (44%). The three most common of *EGFR* mutations in Thai patients are L858R (39.6%), exon 19 deletion (34.1%), and compound heterozygous of exon 19 deletion and T790M (6.7%), respectively. Accurate detection of *EGFR* mutations in tumor tissue is key for offering personalized treatment to cancer patients.

PA-20

SERUM AND URINARY GALECTIN-1 AS A BIOMARKER OF CHRONIC KIDNEY DISEASE PROGRESSION

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Galectins are a family of carbohydrate-binding proteins with an affinity for β-galactoside sugars. In humans, Galectin-1 is encoded by the LGALS1 gene and plays a crucial role in various biological processes, including immune response, inflammation, and cell death. While several studies have highlighted the correlation between Galectin-1 and various diseases, its relationship with chronic kidney disease (CKD) remains unexplored. This study investigated the potential of Galectin-1 as a biomarker for CKD progression by quantifying serum and urine samples from 250 participants in Khon Kaen province as part of the Chronic Kidney Disease Prevention in the Northeast of Thailand (CKDNET) project. The detection was carried out using indirect enzyme-linked immunosorbent assay (I-ELISA). Both serum and urine concentrations of Galectin-1 showed a significant negative correlation with the kidney function estimator, estimated glomerular filtration rate (eGFR) ($r = -0.4845$ and $r = -0.6455$, respectively, $p<0.0001$). Additionally, when comparing late-stage CKD (G4-G5) to early-stage CKD (G1-G3), Galectin-1 levels were higher ($p<0.0001$) in late-stage CKD, indicating promising diagnostic performance in identifying CKD stages. However, when comparing Galectin-1 levels between serum and urine, no significant correlation was found ($r = -0.01352$, $p = 0.8319$). In conclusion, we found a significant association between eGFR and Galectin-1 levels in both serum and urine, suggesting that Galectin-1 could serve as a potential biomarker for CKD progression, with both serum and urinary levels reflecting disease severity.

PA-21

VDA, VLE, AND VLI DEMONSTRATED ANTI-TUMOR ACTIVITY AGAINST A549 BY TARGETING THE JAK2/STAT3 PATHWAY

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Lung cancer remains a leading cause of cancer-related death worldwide in both men and women, largely due to drug resistance and disease recurrence. In addition, lung cancer is clinically silent and thus most patients are at an advanced stage at the time of diagnosis. The limited efficacy of current chemotherapies underscores the need for novel anticancer agents. This study demonstrated the anti-proliferative and apoptosis-inducing effects of three sesquiterpene lactones isolated from Gymnanthemum extensum—vernodalin (VDa), vernolepin (VLe), and vernolide (VLi)—on A549 human lung cancer cells. Sub-cytotoxic doses (maintaining >75% cell viability) of VDa, VLe, and VLi caused G0/G1 phase arrest in A549 cells, while cytotoxic doses led to G2/M phase arrest and apoptosis. Mechanistic studies suggest that the anti-tumor effects of VDa, VLe, and VLi are mediated via the JAK2/STAT3 pathway. Additionally, molecular docking confirmed hydrogen bonding interactions between the compounds and the FERM domain of JAK2 protein. These findings highlight the therapeutic potential of VDa, VLe, and VLi as promising candidates for lung cancer treatment.

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PA-22

THE NOVEL Q500X AND R619X IDUA MUTATIONS IN THAI PATIENTS AND THEIR POTENTIAL TREATMENT THROUGH AMINOGLYCOSIDE INDUCED PREMATURE STOP CODON READ-THROUGH MUTATIONS

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Hurler syndrome is a severe lysosomal storage disorder of Mucopolysaccharidosis type I (MPS I) caused by a deficiency of alpha-L-iduronidase (IDUA), leading to incomplete breakdown of the glycosaminoglycans heparin and dermatan sulfate in organs and tissues. In this study, we identified IDUA mutations in two unrelated Thai MPS I patients. The first patient was homozygous for the novel nonsense mutation c.1855C>T (p.R619X), while the second was compound heterozygous with the missense mutation c.223G>A (p.A75T) and the novel nonsense mutation c.1498G>T (p.Q500X). We investigated the potential effects of gentamicin, an aminoglycoside antibiotic, in COS-7 cells expressing IDUA with nonsense mutation transcripts. Premature stop mutations are the most common IDUA mutations, resulting in truncated protein production and consequent loss of activity. IDUA expression constructs containing the two nonsense mutations from this study (p.Q500X and p.R619X) and five previously reported mutations (p.Q70X, p.E299X, p.W312X, p.Q380X, and p.W402X) were expressed, and IDUA protein and activity were examined. Western blot and enzyme activity analysis revealed that gentamicin treatment significantly enhanced full-length IDUA expression and restored IDUA activity, compared to untreated cells, only in cells expressing cDNA with p.W312X, p.Q380X, p.W402X, and p.R619X mutations. This restoration by gentamicin was dose-dependent. Additionally, RNA sequence and secondary structure prediction suggested that the increased IDUA activity in gentamicin-treated COS-7 cells expressing these specific nonsense mutations may be associated with the surrounding mRNA sequence context and RNA secondary structure of mutant IDUA transcripts. Our findings demonstrate that gentamicin treatment of IDUA variants with nonsense mutation transcripts may be a promising option for personalized medicine through nonsense suppression in MPS I.

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PA-23

CHARACTERIZATION AND FUNCTIONAL STUDY OF A NOVEL CTSA VARIANT IN A FAMILY WITH LATE-INFANTILE GALACTOSIALIDOSIS ASSOCIATED WITH T-CELL DEFECTS

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Galactosialidosis (GS, OMIM# 256540) is a rare lysosomal storage disease with variable onset caused by a defect in protective protein/cathepsin A (PPCA) resulting in a combination of lysosomal β-galactosidase (GLB1; EC 3.2.1.23) and neuraminidase (NEU1; EC 3.2.1.18) deficiency. The late-infantile onset is characterized by developmental delay, visceromegaly, coarse facies, and cherry-red macula. Here, we present cases of late-infantile GS patients in Thailand, with affected members initially presenting with recurrent infections due to T-cell defects. The clinical features of LSD and cherry-red macula led us to perform lysosomal enzyme assays. Measurement of leukocyte enzyme showed impair activity of beta-galactosidase and neuraminidase. PPCA activity was undetectable and CTSA gene sequencing was identified a novel homozygous missense variant c.1307A>G resulting in substitution of glutamine by arginine at position 436. Exome sequencing did not identify variants causing primary immunodeficiencies. In vitro functional analysis was carried out to confirm the pathogenicity of the variant and explain its molecular mechanism.

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PA-24

THE ROLE OF ANKRD1-NF-KB-MAGE-A6 IN METASTATIC BREAST CANCER

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At present, there appears to be a gradual increase in the incidence of breast cancer over time. Cancer metastasis is the most serious problem of breast cancer and the estimated survival rate of patients after diagnosis of metastatic cancer is only 10–20%. Metastatic tumors are often diagnosed at late stages when the primary tumor has already started metastasis and become large in size. This study aims to investigate the novel biomarkers or candidate targets of metastatic breast cancer. We performed the experiments by using a microarray to compare low (MCF-7) and highly (MDA-LM-2 (LM-2)) metastatic breast cancer cell lines. The increasing of Ankyrin Repeat Domain 1 (ANKRD1) level in LM-2 cells was detected. Then we did both overexpression and knockdown of ANKRD1 in MCF-7 and LM-2 cell lines, respectively. The results showed that in ANKRD1-overexpressing cells, the ability of cell migration and invasion was increased, while the ANKRD1-knockdown cell line was reduced. These studies demonstrated that ANKRD1 is essential for both cancer migration and invasion. For In vivo studies, we did subcutaneous and tail vein injection of ANKRD1-knockdown and control cells in NOD.SCID mice, the results showed less cancer metastasis from the primary to the secondary site after injecting ANKRD1-knockdown cells. We performed immunohistochemistry using a human breast tissue array and found that high-grade tumors expressed high levels of ANKRD1 compared to low grade. To elucidate the underlying mechanism of ANKRD1, Next-Generation Sequencing, mass spectrometer, and immunoblotting in ANKRD1-knockdown and control cell lines were performed. The results indicated that the NF-κB and MAGE-A6 act downstream of ANKRD1. The present studies reveal an aspect of the precise molecular mechanism of ANKRD1 in cancer metastasis. These findings suggest that ANKRD1 might be a candidate biomarker as well as a targeted therapeutic for highly metastatic breast cancer.

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PA-25

IDENTIFYING AUTOANTIBODIES AGAINST NDE1, PYCR1, AND VIM COMBINED WITH CA19-9 FOR ENHANCED ICCA DIAGNOSIS

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Diagnosing intrahepatic cholangiocarcinoma (iCCA) is challenging due to its reliance on multiple invasive methods. This study aims to identify non-invasive autoantibody biomarkers that signal early immune responses, differentiating iCCA patients from healthy individuals (CTRs). We profiled autoantibodies from 26 serum samples (16 iCCAs and 10 CTRs) using protein microarrays containing 1,622 functional proteins. Statistically significant candidate autoantibodies were validated through a public RNA-seq dataset. Machine learning classification identified a diagnostic biomarker signature capable of distinguishing between the two groups. Differentially expressed autoantibodies were further evaluated for biological relevance through pathway analysis. We detected a range of autoantibodies that effectively differentiate iCCA from CTR. Using machine learning techniques, we identified a signature comprising three autoantibody biomarkers (NDE1, PYCR1, and VIM) in conjunction with the conventional carbohydrate antigen 19-9 (CA19-9) marker for iCCA detection. This combined signature demonstrated superior diagnostic performance with an AUC of 96.9%, outperforming CA19-9 alone (AUC: 83.8%). Our machine learning pipeline establishes a non-invasive blood-based biomarker signature by combining novel autoantibody biomarkers with CA19-9, aiming to improve the diagnostic accuracy of iCCA. This study introduces autoantibodies as complementary tools for routine iCCA screening, potentially revolutionizing iCCA diagnosis by increasing accuracy, reducing invasiveness, minimizing delays, and lowering mortality rat

PA-26

IDENTIFICATION OF NDRG1 AS A POTENTIAL TARGET FOR ANOIKIS RESISTANCE IN BREAST CANCER METASTASIS

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Metastasis is the primary cause of mortality in cancer patients, driven by mechanisms that enable cancer cells to survive after detachment from the extracellular matrix, a process known as anoikis resistance. Understanding the molecular mechanisms underlying anoikis resistance in cancer cells is critical for identifying potential therapeutic targets or biomarkers. In this study, we investigated the mechanisms of anoikis resistance in breast cancer by utilizing both attached and detached breast cancer cell culture models. We employed integrative transcriptomic and proteomic approaches to identify key genes and proteins showing significant changes in response to anoikis induction. While it is commonly observed that transcriptomic and proteomic datasets often show limited correlation, our analysis identified a consistent upregulation of N-myc downstream-regulated gene 1 (NDRG1) across both datasets. The upregulation of NDRG1 in anoikis-resistant breast cancer cells was further validated by Western blot analysis. Our findings suggest that NDRG1 may serve as a potential therapeutic target or biomarker for breast cancer metastasis. Further studies are needed to explore the functional role of NDRG1 in cancer progression and its potential for clinical applications.

PA-27

ELUCIDATING THE MOLECULAR MECHANISM OF IMPDH2 IN OSTEOSARCOMA METASTASIS

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Metastasis is the primary cause of death in osteosarcoma patients. A significant proportion of osteosarcoma patients who demonstrate resistance to chemotherapy tend to develop metastasis within a few years after their initial diagnosis. Anti-metastatic therapy represents a promising class of oncology drugs aimed at preventing metastasis, thereby potentially reducing the mortality rate in patients who do not respond to conventional treatment. However, the development of such therapies is hampered by the complex mechanisms of metastasis, which are specific to each cancer type. Therefore, an important step in developing effective anti-metastatic drugs is the identification of appropriate molecular targets. Our previous study identified the enzyme IMPDH2 as a prognostic marker associated with shorter metastasis-free survival in osteosarcoma. In the current study, we aimed to elucidate the molecular functions of IMPDH2 associated with metastatic processes. Our findings demonstrated that IMPDH2 knockout cells exhibit reduced rates of invasion, migration, and anchorage-independent growth. These effects were not observed in IMPDH1 knockout cells, suggesting a unique role for the IMPDH2 isoform in the metastasis of osteosarcoma. Additionally, treatment with exogenous guanosine did not rescue the effects observed in IMPDH2 knockout cells, suggesting a novel role for IMPDH2 beyond its primary function in GTP biosynthesis. Proteomic profiling of IMPDH1 knockout, IMPDH2 knockout, and wild-type cells further revealed the activation of the integrin signaling pathway specifically in IMPDH2 knockout cells. In conclusion, this study provides evidence for the significant role of IMPDH2 in osteosarcoma metastasis, mediated at least in part through the integrin signaling cascade. Further investigation into the therapeutic potential of targeting IMPDH2 may justify clinical trials to explore its efficacy as an anti-metastatic agent in osteosarcoma.

PA-28

LABEL-FREE IMPEDIMETRIC IMMUNOSENSORS FOR DETECTION OF CERVICAL CANCER BIOMARKERS

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Cervical cancer (CC) is the fourth-leading cause of cancer-related death in women worldwide. The highest rates of CC incidence and mortality are in low- and middle-income countries that poor access to screening programs. The current screening methods for CC are mainly based on cytology and HPV testing, both of which have some limitations; an invasive, low sensitivity, and specificity. Therefore, there is a need to establish the minimally invasive approaches for diagnosis. Biomarkers for the detection of CC may improve patient outcomes and screening accuracy. This pilot study explored the feasibility of biomarker proteins in CC serum samples using a label-free quantitative proteomics and electrochemical immunosensors. Data analysis revealed 23 proteins that were significantly different ($p<0.05$) in CC patients compared to the normal control subjects. Among these proteins with more than 1.5 fold expressions, 8 up-regulated and 15 down-regulated proteins were found. The detection of possible biomarkers was carried out by measuring changes in electrical parameters when the serum protein is bound to an antibody immobilized on the screen-printed electrode. The results showed Protein A significantly increased in advanced stage CC. The immunosensors may be applied to detect biomarkers in human serum and may provide a new technological platform for developing high sensitivity, and specificity diagnosis. The validation will need to be investigated further.

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PA-29

LABEL-FREE QUANTIFICATION MASS SPECTROMETRY REVEALS METASTATIC PROTEINS AND ADVANCED-STAGE PROGNOSTIC BIOMARKERS IN CHOLANGIOCARCINOMA

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In Southeast Asia, cholangiocarcinoma (CCA) remains a serious public health issue, especially in Thailand, where the disease has the greatest incidence worldwide. Furthermore, there has been an increase in CCA mortality and incidence in Thailand. “Metastasis” describes how a cancer spreads to various areas of the body from where it first developed. The deep physical location of the bile ducts, the lack of identifiable early-stage symptoms, and the lack of accurate biomarkers all contribute to the difficulty of early identification. Our research focuses on identifying protein alterations for biomarkers discovery between low (HuCCA-1) and high-metastatic cells (M213-D5) to enhance therapeutic outcomes for CCA. The invasiveness of CCA cells was evaluated using migration and invasion assay. Results show that M213-D5 is significantly increased in both migration and invasion compared to HuCCA-1. The epithelial-mesenchymal transition (EMT) markers were performed using E-cadherin and N-cadherin to confirm the cancer aggressiveness. The results showed higher expression levels of E-Cadherin compared to M213-D5 cells, conversely, N-Cadherin was lower in HuCCA-1 cells as expected. Mass spectrometry (LC-Timtof Pro2) has been used to identify the differential protein expression involved in cancer aggressiveness. A total of 3,445 proteins were identified. Targeted proteins that changed more than 1.5 fold were validated using Realtime-PCR and western blot analysis. Results showed that more than 6 proteins were significantly increased in M213-D5 as compared to HuCCA-1. Moreover, low-grade and high-grade patient plasma samples were used to validate by western blot to confirm the results. Lastly, we found proteins that could be possible biomarkers for highly metastatic CCA, which might help to predict the disease and lead to the development of more precise CCA therapies.

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PA-30

INVESTIGATION OF THE INHIBITORY EFFECTS OF PROTEIN O-GLCNACYLATION ON SW620 METASTATIC COLON CANCER CELLS

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Colorectal cancer (CRC) is a predominant source of cancer-associated morbidity and mortality, complicating efforts in treatment and management strategies. O-GlcNAcylation, a dynamic post-translational modification of N-acetylglucosamine (GlcNAc) at serine/ threonine residues of cytoplasmic and nuclear proteins, has been reported to be up-regulated and associated with tumor progression in numerous types of cancer. Despite this, its function in influencing chemotherapy responses, especially in CRC, is still inadequately investigated. The aim of this study was to evaluate the impact of O-GlcNAcylation on the efficacy of oxaliplatin (OXA), a first-line chemotherapy for CRC patients, using the SW620 metastatic CRC cells as an in vitro model study. SW620 cells were treated with varying concentrations of OXA to evaluate its effects on O-GlcNAcylation levels and cell viability. OXA treatments caused a reduction in cell viability with IC_{50} at 10 μM OXA and an increase in O-GlcNAcylation levels by dose and time dependent manners. To reduce O-GlcNAcylation, SW620 cells were treated with OSMI-1, a potent inhibitor of O-GlcNAc transferase (OGT) and OGT knockdown (siOGT). As a result, it was noticed that both OSMI-1 and siOGT treatments dramatically increased the sensitivity of SW620 cells to OXA, thereby enhancing its cytotoxic effects through the reduction of O-GlcNAcylation levels. The findings indicate that diminishing O-GlcNAcylation using either OSMI-1 inhibitor or OGT knockdown may enhance SW620 responses to chemotherapy, suggesting a possible combining strategy for the CRC treatment efficacy.

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PA-31

MULTIDRUG-RESISTANT LUNG CANCER CELLS ARE HIGHLY SUSCEPTIBLE TO FERROPTOSIS

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Lung cancer is one of the most common cancers worldwide. Multidrug resistance (MDR) is a major problem conferring chemotherapeutic failure in lung cancer patients. In consequence, this necessitates further research and treatment development. Ferroptosis is a newly discovered type of non-apoptotic programmed cell death, and kills cells through iron-dependent generation of reactive oxygen species and membrane lipid peroxidation. Ferroptosis induction has received attention as a new strategy for treating lung cancer. This study aims to explore ferroptosis sensitivity in a human MDR lung cancer cell line (A549RT-eto) compared to its parental cell line (A549). MTT assay was used to determine cell viability after treatment with test compounds for 48 h. Western blot analysis was employed to detect expression of multidrug resistance protein1 (MDR1 or ABCB1 or P-glycoprotein) and antioxidant enzymes including catalase, glutathione peroxidase1 (GPX1), and glutathione peroxidase4 (GPX4). A549RT-eto and A549 cells showed different drug sensitivity profiles. Compared to A549 cells, the A549RT-eto cells were resistant to etoposide (by 11.9–fold), doxorubicin (by 6.1–fold), and paclitaxel (by 1.8–fold), correlated with increased MDR1 level in the MDR cells. However, both cell lines exhibited similar sensitivity to H2O2. In contrast, A549RT-eto cells were sensitive to cytotoxic effect of a ferroptosis inducer erastin by more than 1.9-fold, compared to the parental cells, and correlated with a lesser GPX4 expression level in the MDR cells. Pre-treatment with a glutathione precursor N-acetylcysteine (NAC) could ameliorate cytotoxic effect of erastin in A549RT-eto cells. In conclusion, our results demonstrated ferroptosis vulnerability in the MDR1-overexpressing lung cancer cells. Therefore, ferroptosis induction has a potential to target MDR cells for overcoming therapeutic resistance in lung cancer therapy.

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PA-32

GLYCOPROTEOMICS OF PLASMA IMMUNOGLOBULIN A IN PATIENTS WITH COLORECTAL CANCER

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Analysis of intact glycopeptide and glycoproteins remains challenging because of the complexity of sugar attachment and difficulties in data interpretation. Immunoglobulin A (IgA), the second most antibody in blood, has two subclasses (IgA1 and IgA2) which contains two (N144 and N340) and five (N47, N92, N131, N205, and N327) N-liked glycosylation sites, respectively. In this study, we developed a strategy for differential analysis of the intact site-specific N-glycopeptides of serum IgA between colorectal cancer (CRC) and healthy controls (HC). IgA was purified using Peptide-M from individual samples of patients with non-metastatic (NM-CRC, n=10), metastatic CRC (M-CRC, n=10), and HC (n=10), and followed by in-solution tryptic digestion. The digested peptide samples were subjected to a nano liquid chromatography coupled tadem mass spectrometer with Orbitrap Mass Spectrometer using higher-energy collisional dissociation (HCD) for MS/MS fragmentation. Identification and quantitation of site-specific of N-glycopeptides were performed by label-free analysis of Progenesis QI and Byonic software using an in-house human IgA and N-glycan database containing 148 glycan forms. In total, several glycoforms in each site were identified including 41 (N144/N131), 8 (N340), 6 (N92), 6 (N205), 18 (N131), 5 (N47) and 2 (N327) glycoforms. Heterogeneity of N-glycans were observed among these sites. Sialylated N-glycans were majority presented in all sites among three sample groups. The relative N-linked glycopeptides dominantly existed at N144/N131 which taken almost 50% of all observed N-linked sites. Among 41 glycoforms at N144/N131, differential quantitation analysis showed that the level of HexNAc(5)Hex(3) was increased in a disease-dependent manner with statically different among sample groups ($p<0.05$). This strategy enables us to discover the detailed profiles of N-glycopeptides and the relative quantitation to determine the level changes in site-specific N-glycopeptides of patients with CRC.

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PA-33

CHARGE-BASED DIAGONAL CHROMATOGRAPHY FOR ISOLATING O-GLCNAC-MODIFIED PEPTIDES IN HELA CELL EXTRACTS

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O-GlcNAcylation, a dynamic post-translational modification, plays a crucial role in multiple cellular processes. Changes in O-GlcNAcylation have been associated with a plethora of human diseases, particularly diabetes, neurodegenerative diseases, and cancers. Deciphering O-GlcNAcylation is an important challenge due to its low abundance and limited detection technologies. Herein, diagonal strong cation exchange chromatography was applied to enrich the O-GlcNAc-modified peptides prior to electron transfer dissociation mass spectrometry (ETD-MS) analysis. In this strategy, the O-GlcNAc-modified peptides were first enzymatically labeled with N-azidogalactosamine (GalNAz), and fractionated by strong cation exchange chromatography. Subsequently, the free azido group in GalNAz was reduced to a primary amine group using tris-(2-carboxyethyl)phosphine (TCEP) and separated by charge-based fractional diagonal chromatography. A total of 250 O-GlcNAcylation sites on 215 proteins were identified by ETD-MS/MS analysis. Based on O-GlcNAcAtlas database, 123 proteins were previously reported for O-GlcNAcylation, e.g., transitional endoplasmic reticulum ATPase, tight junction protein ZO-2, basement membrane-specific heparan sulfate proteoglycan core protein and basigin, while 92 proteins were never reported. Additionally, according to O-GlcNAcPRED algorithms, the identified O-GlcNAcylation sites mostly showed a high-confidence score (> 0.5). Therefore, this novel method could be a potential tool for enrichment and site mapping of O-GlcNAcylation in complex biological samples.

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PA-34

CELIVER: A NOVEL CELL-FREE DNA FRAGMENTATION-BASED PREDICTION MODEL FOR EARLY HEPATOCELLULAR CARCINOMA

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Hepatocellular carcinoma (HCC) detection often relies on conventional biomarkers like alpha-fetoprotein (AFP) combined with ultrasound, which may fall short in early diagnosis. This study introduces a groundbreaking model, “CEliver” (CfDNA-based Automated Capillary Electrophoresis method for Liver cancer screening), a blood based non-invasive technique designed to enhance early HCC detection. The CEliver model leverages the distinctive fragmentation patterns of cell-free DNA (cfDNA), which differ significantly between cancer patients and healthy individuals, with cancer patients typically exhibiting shorter cfDNA fragments. Through our innovation, more than 300 cfDNA features; called “CF-2D” (cfDNA Concentration, main cfDNA Fragment size, 2D features, and F-ratio), were generated from automated capillary electrophoresis data using a feature engineering process. By integrating CF-2D features with clinical parameters, the predictive CEliver model was developed using Light Gradient Boosting Machine (LGBM)-based machine learning. The CEliver model demonstrated an impressive 99% accuracy in distinguishing high-risk individuals and HCC cases. Notably, CEliver outperformed the traditional AFP method, particularly in the early stages of HCC. Our results underscore the potential of the CEliver model as a cost-effective and innovative approach for early HCC detection in surveillance programs, offering a promising alternative to current screening practices

PA-35

INVESTIGATION OF CANCER COLONY FORMATION AND PROTEOMES OF COLORECTAL CANCER CELL LINES USING 3D CULTURE MODELS

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Cancer stem-like cells are thought to play important roles in cancer colony formation and self-renewal. Our laboratory has established three-dimensional models of colorectal cancer (CRC) cell lines which derived from SW480 (non-metastatic clone) and SW620 (metastatic clone) by soft agar and Matrigel. We aim to determine the ability of colony formation of these cells and compare proteomes of CRC cells cultured between the two-dimensional (2D) and three-dimensional (3D) models using mass spectrometry based proteomics and bioinformatics analysis. We found that, using soft agar and Matrigel cultures, SW620 had an ability to form colony formation higher than that of SW480. The proteomic analysis and KEGG pathways revealed that the 3D Matrigel culture of both cells increased in proteins involving mainly in metabolic shifts of amino acids, glycolysis/gluconeogenesis, and pentose phosphate pathway, whereas it decreased in proteins related to spliceosome, DNA replication, and mismatch repair pathways. These altered proteins in this 3D model might be important pathways associated to tumorigenesis. Interestingly, the 3D model of metastatic clone SW620 displayed the upregulated proteins related to ribosome and proteasome pathways involving in an increase proliferation, migration and invasion but showed the downregulated proteins related to the nucleocytoplasmic transport and oxidative phosphorylation pathways that play important roles in tumor growth and carcinogenesis. Taken together, we demonstrated a 3D model of CRC cell lines and identified proteins and pathways that may play vital roles in cancer stem-like cells, especially in the metastatic CRC phenotype. Further studies are needed to determine the role of identified proteins and pathways involved in CRC progression.

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PA-36

CULTURE SUPERNATANTS FROM CHOLANGIOCARCINOMA CELL LINES INDUCE THE M2 PHENOTYPE OF PRIMARY HUMAN MONOCYTES

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Cholangiocarcinoma (CCA) is a highly aggressive and devastating cancer with no successful therapies and a median survival of less than 24 months. Cancer cells use several mechanisms to escape host immune surveillance, including the secretion of tumor-derived factors to create both local and systemic immunosuppressive conditions. Furthermore, the accumulation of inflammatory macrophages (M2) within the tumor microenvironment is associated with poor prognosis. This study aims to investigate the effect of secreted soluble factors from CCA cell lines on the induction of M2 phenotypes. The profile of cytokines, chemokines, and growth factors was first characterized in cell culture supernatants from four selected CCA cell lines (RMCCA-1, HuCCA-1, HuCCT-1, and TFK). Then, the effect of supernatants from different CCA cell lines on the induction of M2 phenotype was investigated using primary human monocytes. Supernatants from all studied CCA cell lines showed high levels of IL-8 and TGF-β1. In contrast, level of IP-10 was high only in supernatant from RMCCA-1, while level of IL-6 was elevated only in supernatant from HuCCT-1 and TFK. Among the different CCA cell lines, only monocytes that were cultured with supernatants from RMCCA-1 or HuCCT-1 had higher expression of CD206, a marker of M2 phenotype, by approximately 10 folds compared to those cultured in regular medium. In addition, the increased surface CD206 expression was associated with the morphological characteristics of an M2-like macrophage with an elongated, rod-shaped structure. These findings showed that each CCA cell line exerted a unique profile of secreting factors, which may contribute to its different ability to induce M2 phenotype.

PA-37

ACTIONABLE GENETIC ALTERATIONS IN NON-SMALL CELL LUNG CANCER IDENTIFIED THROUGH MULTI-GENE PANEL TESTING

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Non-small cell lung cancer (NSCLC) remains one of the leading causes of cancer-related mortality worldwide. Despite significant advances in targeted therapies and immunotherapies, single genetic testing is routinely practiced at our institution. In this study, we report a comprehensive investigation into actionable genetic alterations in patients with NSCLC. Molecular testing, including polymerase chain reaction (PCR) and next-generation sequencing (NGS), was performed on tissue and liquid biopsies from 88 patients with primary lung cancer, collected between September 30, 2019, and September 8, 2024. Of these, 57 patients were identified with multi-gene panel mutations, and their clinical information was gathered for analysis. Among the 57 patients with NSCLC, molecular testing was performed on formalin-fixed paraffin-embedded (FFPE) tissue samples in 37 patients (64.9%) and plasma samples in 20 patients (35.1%). PCR techniques were employed for 35 patients (61.4%), while NGS was utilized for 22 patients (38.6%). The most frequently observed genetic alterations were EGFR mutations (28.1%), KRAS mutations (12.3%), ROS1 fusion (5.3%), ALK fusion (3.5%), HER2 Exon 20 insertions (3.5%), MET exon 4 skipping mutations (3.5%), and BRAFV600E mutations (1.8%). Among the patients with EGFR mutations, 41.2% had an Exon 19 deletion, and 41.2% had an Exon 21 L858R mutation, followed by Exon 20 insertions (11.8%) and Exon 20 S768I mutations (5.9%). Notably, one patient was identified with an EGFR co-mutation (Exon 21 L858R and Exon 20 insertion), detected via PCR on FFPE samples. This retrospective study revealed a lower frequency of EGFR mutations compared to previous reports that utilized comprehensive genomic profiling with NGS. Future studies incorporating both single genetic testing and broader genomic approaches could offer a more detailed understanding of EGFR mutation patterns and actionable genomic alterations in patient with NSCLC.

PA-38

THE EFFECT OF PASSIVE SMOKING ON INFLAMMATORY FACTORS IN INFERTILE WOMEN IN SOUTHERN CHINA

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To investigate the impact of passive smoking on the reproductive immunity of women undergoing in vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) treatment for infertility. Design: Retrospective cohort study. Setting: A single, university-affiliated infertility practice. Patient(s): The study included 445 females who underwent IVF/ICSI cycles from January 2022 to June 2023. Main Outcome Measure(s): Serum cotinine levels, serum TNF levels, serum IGF-1 levels, serum IGF-2 levels, serum IL-6 levels. The average plasma level of these patients is 33.4 ± 5.2 years old, the average BMI is 22.4 ± 3.8 kg/m^2, the average serum cotinine level is 914.9 ± 480.0 ng/μL, the average serum TNF level is 29.9 ± 24.4 pg/mL, the average serum IGF-1 level is 62.0 ± 32.3 pg/mL, the average serum IGF-2 level is 57.3 ± 22.8 pg/ mL, the average serum IL-6 level is 18.8 ± 10.2 pg/mL. Adjusted for age and BMI, for increase of 1 ng/μL serum cotinine, the TNF levels was observed increased 0.06 pg/mL (β0.06, [95% CI, 0.06–0.06], p<0.0001), the IGF-1 levels was observed increased 0.04 pg/mL (β0.04, [95% CI, 0.03–0.05], p<0.0001), the IGF-2 levels was observed increased 0.03 pg/mL (β0.03, [95% CI, 0.03–0.04], p<0.0001), the IL6 levels was observed increased 0.02 pg/mL (β0.02, [95% CI, 0.02–0.02], p<0.0001). Passive smoking related chemicals may damage immune environment of pregnancy. Whether pregnancy outcomes was affected requires further studies to confirm.

PA-39

NURSE SONOGRAPHER COMPETENCY IN LIVER AND BILE DUCT CANCER SCREENING: AN ALTERNATIVE TO RADIOLOGIST ASSESSMENT

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Liver and bile duct cancers are the leading causes of cancer-related deaths in Thailand. Screening with ultrasonography can help detect these cancers at earlier stages, offering a survival benefit. However, the shortage of radiologists in remote areas presents a barrier to widespread screening. This study aims to evaluate the competence of nurse sonographers (7 nurses with 3–8 years of experience) in performing liver and bile duct screenings using ultrasonography, compared to experienced radiologists, in a liver and bile duct cancer screening and surveillance program for an at-risk population in Phranomphrai province, Roi Et, Thailand. A retrospective analysis was conducted on 1,000 individuals participating in the cohort program (29% male, with a median age of 56). Abnormal findings in the liver and biliary system were identified in 505 cases (50.5%), including two cases of cancer (1 hepatocellular carcinoma and 1 case of liver metastasis). The reported abnormalities include liver issues (fatty liver, liver parenchymal disease, cirrhosis, masses/nodules, hepatomegaly, cysts, and calcifications) as well as bile duct abnormalities (bile duct dilatation, gallstones, and gallbladder polyps). All ultrasound images and reports from nurse sonographers were reviewed by two radiologists, who were blinded to the original interpretations. Inter-reader agreement between nurse sonographers and radiologists was evaluated using Cohen's kappa test, along with sensitivity, specificity and predictive values. The results showed substantial overall agreement between the two groups (Kappa 0.61, $p<0.001$), with almost perfect agreement in biliary system findings (Kappa 0.81, $p<0.001$). Nurse sonographers demonstrated a sensitivity of 76%, specificity of 85%, positive predictive value of 80%, and negative predictive value of 78% compared to radiologists. These findings suggest that nurse sonographers, with adequate training, can perform liver and bile duct screenings with accuracy comparable to radiologists. Expanding training programs could enhance early cancer detection in underserved regions of Thailand.

PA-40

THE ASSOCIATION OF GUT MICROBIOME IN THAI COLORECTAL ADENOMAS

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Colorectal adenomas are the most common precursor to colorectal cancer (CRC) and are influenced by the gut microbiome. Colorectal adenomas arising from the conventional adenoma-carcinoma sequence (e.g., tubular adenomas) have greater malignant potential than those from the serrated polyp pathway (e.g., hyperplastic polyps). Investigating the gut microbiome associated with colorectal adenoma development could shed light on the mechanisms linking specific microbes and metabolic pathways to colorectal carcinogenesis. Our study examines the characterization, functions, and interactions of the gut microbiome in patients with hyperplastic polyps and tubular adenomas. The full-length 16S rRNA sequence was used to identify the gut microbiome in stool samples from participants without polyps (control, n=40), patients with hyperplastic polyps (HP, n=52), and patients with tubular adenomas (TA, n=60). Differential abundance analysis, co-occurrence network analysis, and differential pathway analysis were applied. The results showed a significant increase in the abundance of *Mediterraneibacter gnavus* in both HP and TA. In contrast, the abundance of Bacteroides fragilis and *Streptococcus gallolyticus* significantly increased in TA. Functional analysis revealed exclusive enrichment of the sulfur oxidation pathway in HP, whereas TA showed dominance in pathways related to secondary metabolite biosynthesis (e.g., mevalonate). The co-occurrence network analysis indicated co-occurrence of dysbiosis-associated bacteria in HP, whereas CRC-associated bacteria were dominant in TA. Moreover, the co-occurrence of SCFA-producing bacteria was lower in TA than in HP. This study identified distinct gut microbiome signatures associated with colorectal adenoma formation. Understanding the role of the gut microbiome in colorectal adenomas could aid in developing non-invasive screening methods and targeting the gut microbiome to prevent CRC carcinogenesis.

PA-41

HEALTH LITERACY REGARDING LIVER AND BILE DUCT CANCER IN HIGH-RISK GROUPS

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Health literacy is a critical aspect of cancer care management, with low health literacy strongly linked to poorer health outcomes. In Thailand, there is limited data on health literacy regarding liver and bile duct cancer—the leading cause of cancer deaths—particularly among at-risk individuals. This study used a validated 26-item health literacy questionnaire, covering six sections: (1) general knowledge of liver and bile duct cancer, (2) sources of health information/health services and their accessibility, (3) comprehension of accurate and sufficient information, (4) methods for verifying health information accuracy, (5) health information retrieval and sharing, and (6) personal health management to prevent liver and bile duct cancer. Health literacy was classified into three levels: Low (< 60% score), Moderate (60%-80%), and High (> 80%). A cross-sectional survey was conducted with 400 participants from four subdistricts (Pa Kha Luang, Suat, Ban Pha, and Ban Phi) in Banluang district, Nan, Thailand, a region with one of the world's highest incidences of liver and bile duct cancer. Sixty percent of participants were male, with a median age of 56 years. Half had completed only primary education. Low health literacy was observed in 20-51% of participants across the questionnaire's sections, notably in sources of health information/health services and accessibility (51%), general knowledge of liver and bile duct cancer (43%), and methods for verifying health information accuracy (41%). In contrast, 79% of participants showed moderate-to-high literacy in personal health management to prevent liver and bile duct cancer. Additionally, individuals over the age of 60 were at higher risk of having low health literacy. The findings suggest that overall health literacy about liver and bile duct cancer is inadequate in this high-risk population. Addressing barriers to health literacy will be essential in designing effective interventions to improve outcomes for those at risk of developing these cancers.

PA-42

PREDICTOR OF ADVANCED COLORECTAL NEOPLASIA DEVELOPMENT AFTER POLYPECTOMY

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Colorectal cancer (CRC) is one of the leading causes of cancer-related deaths worldwide. Early detection and removal of colon polyps through endoscopic resection can significantly reduce both the incidence and mortality of the disease. However, polyp recurrence can still occur after colonoscopic polypectomy, depending on various patient-related risk factors. This study aims to assess the polyp recurrence rate following colonoscopic polypectomy and to identify patient characteristics and polyp features associated with the development of advanced colorectal neoplasms (ACN). This cohort study included data from 1,347 Thai participants aged 50-65, among whom 387 (29%) had 511 polyps detected during their initial colonoscopy. Of these, 359 patients (59% female) underwent repeat colonoscopies 3-5 years later. During follow-up, 479 polyps were detected in 183 patients, resulting in a polyp recurrence rate of 51%. Among these, 14 patients had 24 polyps classified as ACN, including two cases of CRC. A univariate mixed-effects proportional hazards regression analysis was performed to evaluate baseline clinical factors and polyp characteristics influencing the development of ACN. These factors included gender, age, polyp size, polyp location, and histology of the colorectal polyps. Only polyp size and histology were found to significantly affect the development of ACN. A polyp that increased in size by 1 mm was associated with a 5.4-fold increase in the risk of ACN (OR 5.40 [95% CI: 2.09–13.94]; $p < 0.001$), while tubulovillous or villous adenoma histology was associated with a 25.77-fold increased risk of ACN (OR 25.77 [95% CI: 2.88–230.34]; $p = 0.004$). Due to the limited number of ACN cases, only univariate analysis was conducted. Therefore, patients with large polyps or histology indicating tubulovillous or villous adenoma should undergo repeat colonoscopy surveillance, in accordance with standard colorectal polyp surveillance guidelines.

B.
DRUG DEVELOPMENT FOR PREVENTION AND TREATMENT

PB-01

SYNERGISTIC ANTIBACTERIAL ACTIVITY OF 1-METHYL ESTER-NIGERICIN AND METHYL 5-(HYDROXYMETHYL) FURAN-2-CARBOXYLATE AGAINST *PROTEUS* SPP

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1-methyl ester-nigericin (1) and methyl 5-(hydroxymethyl) furan-2-carboxylate (2) were isolated from *Streptomyces hygroscopicus* BRM10, an endophyte in *Alpinia galanga*, and *Streptomyces zerumbet* W14, an endophyte in *Zingiber zerumbet,* respectively. Both compounds showed great antibacterial properties. Compound 1 is a carboxylic ionophore that can intercalate into intracellular organelle membranes and exchange protons for K+ or Na+. Compound 2 is a furancarboxylate which has antibiofilm property and leads to bacteriolysis by causing cell wall damage. Synergistic combinations of antimicrobial agents with different mechanisms of action are successful approaches for combating bacterial infections. This study aimed to evaluate the synergistic effect of compounds 1 and 2 on the growth inhibition and biofilm elimination of two reference strains of *P. mirabilis* and two clinical isolates of multidrug-resistant (MDR) *P. vulgaris*. The synergistic antimicrobial activity of the compounds was tested by the checkerboard method and time-kill curves. To estimate the interaction between the compounds, the fractional inhibitory concentration index (FICI) of the combination was calculated. The cytotoxic activity of the compounds in combination was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay on LLC-MK2 cell lines. The reduction percentage of biofilms was obtained using the colourimetric method. The MIC values for compounds 1 and 2 against test bacteria ranged from 39.06 to 78.12 µg/ml, and from 78.12 to 156.25 µg/ml, respectively. The MIC was reduced to one-eighth as a result of the combination of compounds 1 and 2. After 4 to 24 h of treatment with ½ MIC of compounds 1 and 2, the killing rate (in cfu/ml) increased to a greater degree than observed with either test compound alone. The combination of compounds 1 and 2 showed a synergistic effect with FICI of 0.50 and 0.28. The synergistic combination of compounds 1 and 2 was effective on the biofilm reduction of Proteus vulgaris NP16 (85.72%) and NP47 (89.14%). This study recommends compounds 1 and 2 in combination as a potential alternative treatment agent for *Proteus* spp infections.

PB-02

ADENOSINE: ITS MOLECULAR MECHANISMS AND POTENTIAL IN CHOLANGIOCARCINOMA TUMOR INHIBITION *IN VIVO*

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Cholangiocarcinoma (CCA) is one of the lethal cancers requiring new therapeutic drugs. Adenosine is a natural molecule that inhibits CCA cell growth but has less effect on cholangiocytes, while other drugs such as cisplatin can kill cholangiocytes more than CCA cells. Adenosine typically governs cellular functions via adenosine receptors which are coupled to G-protein αs or αi. Activation of G-protein αs leads to cAMP production via activation of adenylcyclase. Adenosine does not increase cAMP level in CCA but elevates cAMP level in cholangiocytes. In cholangiocytes, adenosine binds to receptors on cell surface and activates cAMP production, but with CCA cells, adenosine enters through equilibrative nucleoside transporters (ENTs). Inhibition of these transporters significantly reduces the inhibitory effects of adenosine on CCA cells. After adenosine enters CCA cells, it is changed into AMP but not inosine, indicating the metabolic pathway of adenosine in CCA cells. Therefore, AMP level inside CCA cells was increased. This leads to an imbalanced ratio of AMP:ATP inside the cells. Then, when the ratio of AMP:ATP increases, this triggers the low-energy sensor, AMPK. CCA cells then behave as if they are under low energy state, halting cell growth and undergoing autophagy. However, cell death is not observed, but a combination of adenosine and autophagy inhibitors leads to significant cell death in CCA cells. Similar results are obtained from in vivo experiments in nude mice where adenosine slows down CCA tumor growth, leading to a reduction of tumor mass. Normally, activation of autophagy involves an activation of AMPK pathway and a deactivation of mTOR pathway, which is a positive regulator of cell proliferation. Interestingly, in CCA cells, autophagy induced by adenosine is distinct from typical autophagy. Both AMPK pathway and mTOR pathway are upregulated in adenosine-induced autophagy in CCA cells.

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PB-03

PIPER NIGRUM EXTRACT REDUCES BREAST CANCER INCIDENCE BY MODULATION OF CANCER CYTOKINES/CHEMOKINES AND T CELLS

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Piper nigrum, black pepper, has been widely used in traditional medicine for many diseases including cancer. This study aimed to evaluate the breast cancer-preventive effect of a low piperine *P. nigrum* extract, namely PFPE or LPPE. In order to study the action of PFPE on immune boosters, female Sprague-Dawley rats were induced mammary tumors by intraperitoneally (i.p.) injection with carcinogen N-nitroso-N-methylurea (NMU) followed by administration with PFPE at doses of 50, 100, 150 mg/kg BW. We found that the incidences of tumors in the control, vehicle, PFPE 50 mg/kg BW and PFPE 100 mg/kg BW groups were 70, 50, 20, 20 and 0%, respectively. Remarkably, no cancerous were found in the PFPE at dose of 150 mg/kg BW at approximately three months. Moreover, PFPE at dose of 100 mg/kg BW suppressed cancer cytokines/chemokines including sICAM-1, CX3CL1, CXCL7, VEGF, TIMP-1, CCL5, CCL20, CXCL1, CXCL5, CXCL9 and IL-1β. PFPE at doses of 50, 100 and 150 mg/kg BW stimulated IFN-γ and promoted Th1 cells while all these doses of PFPE inhibited Th2 and Treg cells. In the tumor tissue, PFPE at doses of 50 and 100 mg/kg BW inhibited cancer related proteins including ER-α and NF-κB and increased IL-6R and STAT3. Our findings indicate that PFPE has the potential to reduce breast tumor incidence and retardation of tumor growth by modulating Th1/Th2/Treg cells and cytokines/chemokines, and decreasing cancer related proteins.

PB-04

LYCOPODIUM ALKALOIDS: ISOLATION, SEMI-SYNTHESIS, ANTIACETYLCHOLINESTERASE ACTIVITY AND NOVEL PROTECTIVE APPLICATION AGAINST NEURODEGENERATIONS

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The *Phlegmariurus* genus (Lycopodiaceae) is cultivated as ornamental plants in Thailand, with extracts serving as a rich source of Lycopodium alkaloids (Ngernnak et al., 2021). This presentation encompasses the isolation, structure elucidation, semi-synthesis, and biological evaluation of isolated Lycopodium alkaloids from Thai clubmoss cultivars. Eleven unprecedented Lycopodium alkaloids, namely phlegcarines A-C and nummulines A-H, were isolated from *Phlegmariurus carinatus* (Desv. ex Poir.) Ching. (Thamnarak et al., 2023) and *P. nummulariifolius* (Blume) Ching (Thamnarak et al., unpublished), respectively. The structural assignments were established through comprehensive spectroscopic techniques, including HR-ESI-MS, NMR techniques, chemical correlations, and further confirmed by X-ray analysis specifically for nummuline D. While the isolated Lycopodium alkaloids were evaluated for anti-acetylcholinesterase activity, none demonstrated efficacy surpassing that of huperzine A (HupA, IC_{50} eeAChE = 0.03 μM). Huperzine A derivatives were further synthesized to enhance potency (Anukanon et al., 2021). HupA and derivatives significantly suppressed the Aβ25-35 cytotoxicity and showed recovery effects against arsenic-induced AD pathologies including reactive oxygen species generation, neurite outgrowth shortening, amyloid precursor protein suppression and the elevation of β-secretase, endogenous Aβ peptide, and Tau and neurofilament light proteins (Homkajorn et al., submitted).

PB-05

PIPER NIGRUM EXTRACT REDUCES BREAST CANCER INCIDENCE BY MODULATION OF CANCER CYTOKINES/CHEMOKINES AND T CELLS

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Polyphenolic glycosides have traditionally been used as medicinal remedies for various diseases. Mangiferin (MGF), a natural C-glucosyl xanthone, is distributed in several plants and exhibit various biological activities, including anti-inflammmatory, antioxidant, antiviral, antidiabetic and neuroprotective effects. *Hypodematium* sp., a rare fern in the Hypodermatiaceae family has no prior phytochemical report. Our preliminary results showed that the crude extract of this plant has been found to be rich in polyphenolic glycosides, including MGF. This study is the first report on the isolation of an unprecedented polyphenolic glycoside from *Hypodematium* sp., namely hypodemathone. Additionally, five known bioactive compounds were obtained and MGF was the major component. The structures were elucidated using HR-ESI-MS, NMR techniques, and chemical correlation. Biological evaluations for antioxidant and anticancer activities were conducted. Furthermore, this study presents the first validated method for the quantification of polyphenolic glycosides such as MGF from the leaves and rhizome of *Hypodematium* sp. Five different extraction techniques were explored including maceration-shaker, maceration-stirrer, ultrasonic, microwave and conventional heating extraction. Maceration-shaker and conventional heating extraction yielded the highest concentrations of MGF at 0.48 and 0.40 g per 10 g of rhizome, respectively. In summary, the rhizome of Hypodematium sp. provides greater yields of MGF than the leaves and both extraction techniques offer promising approaches for downstream processing.

PB-06

BASE-MEDIATED AND SILVER-CATALYZED DIVERGENT SYNTHESIS OF NAPHTHALENE DERIVATIVES FROM ENONE-OXAZOLONE

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An efficient divergent approach to functionalized naphthalene derivatives, the naphthalenamides, via base-mediated and silver-catalyzed cyclization has been developed using enone-oxazolones as the precursors. This protocol utilized base in methanol with heating to construct the corresponding hydroxynaphthalenamides 2 by a C–C bond formation, oxazolone ring-opening, and aromatization in good yields. On the other hand, phosphorylated dihydronaphthylamides **3** were generated by using H-phosphonate as the phosphonating reagent in a silver-catalyzed cyclization involving the phospha-1,4-addition/intramolecular ring closure with concomitant C–P/C–C bond formation in good yields. Their biological activity of compounds **2** and **3** were evaluated. Interestingly, compounds **2l-m** and **3n** showed a more potent cytotoxic activity against to the HepG2 cell line than the positive control, etoposide.

PB-07

SYNTHESIS AND EVALUATION OF TACRINE-α-ONOCERIN HYBRIDS AS CHOLINESTERASE INHIBITORS AGAINST ALZHEIMER'S DISEASE

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Alzheimer's disease (AD) is a prevalent form of dementia that affects a growing number of elderly individuals, with current treatments offering only symptomatic relief. Given the urgent need for more effective therapies, this research focused on the synthesis of new tacrine-α-onocerin hybrids, named tacrinocerins (**4a**-**4w**), derived from α-onocerin isolated from the Thai club moss Phlegmariurus nummulariifolius (Lycopodiaceae) via the Friedländer reaction. These compounds were biologically evaluated for their inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). Notably, tacrinocerins **4c** and **4u** exhibited strong inhibition of eeAChE, with IC_{50} values of 1.93 ± 0.13 μM and 60.58 ± 0.05 μM, respectively. Molecular docking studies revealed that tacrinocerin **4c** showed enhanced binding affinity with hAChE, although it demonstrated significant neurotoxicity at 5 μM. In contrast, **4u** exhibited low neurotoxicity and hepatotoxicity. Consequently, both **4c** and **4u** can be considered as a promising lead compound for further investigation in the treatment of AD.

PB-08

TWO UNPRECEDENTED CLASSES OF BIFLAVONOID LINKAGE FROM SELAGINELLA PLANTS

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Selaginella, also known as spike mosses, is the single genus of the Selaginellaceae family; it consists of over 700 species with a worldwide distribution and about 30 species have been found in Thailand. The isolation and purification of the aerial parts of *Selaginella* plants gave four new bioflavonoids, namely neoamentoflavone, 7-methoxyneoamentoflavone, 2'-methoxyneoamentoflavone with C5' - C8'' bond linkage and neorobustaflavone with C5' - C6'' bond linkage. This was the first report of two unprecedented classes of these biflavonoids linkage together with four known biflavonoids including amentoflavone, sequoiaflavone, robustaflavone and isocryptomerin. The chemical structures of new compounds were established through comprehensive spectroscopic techniques and chemical correlations. The new compounds showed the identical structure with amentoflavone and robustaflavone except for the unusual connection of the hydroxy group at C2' on ring B instead of the hydroxy group at C4'.

PB-09

PHOTOINDUCED CASCADE REACTION OF ENE-YNE-OXAZOLES WITH TMSN3 AND NIS: SYNTHESIS OF AZIDYL SPIROINDENOXAZOLONES

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An intriguing photoinduced azidation cyclization of ene-yne-oxazolones using trimethylsilyl azide (TMSN3) was investigated in the presence of N-iodosuccinimide (NIS) as the iodine source. Simple and mild metal-free conditions were carried out using LED blue light (450-500 nm) to activate the azidyl radical in the reaction. A plausible mechanism may proceed via aza-1,4-addition followed by 5-exo-dig cyclization, leading to multiple bond formations of C-C, C-N, and C-I bonds. Finally, the desired product, iodo-benzylidene spiroindenoxazolones was obtained in good yields in 1:1 diastereomeric ratio (d.r. ratio).

PB-10

PHOTOSENSITIZER-LOADED POLYMER-LIPID HYBRID NANOPARTICLES; SYNTHESIS, PHOTOPHYSICAL PROPERTIES AND IN VITRO PHOTODYNAMIC THERAPY WITH THYROID CANCER CELLS

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Photodynamic therapy (PDT) is a cancer treatment that employs a specific wavelength of light to activate photosensitizer (PS) in the presence of oxygen to produce reactive oxygen species for killing cancer cells. However, almost currently used PS are hydrophobic, limiting their distribution in the body. Two types of polymer-lipid hybrid nanoparticles with different core polymers encapsulating hydrophobic PS have been successfully formulated by using nanoprecipitation technique. The biodegradable polymers, poly(D,L-lactide-coglycolide) (PLGA) or poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) were wrapped with mixed lipid shell, in order to overcome the limited water solubility of PS. The resulting nanoparticles had an average particle size of 89 nm for PLGA and 215 nm for PHBV, with a core-shell structure that observed under transmission electron microscope. X-ray diffraction analysis confirmed the successful encapsulation of PS within the nanoparticles. The PS molecules encapsulated within both PLGA and PHBV polymeric cores had improved photophysical properties in terms of singlet oxygen generation in water. In vitro photocytotoxic effect against FTC-133 human thyroid carcinoma cells of both nanoparticle types was greater than free PS, improving PDT effect. Overall, both PLGA and PHBV hybrid nanoparticles could improve photophysical properties of entrapped hydrophobic PS molecules and increased its phototoxicity.

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PB-11

TROLOXAMIDE DERIVATIVES AS POTENT ACETYLCHOLINESTERASE INHIBITORS

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Alzheimer's disease (AD) is a progressive disorder leading to memory and cognitive decline. The main USFDA-approved treatments are acetylcholinesterase inhibitors (AChEIs) like donepezil, rivastigmine, and galantamine. Acetylcholinesterase (AChE) is an enzyme in the synaptic cleft that breaks down the neurotransmitter acetylcholine (ACh), a process that can lead to decreased ACh levels in the brain, contributing to Alzheimer's disease. AChE has two key active sites: the catalytic anionic site (CAS), which contains catalytic triad of amino acid residues for ACh hydrolysis, and the peripheral anionic site (PAS), with aromatic residues . Donepezil binds to both CAS and PAS due to its aromatic structure and indanone ring, enhancing its efficacy through π-π stacking interactions. Trolox, a water-soluble vitamin E derivative, is a potent antioxidant with a structure similar to α-tocopherol, featuring a chroman ring with hydroxyl and carboxyl groups. Its ability to neutralize free radicals is beneficial for neurodegenerative diseases. Trolox can also serve as a scaffold for designing new acetylcholinesterase inhibitors by facilitating π-π stacking at the PAS of AChE, potentially aiding in AD treatment. Six troloxamide derivatives (**2a-2f**) were designed with piperidine, pyrrolidine or dimethylamine moieties linked to trolox through a methylene chain (n=2, 3), aiming to interact with the CAS. All compounds were synthesized via HATU coupling in the presence of DIEA. IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry characterized all synthesized compounds. To evaluate acetylcholinesterase inhibitors, we conducted the AChE inhibition assay, cytotoxicity studies, neuroprotective effect evaluations, ADMET profile analysis, and enzyme binding interaction investigations through molecular docking. The IC50 values of compound **2a-2f** ranged from 0.22-7.45 μM which showed potent to moderately AChE inhibition activity. Compound **2a** (n=2, R= N,N-dimethylamine) exhibited the most potent AChE inhibition activity in the series (IC_{50} = 0.22±0.09 μM), which is higher potency than the standard drugs rivastigmine and tacrine The compounds were predicted to have ADMET properties, which all followed Lipinski's rule of five, achieving both high BBB permeance and good GI absorption. Molecular docking studies confirmed binding interactions between **2a** and the enzyme at both CAS and PAS sites. All tested compounds were non-toxic to the SH-SY5Y cells (IC_{50} > 200 μM) and showed significant neuroprotective effects. These findings suggest the potential of troloxamide derivatives as effective AChE inhibitors for Alzheimer's disease therapy, paving the way for further drug development.

PB-12

THE UTILIZATION OF A COMBINED PROTEIN SELECTION STRATEGY TO ISOLATE ANTI-PCSK9 NANOBODIES FROM SYNTHETIC LIBRARIES

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Nanobodies (Nbs) have the potential to replace conventional antibodies in various medical applications owing to their small size, stability, and broad range of potential antigens. We have developed a reliable genetic selection strategy combining magnetic-activated cell sorting (MACS) and functional ligand-binding identification by Tat-based recognition of associating proteins (FLI-TRAP) to screen Nbs against proprotein convertase subtilisin/kexin type 9 (PCSK9) from a synthetic library. PCSK9 is known to be a major cause of hypercholesterolemia, a leading cause of atherosclerosis and heart failure. Currently, evolocumab and alirocumab are FDA-approved mAb drugs and are commercially available to treat hypercholesterolemia. Nonetheless, Nbs may represent an alternative treatment. An initial screening using MACS biopanning was followed by FLI-TRAP, which leverages the Tat system's ability to co-translocate noncovalent polypeptides from the cytoplasm to the periplasm. This process resulted in 12 out of 40 clones being positively selected (17.5%). These Nbs showed highly specific interactions with wild-type (wt) and mutant (gain-of-function D374Y) PCSK9 in ELISA and Nb polyreactivity prediction. Three Nbs with the strongest ELISA signals were further analyzed using surface plasmon resonance, which revealed that they interacted with wt-PCSK9 with an affinity in the sub-nanomolar range. Additionally, all three Nbs demonstrated neutralization activity against the interaction between wt/D374Y-PCSK9 and the low-density lipoprotein receptor (LDLR). This new high-throughput selection process from a synthetic library successfully identified potent Nbs using MACS and FLI-TRAP, inexpensive and straightforward separation methods that can be executed in basic laboratory settings without specialized equipment such as cell sorters. We expect our strategy will be valuable in developing Nbs for various applications.

PB-13

NOVEL SULFONAMIDE DERIVATIZED ZN(II)-DIPICOLYLAMINE COMPLEXES AS POTENTIAL ANTIBACTERIAL DRUG LEADS

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The existing antimicrobial agents face the challenge of increasing antimicrobial resistance which threatens the effective treatment of different infections. Development of antimicrobial resistance has led scientists to discover new antimicrobial agents to overcome this global health problem. The synthesis of zinc complexes is an evolving research area due to the presence of vital biological properties of Zn metal. The objective of this study was to synthesize, characterize and evaluate the antibacterial properties of Zn(II)-dipicolylamine complexes. Three novel Zn(II) complexes; $Zn(N(SO_2pyridine)dpa)Cl_2$ (C1), $Zn(N(SO_2tmb)dpa)Cl_2$ (C2) and $Zn(N(SO_2chlorobenzene)dpa)Cl_2$ (C3) derived from dipicolylamine derivatized sulfonamide ligands; $N(SO_2pyridine)dpa$ (L1), $N(SO_2tmb)dpa$ (L2) and $N(SO_2chlorobenzene)dpa$ (L3) have been synthesized in good yield and characterized by single crystal X-ray diffraction analysis, FT-IR, UV-Visible and fluorescence spectroscopic measurements. Structural data revealed that all three complexes crystallized in monoclinic form and possess a distorted tetrahedron structure. In FT-IR spectroscopy, the S-N bond stretching vibrations of the free ligands have shifted to a lower wave number in the corresponding Zn complexes. High energy absorption bands observed in the region of 200 nm to 300 nm in UV-Visible spectra, are attributed to intraligand л-л* and n-л* transitions. Three complexes displayed lower fluorescence intensities compared to their free ligands and this lowered fluorescence may be due to the quenching of fluorescence upon coordinating of the ligand with Zn metal. Synthesized Zn complexes were docked with glucosamine-6-phosphate synthase (PDB ID: 2VF5) to identify the possible antibacterial properties. C1, C2 and C3 showed MolDock scores of -112.506, -125.708 and -117.176, respectively for glucosamine-6-phosphate synthase. Taking into consideration the results of molecular docking, *in vitro* antibacterial activity of Zn complexes was evaluated against Gram-positive *Staphylococcus aureus* ATCC® 25923 and Bacillus cereus ATCC® 11788 and Gram-negative *Escherichia coli* ATCC® 25922 and Pseudomonas aeruginosa ATCC® 27853 bacterial species. The outcome of molecular docking study was further confirmed by the findings of antibacterial assay, where C2 and C3 displayed moderate antibacterial activity against all four microorganisms tested while C1 displayed moderate antibacterial activity only against Escherichia coli. Hence, this study proposes the possible way forward to develop these Zn complexes as potential antibacterial drug leads.

PB-14

THE APPLICATION OF BORON NITRIDE NANOSTRUCTURES IN ANTI-CANCER AND ANTI-INFLAMMATORY DRUGS DELIVERY

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The delivery of anticancer drugs is emerging as a complex challenge for researchers. This method facilitates precise drug administration directly to cancerous cells, significantly reducing systemic exposure and minimizing unwanted side effects. The exploration of diverse nanocarriers for targeted drug delivery is crucial for enhancing therapeutic efficacy while minimizing adverse effects, ultimately improving treatment outcomes across different medical conditions. Various boron nitride (BN) nanostructures, including nanoparticles, nanotubes, nanosheets, nanocones, and fullerene-like cages, have emerged as promising one- and zero-dimensional materials for biomedical applications due to their unique structural and chemical properties, as well as their demonstrated nontoxicity, biocompatibility, and chemical or biological inertness. We investigate the potential of BN nanostructures as drug delivery vehicles for anticancer (doxorubicin, penicillamine, 5-fluorouracil, and 5-aminolevulinic acid) and anti-inflammatory (sulfasalazine, ibuprofen, celecoxib, and naproxen) drugs, utilizing computational techniques such as Density Functional Theory, molecular docking, and Molecular Dynamics simulations. The evaluation of computational techniques demonstrated that BN nanostructures can function as promising carriers for improving the encapsulation, transportation, and release of anticancer and anti-inflammatory drugs through electrostatic and covalent interactions in both vacuum and aqueous environments, utilizing the B3LYP, PBE0, M06-2X, and CAM-B3LYP methods. The loading of anticancer and anti-inflammatory drugs with physisorption and chemisorption behaviors onto and into BN nanostructures results in alterations to the optical and electronic characteristics of the carriers. The MD and Molecular docking simulations indicate that BN nanostructures loaded with anticancer and anti-inflammatory drugs exhibit enhanced inhibition of pro-inflammatory and anti-cancer cytokines compared to free drugs.

PB-15

PREPARATION AND EVALUATION OF SPRAYABLE HYDROGEL FORMULATIONS FOR WOUND DRESSING

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Creating a sprayable hydrogel with quick in situ gelation presents a viable method of healing wounds and offers novel opportunities for biomedical applications. They are able to adapt to uneven surfaces, produce a moist environment that promotes the formation of new tissue, and offer protection against infections. Herein, we develop therapeutic hydrogel dressings that are easy to use and can be applied with a normal spray bottle. The hydrogels were developed by the preparation of Pluronics (F127) and N-succinyl chitosan (NSC) as thermo- and pH-responsive polymers, respectively. NSC was prepared by modification of chitosan with succinic anhydride, as confirmed by Fourier-transform infrared and proton nuclear magnetic resonance spectroscopy. To achieve sprayability, we evaluated the concentration of F127 within a range of 18-20% (w/v) and then incorporated different NSC concentrations in a range of 0.25-0.50% (w/v). Various tests were conducted to investigate the effect of polymer concentration on the sprayability, such as physical appearance, sol-gel phase transition, pH determination, gelling capacity, and spreadability. Furthermore, the sprayability of F127/NSC solutions was investigated in terms of spray amount and sprayed area to evaluate their potential application as sprayable dressings. Results showed that the gelation temperatures of all the formulations were within the range of 29-34°C, upon varying pH of 5-9.5. The phase transition depended on the F127 and NSC content so that the gelation could be manipulated. On the basis of these properties, it can be concluded that these F127/NSC sprayable hydrogels show considerable potential for use in wound dressing applications.

PB-16

REPURPOSING CEFTRIAXONE: A POTENTIAL THERAPY FOR ACQUIRED EPILEPSY IN ALZHEIMER'S DISEASE: FINDINGS FROM *IN VIVO* AND *IN VITRO* STUDIES

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The pathological relationship between Alzheimer's disease (AD) and epilepsy is complex and bilateral. However, the specific mechanisms underlying this connection remained unclear. This study aimed to explore the role of the glutamate-glutamine cycle in this relationship and evaluate the potential of ceftriaxone, a glutamate transporter-1 (GLT-1) enhancer, to reduce seizure susceptibility in the Tg2576 mouse model of AD and improve cell viability in human astrocytes. In vivo model, we measured the expression of glutamate transporter-1 (GLT-1) in Tg2576 mice (n=7) and wild-type littermates (n=7), and subsequently in the kindling model of epilepsy (n=6) and sham (n=6). Then, kindling susceptibility was divided into three groups: 200mg/kg ceftriaxone-treated Tg2576 (Tg-Ceft, n=9); saline-treated Tg2576 (Tg-Sal, n=9); and saline-treated wild-type (WT-Sal, n=15). *In vitro* model, effects of ceftriaxone on cell viability of amyloid-beta-treated human astrocyte were measured by MTT assay. Tg2576 mice exhibited lower levels of GLT-1 ($p=0.0093$), compared to wild-type mice. Kindling increased GLT-1 (cortex: $p<0.0001$, hippocampus: $p=0.0075$) protein levels, compared to sham. Both Tg-Ceft and WT-Sal displayed Class IV seizures in response to the first stimulation ($p>0.99$), while Tg-Sal displayed Class V seizure ($p=0.0212$ vs WT-Sal). Seizure susceptibility of Tg-Ceft was similar to Tg-Sal ($p>0.05$), and kindling rates did not differ between groups. However, a protective trend was observed in in vitro study where non-cytotoxic concentration of ceftriaxone (0.3 and 1.25 μM) improved the viability of astrocytes against Aβ toxicity. Impairments in the glutamate-glutamine cycle are identified in models of AD and epilepsy. While ceftriaxone treatment restored GLT-1 expression in Tg2576 mice, it did not influence seizure susceptibility. This suggested that increasing GLT-1 levels alone may not be sufficient to prevent seizures in AD, or a higher dosage may be necessary. Meanwhile, preliminary findings in human astrocyte indicate that ceftriaxone may offer protection against Aβ-induced cell toxicity.

PB-17

THE ACTIVE CONSTITUENT ROLES IN ANTI-CANCER ACTIVITY OF *BULBOPHYLLUM* ORCHID

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Thailand is one of the original bases for the diversity of tropical orchid species. Some of them have become excellent sources of bioactive compounds with potential chemotherapeutic applications. Among these, Bulbophyllum orchid, the second largest genus after the Dendrobium orchid, contain high concentrations of phenolic and aromatic compounds, such as phenanthrenes, bibenzyls, and phenylpropanoids. These chemicals suspected acting as anti-cancer, anti-miotic, anti-angiogenic, anti-oxidant, anti-inflammatory and immunomodulatory activities. Antioxidant analysis through DPPH radical scavenging activity revealed higher non-enzymatic antioxidant contents in *Bulbophyllum* orchid than that of the *Dendrobium* orchid. Most of pseudobulb tissues samples contain high level of polyphenolic compounds, including total polyphenols, phenolic acids, tannins, and flavonoids. Furthermore, transcriptomic analysis showed high abundant (max. 118-fold) of isoflavone reductase (IFR) transcripts, belonging to the SDR superfamily, in pseudobulb tissues of *Bulbophyllum* species compared with the IFR of *Dendrobium* control plant. Quantitative phytochemical analysis, ten compounds were isolated from *Bulbophyllum blepharistes* Rcbh.f.. Lusianthridin (compound 2) showed cytotoxicity to H69AR, HeLa, HL-60, HepG2, and MOLT-3 cells. Compounds 1 and 5 (tristin) significantly inhibited the growth of MOLT-3. The rhizome of B. blepharitis appears to be a promising source of potential anti-cancer agents (i.e. lusianthridin). In addition, the ethanol extracts from rhizomes of *B. blepharitis* demonstrated higher cytotoxicity than extracts from the leaf and pseudobulb tissues. Recently, small peptides derived from the protein hydrolysate of root, leaf, pseudobulb and rhizome tissues of *Bulbophyllum* exhibited anti-cancer activity. Specifically, peptides from *Bulbophyllum* roots inhibited the growth of skin cancer, tongue cancer, leukemia higher than the protein hydrolysate from Vanda orchids. These finding suggest that *Bulbophyllum* orchids, particularly in Thailand, might be useful for the development of novel anti-cancer therapies.

PB-18

SYNTHESIS OF CAERULOMYCIN ANALOGUES FOR IMPROVE ANTICANCER ACTIVITY

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Caerulomycin A, a bipyridinic core with a substituted oxime functional group derived from Streptomyces caeruleus, represents a marine natural product with notable antibiotic, antifungal, and cytotoxic activities. However, its clinical utility is limited due to both its low natural abundance and the high cost of commercially available Caerulomycin. In this study, we synthesized caerulomycins and their analogues through a five-step process utilizing commercially available reagents. We achieved the total synthesis of Caerulomycin A and successfully modified its molecular structure to generate four series of analogues. These novel analogues were evaluated for their cytotoxic activity against seven cancer cell lines. Importantly, the series of benzyl ether caerulomycin derivatives exhibited higher cytotoxic activity compared to the parent Caerulomycin A. This research highlights the potential of synthetic Caerulomycin derivatives as promising candidates for future anticancer drug development.

PB-19

SYNTHESIS AND CHARACTERIZATION OF SYRINGIC ACID-LOADED MIL-100(FE) METAL-ORGANIC FRAMEWORK FOR POTENTIAL THERAPEUTIC APPLICATIONS

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The research objectives are to synthesize MIL-100(Fe) and utilize MIL-100(Fe) as the drug carrier for syringic acid. Characterization will be done to confirm the presence of syringic acid in the framework. The metal-organic framework used in the study is MIL-100(Fe). The synthesis method of MIL-100(Fe) was derived from the works of Luo and coworkers (2020). The de novo synthesis of MIL-100(Fe) using syringic acid in exchange for trimesic acid as the organic ligand was done at varying molar concentrations. For both the pristine and modified MIL-100(Fe), syringic acid is entrapped through a simple drug impregnation technique at 12, 24, 36, and 48 hours at a 1:1 and 1:2 ratio of syringic acid and MIL100(Fe). The samples were characterized using PXRD, Nitrogen Adsorption-Desorption Technique, and Fourier Transform Infrared Spectroscopy. Independent variables such as loading time, syringic acid: MIL-100(Fe) ratio, and modification were found to have a s significant effect on the mean drug loading percentages at a p-value of less than 0.001. It is noted that the significantly highest mean drug loading percentage was at 12 hours (64.42±0.03%) for 1:1, 36 hours (26.38±0.02%) for 1:2 for the unmodified MIL-100(Fe) while 36 hours (33.70±0.00% and 66.85±0.00%) for 1:1, and 1:2 ratio, respectively, for modified MIL-100(Fe) at p-values of less than 0.001. Successful loading was confirmed by the pristine and after-loading MIL-100(Fe) results of PXRD, Nitrogen Adsorption-Desorption technique, and FTIR. The study successfully synthesized and characterized both pristine and modified MIL-100(Fe) frameworks, confirming the entrapment of syringic acid through drug impregnation techniques. The use of porous metal-based nanoparticles such as MIL-100(Fe) demonstrates significant potential as a drug carrier for syringic acid, primarily due to its high surface area and versatile structural properties.

PB-20

PHYTOCHEMICAL AND ANTIMICROBIAL POTENTIAL OF ENDEMIC PHILIPPINE VANOVERBERGHIA SPECIES: UNVEILING THEIR MEDICINAL VALUE

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Vanoverberghia sepulchrei, locally known in the Philippines as “barapat,” and Vanoverberghia vanoverberghii, called “kalawin,” are two perennial plants endemic to the Philippines and part of the ginger family (Zingiberaceae). Despite their traditional use, particularly their fruits being considered delicacies, their potential medicinal properties have not been thoroughly explored. This research aims to bridge that gap by investigating the chemical components and bioactive properties of these plants, with a focus on their potential for drug development and treatment. The research group extracted various parts of the plants (leaves, stems, rhizomes, roots, and fruits) using methanol (MeOH) and ethanol (EtOH) and analyzed them through phytochemical screening, ^{1}H NMR spectroscopy, antimicrobial testing, and assays for antioxidant properties like total phenolic and flavonoid content. The analysis identified compounds such as tannins, saponins, free fatty acids, and flavonoid-related molecules. ^{1}H NMR results also pointed to the presence of sugars, aglycones, and free fatty acids. In antimicrobial tests, all extracts were effective against *Bacillus subtilis* and *Staphylococcus aureus*, with the fruit extracts showing especially potent activity against Escherichia coli and *Salmonella typhimurium*, even surpassing the antibiotic Amikacin. These findings suggest significant antimicrobial potential, making these plants promising candidates for developing new treatments. This study is the first to detail the bioactive compounds of *V. sepulchrei* and *V. vanoverberghii*, highlighting their potential as sources of new drugs for combating bacterial infections. By further investigating these bioactive compounds, we can pave the way for novel treatments that may be used in tackling antibiotic-resistant infections and other medical conditions. Continued research will be crucial in isolating these compounds and understanding their mechanisms of action, with the goal of integrating them into modern pharmaceutical applications.

PB-21

DEVELOPMENT AND VALIDATION OF ELISA FOR THE MEASUREMENT OF TRASTUZUMAB ANTI-DRUG ANTIBODY IN RAT SERUM

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During nonclinical studies of biologics, pharmacokinetic (PK) analysis is crucial for demonstrating product quality. Anti-drug antibodies (ADA) produced by the host species during PK studies can influence the interpretation of results. Therefore, developing an ADA detection method for specific biologics is essential for accurate PK analysis. In this study, we developed and validated an ADA detection assay for trastuzumab in rat serum using a bridging ELISA with a commercial anti-trastuzumab antibody. The validated parameters included an LLOD of 100 ng/mL and a ULOQ of 405 ng/mL, meeting FDA-recommended ADA detection levels (250-500 ng/mL). The assay's drug tolerance was determined to be no more than 100 ng of trastuzumab in rat serum samples. The validated technique was applied to rat serum samples from animals that received trastuzumab in PK study. Results showed no ADA-positive serum in animals that received five doses of trastuzumab over two weeks. These findings correlated with the drug serum levels observed in all animals. A commercial trastuzumab detection kit was also used to detect ADA in rat serum, producing results consistent with the in-house ADA detection method. Thus, we conclude that the validated trastuzumab ADA detection method was successfully applied in nonclinical studies.

PB-22

BIOACCESSIBILITY AND BIOACTIVITY OF MICROENCAPSULATED CALAMANSI WASTE EXTRACT DURING SIMULATED DIGESTION

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Calamansi (*Citrus microcarpa*) is a widely cultivated fruit in Southeast Asia. Commercial processing of calamansi generates significant byproducts, such as peels and seeds, which are often underutilized. Calamansi waste is rich in valuable bioactive compounds, including phenolics and flavonoids, which are known for their various health benefits. However, these compounds are susceptible to degradation during digestion, reducing their bioavailability. Encapsulation by spray drying has emerged as an effective technique to protect these compounds and enhance their stability during digestion. Despite its potential, limited research has explored the bioavailability of microencapsulated calamansi waste extract during gastrointestinal digestion. Understanding the effects of encapsulation on the bioavailability of these compounds is essential to maximizing their potential as functional food ingredients. Therefore, this study aims to evaluate the bioaccessibility and bioactivity of bioactive compounds in microencapsulated calamansi waste extract powder (MCWEP) during simulated gastrointestinal digestion. The results showed that at least 30% of bioactive compounds were bioaccessible at the intestinal phase. Phenolic compounds (PCs) exhibited the highest bioaccessibility during the oral phase (81.17%) but dropped to 32.77% in the intestinal phase. The degradation of phenolic compounds is primarily caused by their interaction with digestive enzymes and the changes in pH levels throughout the digestion process. In contrast, flavonoids exhibited their highest bioaccessibility (40.22%) in the intestinal phase, likely due to hydrolysis and depolymerization of flavonoid induced by pancreatin and rising pH levels. The bioactivities of MCWEP after digestion varied depending on the antioxidant assays employed. DPPH radical scavenging activity was significantly reduced by 77% after digestion, with a strong correlation to PCs ($r = 0.701$), suggesting that phenolics, which are more prone to degradation during digestion, are the primary contributors to DPPH activity. Ferric reducing antioxidant power retained moderate activity after digestion, showing only a 21% reduction, with a strong correlation to PCs ($r = 0.898$), indicating that phenolics also contribute to reducing power. Total antioxidant capacity decreased substantially during the gastric phase but increased by 353% in the intestinal phase, likely due to the higher stability and bioavailability of flavonoids at this stage ($r = 0.858$). Ferrous ion chelating activity remained stable through the oral and gastric phases. It increased by 201% after intestinal digestion, aligning with the enhanced bioavailability of bioactive compounds under alkaline conditions, which promote ferrous ion chelation. ABTS radical scavenging activity increased by 254% after digestion, suggesting that MCWEP showed greater efficacy in scavenging ABTS radicals than DPPH radicals. This study demonstrates that microencapsulation by spray-drying effectively protects bioactive compounds in calamansi waste and enhances their bioavailability during the gastrointestinal digestion process. The increased antioxidant activity after digestion suggests that MCWEP could serve as a functional food ingredient, contributing to food security by transforming underutilized fruit waste into health-promoting products.

PB-23

THE EFFECT OF MELATONIN AND SMALL MOLECULES ON CELL REPROGRAMMING IN HUMAN SKIN FIBROBLAST

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Chemically induced neuronal fate reprogramming is another method of direct conversion that uses a set of small molecules to change the intracellular signaling pathway and physiology, as well as improve the effectiveness of conversing for desired cells, including neurons, to lessen the influence on genome modification and instability. Melatonin, a hormone synthesized by the pineal gland, demonstrates neuroprotective effects, especially in regulating neural stem cell (NSC) proliferation and differentiation. In the present study, human skin fibroblasts (HDFs) were seeded in a fibroblast medium before undergoing neural differentiation procedures. Six small molecules can convert human dermal fibroblasts (HDFs) into neuronal cells: valproic acid (VPA), a histone deacetylase inhibitor; CHIR99021, a GSK-3 inhibitor; Forskolin, a cAMP agonist; SP600625, a JNK inhibitor; Y-27632, a ROCK inhibitor; and melatonin, an antioxidant. We uses immunostaining and scanning ion conductance microscopy (SCIM) to investigate the conversion efficiency of small molecules in combination with melatonin during direct neural reprogramming.SCIM was used to analyze the mechanical stiffness of both induced and non-induced cells after 24 hours of culture. Next, we use immunostaining to assess the expression of neuronal markers. The result revealed that melatonin induced the expression of both neuronal markers, including MAP2, DCx, and synaptophysin. The findings indicated that melatonin might help the maturation of neurons that have been directly converted and the expression of synaptic protein.

PB-24

LEGUME-5K PROTECTS SH-SY5Y CELLS FROM ERASTIN-INDUCED NEURITE OUTGROWTH DAMAGE LINKED WITH OTUB1 AND MIDKINE

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As life expectancies rise over the coming decades, the impact of neurodegenerative illnesses would also rise. There is currently no treatment for these conditions—just medications that only mask the symptoms. This study discovered possible usage of legume-5K hydrolysate (PH) for neuroprotective medication as multiple positive effects on the human neuronal cell line SH-SY5Y were shown. MTT assay showed that 24 hours treatment of PH did not impair cell viability of SH-SY5Y cells at concentrations up to 100 µg/mL. ImageJ analysis showed that 24 hours treatment of PH at 1 µg/mL significantly increased neurite length of SH-SY5Y cells by 20% as compared to control. Next, we used a fluorometric technique to measure choline that could result from the destruction of neurotransmitter acetylcholine, an important molecule for neuronal functions such as memory. The result showed that 1 µg/mL PH did not cause acetylcholine breakdown, reflecting acetylcholine remained intact. Thus, it was anticipated that PH would not impair but possibly enhanced neuronal function by facilitating neurite extension. We further investigated the effect of 1 µg/mL PH on erastin-induced neurite outgrowth damage. Twenty-four hours treatment of erastin at 5 µM was considered as sub-lethal concentration in this study as cell viability was impaired less than 20%. However, the neurites of SH-SY5Y cells were already shortened upon erastin treatment by 20-30% as compared to control. Even while neurite length decreased after 5 µM erastin treatment, it remained comparable to the control when PH was present. This result indicated that PH could have mitigated the damage that erastin caused to neurite outgrowth. Differential protein expression associated with neuroprotective effect of PH against erastin neurotoxicity was then investigated using proteomics. Results revealed 288, 155, 140, and 200 proteins presented only in control, PH, erastin, and erastin-PH co-treatment, respectively whereas 2,509 proteins were found in all conditions. Interestingly, PEAK studio analysis (fold change cut-off 1.2 and p-value<0.05) suggested ubiquitin thioesterase OTUB1 (OTUB1), a neurite-related protein stabilizer, and midkine (MK), neurite promotor, may play roles in PH against erastin-induced neurite outgrowth damage. Validation using immunoblotting of OTUB1 and MK were then performed. Results showed that PH induced 1.25 and 1.2-fold increases in OTUB1 and midkine, respectively. Upon erastin treatment, OTUB1 and midkine were 1.2-fold lower and did not alter, respectively; however, both proteins rose by 1.2-fold when PH was co-administered. Thus, PH could promote neurite extension in SH-SY5Y cells and trigger the production of proteins that promote neurite growth. This study indicating that PH might be employed as a treatment for neurodegenerative illnesses.

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PB-25

DEVELOPMENT AND VALIDATION OF AN HPTLC METHOD FOR DETERMINING MITRAGYNINE IN OD-FIN POLYHERBAL EXTRACT

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The purpose of HPTLC method for quantifying the biomarker; Mitragynine in OD-FIN, a polyherbal extract for drug withdrawal, has been validated for routine analysis. The samples were applied on silica gel HPTLC plate using in n-hexane: ethyl acetate (6:4) as a mobile phase the validated method was specific to the compound with R_f values 0.12±0.01. Mitragynine showed acceptable validation parameters within 100-500 ng of the quantitative range. The linear equation and correlation coefficient (R^2) was y = 6E-06x - 9E-05, R^2 = 0.9999. The lowest amount detected and quantified at 222 nm was 11.95. A recovery study has been performed to estimate the accuracy of the analytical procedure at low, medium, and high concentrations with 99.82%±2.68, 101.39%±3.57, and 97.65%±5.18. Intra-day and inter-day precision showed %RSD less than 2.75 and 8.82. The method was robust with less than 10% deviation from minor change of detection wavelengths for the substance. The purpose method was specific for quantification of Mitragynine in the extract which can be used for routine analysis.

PB-26

ALOIFOL I: A NOVEL ANTI-INFLAMMATORY AGENT WITH FAVORABLE CNS SAFETY PROFILE FOR TREATING SICKNESS BEHAVIORS

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Sickness behaviors, characterized by fever, fatigue, and lethargy, are adaptive responses to inflammation and infection. Current treatments like nonsteroidal anti-inflammatory drugs (NSAIDs) often have limitations, highlighting the need for safer and more effective anti-inflammatory therapies. Natural compounds like Aloifol I, a phenanthrene stilbenoid derived from Dendrobium senile, offer promising potential in this regard. This study aimed to investigate the anti-inflammatory effects of aloifol I on sickness behaviors, its impact on peripheral and central immune cells, and its potential CNS side effects. In vitro studies using RAW 264.7 macrophages and BV-2 microglia evaluated the effects of aloifol I on lipopolysaccharide (LPS)-induced proinflammatory cytokine production (IL-6 and TNF-α). In vivo studies in mice assessed the impact of aloifol I on LPS-induced sickness behaviors, fever, and locomotor activity at 12.5, 25 and 50 mg/kg doses (i.p.). Additionally, the study measured proinflammatory cytokines in plasma and brain tissues to assess peripheral and central inflammatory responses. Furthermore, the effects of aloifol I on motor coordination and general behaviors were evaluated using the rotarod test and LABORAS automated home cage monitoring system, respectively. *In vitro* studies revealed the ability of aloifol I to suppress LPS-induced proinflammatory cytokine production (IL-6 and TNF-α) in both RAW 264.7 macrophages and BV-2 microglia cells. *In vivo*, aloifol I effectively attenuated LPS-induced fever in mice, reducing body temperature from 38.3°C to baseline levels (36°C) in a dose-dependent manner. Additionally, aloifol I dose-dependently improved LPS-induced locomotor impairments, increasing locomotion and reducing immobility. Notably, the effects of aloifol I at 50 mg/kg dose on home cage behaviors were comparable to those of the positive control, indomethacin (10 mg/kg, i.p.). Moreover, aloifol I significantly reduced LPS-induced proinflammatory cytokine levels in both plasma and brain tissues. The effects of aloifol I (50 mg/kg) on LPS-induced IL-6 and TNF-α levels in both peripheral and central tissues were comparable to those of indomethacin at 10 mg/kg. Importantly, the CNS safety evaluation demonstrated that the highest effective dose of aloifol I had no effect on motor coordination, long-term locomotor activity, or food and water intake of mice. In contrast, the positive control, chlorpromazine (5 mg/kg, i.p.), significantly impaired these parameters. In conclusion, aloifol I exhibits promising anti-inflammatory properties and a favorable CNS safety profile, suggesting its potential as a therapeutic candidate for inflammation-related disorders, including sickness behaviors. Further research is necessary to elucidate its exact mechanisms of action and explore its clinical applications.

PB-27

FORMULATION AND EVALUATION OF TOPICAL GEL USING SECURINEGA LEUCOPYRUS AND ALOE VERA FOR WOUND HEALING ACTIVITY

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Chronic ulcers are a major problem in health care worldwide. The pathogenesis of chronic wounds is complex, resulting in major difficulties in the management of such wounds. Despite the recent advances in the field of wound management and novel medications for wound healing and skin regeneration, traditional methods based on herbal and natural therapeutics are still known as promising alternative medications. Therefore, the present work aims to formulate and evaluate the *Securinega leucopyrus* based topical gel for wound healing activity. The water extract of Securinega leucopyrus leaves was prepared using the maceration method and incorporated into 04 topical gel formulations at 04 different concentrations (F1-F4), with *Aloe vera*, methyl paraben sodium, propyl paraben sodium, carbopol 934, and triethanolamine as the bases. The prepared gel formulations were characterized for preliminary phytochemical analysis, spreadability, pH, viscosity, homogeneity and extrudability. Antimicrobial activity was evaluated for the 04 gel formulations. The results of preliminary phytochemical investigation showed the presence of alkaloids, carbohydrates, flavonoids, saponins, phytosterols, diterpenes and glycosides. Other parameters such as spreadability, pH, viscosity and extrudability were found to be well within the limits. The antimicrobial activity of optimized formulation (50g) with Securinega leucopyrus 10g and *Aloe vera* 25g concentration was found to be effective in both gram positive (*Staphylococcus aureus*) and gram-negative organisms (*Escherichia coli*). From the present study, it was concluded that the prepared herbal gel formulation showed significant antimicrobial potential and further studies are needed with a clinical trial confirm this.

PB-28

UNCOVERING THE POTENTIAL OF DEAB AS A COMPOUND SELECTIVELY KILLS MULTIDRUG-RESISTANT LUNG CANCER CELLS

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Multidrug resistance (MDR) is a major obstacle in chemotherapy and being a main cause of cancer mortality. MDR is frequently observed in multiple cancer types including non-small cell lung cancer (NSCLC) which is a subtype accounting for 85% of diagnosed lung cancer cases. Several lines of evidence suggest that while drug resistance phenotype is acquired, the cells also possess hypersensitivity towards some compounds, liked an “Achilles’ heel” of MDR cancer cells. This phenomenon is called collateral sensitivity that has been interested as a strategy for selective killing of MDR cancer cells. By using A549 human NSCLC cell line and its MDR sub-line A549RT-eto, a cytotoxicity screening for MDR-selective agent has been established with assessment of cell survival rate after 72 h treatment using MTT assay. We could identify 4-diethylaminobenzaldehyde (DEAB) as a compound that exerted greater cytotoxicity against A549RT-eto cells relative to A549 cells, by 1.7-fold difference in their IC_{50} values. DEAB is a slow competitive substrate for aldehyde dehydrogenases and is used in ALDEFLUORTM assay for determination of stem cells. Aldehyde dehydrogenase catalyzes conversion of aldehydes to carboxylic acids coupling with reduction of NAD+ to NADH. Intracellular levels of NAD^+, NADH, and ATP were determined by using luminescent assay kits. After 15 min treatment, DEAB rapidly decreased intracellular NAD^+ level in A549RT-eto cells with 35% reduction, while a 6% decrease was observed in A549 cells. An increase in NADH level in A549RT-eto cells by DEAB treatment (3.8-fold increase) was greater than that of A549 cells (3.3-fold increase), resulting in elevation of NADH/NAD^+ ratio from 0.28 to 1.57 in A549RT-eto cells, and from 0.21 to 0.69 in A549 cells. DEAB also reduced intracellular ATP level in the cells, 85% ATP depletion was observed in A549RT-eto cells compared to control, while ATP depletion in A549 cells was 49% decrease after 15 min treatment. A glycolysis inhibitor 2-deoxy-D-glucose (2-DG) was used as a positive control to deplete ATP level in the cells. 2-DG treatment for 15 min reduced ATP level in A549RT-eto and A549 cells by 48% and 36% depletion, respectively, compared to control. 2-DG was more cytotoxic to A549RT-eto cells relative to A549 cells, by 1.6-fold difference in their IC_{50} values. In conclusion, the MDR-selective cytotoxicity of DEAB was uncovered and its mechanism of action was elucidated. DEAB has a potential as a lead compound for the development of MDR-targeting drugs.

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PB-29

HMG-COA REDUCTASE AND CHOLINESTERASE INHIBITORS OF CITRUS HYSTRIX: MOLECULAR DOCKING–ENZYMATIC EVALUATIONS

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Citrus hystrix DC, leech lime of the Rutaceae family, is commonly found in Southeast Asia. It is a small plant of about 3-8 m in height. The leaves and fruits of this plant have long been used as an ingredient in several Thai cooking recipes. Recent studies reported the presence of several types of secondary metabolites with diverse skeletons. Among the isolates, the prenylfuranocoumarin–hydroxymethylglutaric acid (HMGA) conjugates, such as the prenylfuranocoumarin–HMGA–flavonol glucoside conjugates and prenylfuranocoumarin-HMGA-1-O-isopropyl-β-D-glucopyranoside conjugates, are of particular interest due to the presence of the HMGA moiety. HMGA is known to be a building block in terpenoid and steroid biosynthesis. In the present study, it was proposed that the HMGA conjugates that share HMG moiety could bind competitively in the HMG-CoA reductase active sites, thus suppressing mevalonate generation, resulting in a decrease of cholesterol formation and consequently alleviate hypercholesterolemia as statins were thought to do. Molecular docking of selected compound structures and assay for HMG-CoA reductase inhibitory activity of selected pure compounds and extracts were investigated. Furthermore, some HMGA conjugates were also reported to possess cholinesterase inhibitory activity, molecular docking and inhibition of AChE and BChE of additional pure isolates were also investigated.

PB-30

DEVELOPING A SYSTEM FOR THE RATIONAL USE OF ANTIBIOTICS IN AGRICULTURE IN A PARTICIPATORY MANNER IN AMNATCHAROEN PROVINCE

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Antibiotics are used in veterinary medicine for treating diseases, preventing outbreaks, and promoting growth. The use of antibiotics before bacterial infection occurs, their widespread and inappropriate use, have led to the development of drug-resistant bacteria in animals. To study and develop a rational antibiotic usage system in the agricultural sector through participatory approaches in Amnatcharoen Province. This research employed a participatory action research approach with network partners. Data was collected through interviews using questionnaires. The sample consisted of 53 animal drug stores, agricultural supply stores, and feed stores. The study was conducted in 2023, and data were analyzed using descriptive and content analysis methods. The majority of the sample population were female (66%), aged 35-45 years (45.3%), operated long-term businesses (39.6%), and sold animal feed (66%). Antibiotics were sold without a license by 65.5% of the stores. There was a lack of legal compliance (69%) and issues with counterfeit or substandard drugs (62.1%) without suitability analysis (55.2%). Operators had knowledge of antibiotic use at 43.39%, with misunderstandings about drug use and residue in animal waste and the environment at 26.42%. Network participation led to the development of guidelines for regulating antibiotic use in feed stores and agricultural product stores. Controlling antibiotic use in agriculture should involve integrated cooperation from all sectors. The scope of the study should be expanded to other regions, and factors affecting antibiotic use should be investigated to address issues appropriately.

C.
ANTIMICROBIAL RESISTANCE

PC-01

TRACKING ANTIBIOTIC RESISTANCE GENES AND HUMAN FECAL MARKER CRASSPHAGE IN HOSPITAL EFFLUENTS

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Antimicrobial resistance (AMR) is a global health challenge, with hospital wastewater acting as a significant source of antibiotic resistance genes (ARGs) that can disseminate through aquatic ecosystems. Hospital wastewater treatment plants (WWTPs) are critical in reducing this risk, but ARGs and other resistant microorganisms may still be released into downstream environments. This study aimed to investigate the presence of ARGs and their correlation with crAssphage, a viral marker specific to human fecal contamination, in a hospital wastewater treatment plant and a receiving canal. CrAssphage is a bacteriophage commonly found in the human gut, making it a reliable microbial source tracking (MST) marker for human fecal contamination. Its detection alongside ARGs can help trace the spread of AMR linked to human waste. Using high-throughput qPCR, 94 ARGs and crAssphage were analyzed across water samples from different stages of the WWTP process. Seven ARGs were further quantified using qPCR. The highest diversity of ARGs was observed in raw wastewater, with significant reductions following treatment, though some ARGs persisted in treated effluent and the receiving canal. Removal efficiency, expressed as log reduction values (LRV), ranged from 0.60 to 3.23 across different ARGs, with *tet*Q showing the greatest reduction and mcr-1 the least. Strong correlations between ARGs and crAssphage concentrations were observed, suggesting that crAssphage could serve as a proxy for tracking ARGs and human fecal contamination in wastewater. These findings underline the importance of including environmental surveillance, particularly in hospital wastewater systems, in global AMR monitoring programs. The use of crAssphage as a human-specific marker alongside ARG monitoring offers a promising approach to understanding the spread of AMR from clinical settings into the broader environment.

PC-02

PROSPECTS OF BACTERIOPHAGES IN DEVELOPING SOLUTIONS TO REDUCE ANTIBIOTIC ABUSE IN AQUACULTURE IN THAILAND

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Tilapia aquaculture is a major revenue source of Thailand's economy and is highly susceptible to bacterial pathogens due to unsustainable stocking densities. The development of environmental antibiotic resistance due to antibiotic abuse in the field is a major health concern. Therefore, an alternative therapy to antibiotic usage for bacterial infectious diseases in fish are urgently needed. This study focuses on isolating bacteriophages against pathogenic *Aeromonas* spp. for use in therapeutic application against infectious fish diseases. Water samples from multiple wastewater treatment plants, animal farms, and a fish farm from different provinces in Thailand was enriched with *Aeromonas* host bacteria isolates (AH_62, 67, 68, and 82) and phages were isolated. Seven selected purified phages were visualized using transmission electron microscopy (TEM) and host specificity was assessed. *In-vitro* performance of eight phage cocktails (A~H), formulated with the above seven phages was evaluated using host growth inhibition curves at the multiplicity of infection values ranging from 0.01 to 01. As for TEM, selected phages morphologically belong to various families, including *siphoviridae*. Each respective phage was only specific to the bacterial host used for enrichment but to no other tested bacteria species. As for overall host growth inhibition curve kinetics of phage cocktails, Cocktail B appeared to be the most ideal cocktail against all 4 bacterial isolates based on coverage and level of effectiveness. Based on the above results, it can be concluded that wastewater can be a valuable source of phages against Aeromonas spp. and a great asset in developing therapeutic solutions for fish in reduction of antibiotic usage associated with aquaculture in Thailand.

PC-03

THE STUDY OF ANTIBIOTIC RESISTANCE MECHANISMS IN *PSEUDOMONAS AERUGINOSA* PCIP SCREENED FROM PAO1 GENOMIC DNA LIBRARY

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Pseudomonas aeruginosa is one of the multidrug-resistant pathogens that is responsible for clinical challenging nosocomial infections. *P. aeruginosa* PAO1 genomic DNA library was constructed aiming to mimic the over-expression of gene(s) by using plasmid mediated system. A ciprofloxacin resistant isolate, PCIP, was screened from the library. Antibiotic susceptibility among various drug groups were determined by disk diffusion assay. PCIP showed the changes in antibiotic susceptibility against quinolones, imipenem, chloramphenicol, trimethoprim, aminoglycosides and certain β-lactams. DNA sequencing revealed that *PA2048*, putative quinol monooxygenase, was laid in the plasmid of PCIP. Sub-cloning of *PA2048* to pBBR1MCS-4 confirmed its effect on the changes in antibiotic susceptibility. Proteomic analysis revealed the mechanisms and network contributing in the changes. MexE, MexF, and OprN were significantly increased in PCIP by 233-, 28- and 93-fold, respectively, compared to the PAO1 wild-type. These up-regulation were confirmed by qRT-PCR. MexEF-OprN is an RND efflux pump system, which comprises of membrane fusion protein (MexE), transporter (MexF), and porin or outer membrane protein (OprN). The over-expression of MexEF-OprN is known to be responsible for against quinolones, chloramphenicol and trimethoprim resistant. Altogether, high level of PA2048 in *P. aeruginosa* results in the up-regulation of MexEF-OprN, which leads to multidrug resistance phenotypes.

PC-04

TRACKING ESBL-PRODUCING E. COLI AND ANTIBIOTIC RESISTANCE GENES IN LIVESTOCK WASTE FOR ONE HEALTH SURVEILLANCE

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Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-*E. coli*) are major contributors to antimicrobial resistance (AMR) and pose a significant public health threat. This study is focused on isolating and characterizing ESBL-*E. coli* from animal manure and farm wastewater collected from various livestock, including pigs, cows, goats, chickens, and ducks. Samples were cultured on MacConkey agar with 4 µg/mL cefotaxime to select for ESBL-producing strains. *E. coli* isolates were confirmed via conventional PCR for species identification. Additionally, PCR assays were used to detect key antibiotic resistance genes, including those conferring resistance to colistin (mcr genes), carbapenems (bla_{NDM}, bla_{VIM}, bla_{KPC}), and extended-spectrum beta-lactamases (ESBLs), particularly the bla_{CTX-M} gene. The findings reveal the presence of ESBL-*E. coli* and associated resistance genes in agricultural environments, highlighting potential reservoirs of AMR within animal farming systems. These results stress the need for continuous surveillance of antimicrobial resistance in livestock as part of One Health approaches, which seek to curb the transmission of resistant bacteria between animals, humans, and the environment.

PC-05

OXIDATIVE STRESS MEDIATED MECHANISTIC INSIGHTS OF SILVER NANOPARTICLES IN BACTERIAL CELLS TO OVERCOME ANTIMICROBIAL RESISTANCE

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One Health emphasizes the interconnectedness of human, animal, and environmental health, recognizing that these domains are deeply intertwined and mutually influential. The challenges inherent in this holistic approach are particularly pronounced when addressing emerging threats such as antimicrobial resistance (AMR). The looming problem of resistance to antibiotics in microorganisms is a global health concern and so it is extremely important to develop not only the therapeutic interventions to curtail the infections but also the strategies to avert the menace of microbial drug resistance. Nanotechnology offers a promising platform for addressing this challenge. Nanoparticles have unique properties that make them highly effective in combating bacterial infections by inhibiting the growth and survival of multidrug-resistant bacteria. Woodfordia fruticosa has several phytochemicals having different therapeutic and pharmacological properties. Thus, the current study investigated the antibacterial activity, and oxidative stress-mediated mechanistic insights of *Woodfordia fruticosa* flower extract-mediated silver nanoparticles (Wf-AgNPs) on gram-positive *Staphylococcus chromogenes* and gram-negative *Pseudomonas aeruginosa*. Here, AgNPs were synthesized using the *Woodfordia fruticosa* flower extract and were characterized using various analytical techniques. The Wf-AgNPs had a UV-Vis absorption peak at 360 nm and 520 nm along with a hydrodynamic size of 121.6 nm and a zeta potential value of -28 mV. Wf-AgNPs had significant antibacterial properties against gram-positive and gram-negative organisms detected by antimicrobial susceptibility test (AST) and minimal inhibitory concentration (MIC). This antibacterial property is due to the induction of oxidative stress in bacterial cells, resulting in a significant increase in key protein regulators viz., lipid peroxidation and protein carbonyl content together with a decrease in the activity of catalase, glutathione reductase, glutathione-s-transferase, superoxide dismutase in the, that results in inhibition of further bacterial growth. Thus, the above results represent conclusive findings on the mechanism of Wf-AgNPs against gram-positive and gram-negative bacteria and demonstrate the promising use of nanoparticles as antibacterial agents.

PC-06

A SYNERGISTIC ANTIBIOTIC COMBINATION AGAINST PULMONARY NTM

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Mycobacterium abscessus is the most common nontuberculous mycobacteria (NTM) implicated in lung diseases. A low rate of successful chemotherapeutic treatment against *M. abscessus* is mainly due to the bacterium being intrinsically drug resistant to most antibiotics. The frontline antibiotic against *M. abscessus* - clarithromycin - is losing its effectiveness as more *M. abscessus* strains carry the erm(41) gene that confers resistance against clarithromycin. We have successfully identified a combination of clarithromycin and rifaximin that is effective against *M. abscessus* regardless of the presence of erm(41) gene. This synergistic combination showed *in vivo* efficacy against *M. abscessus* in a Zebrafish infection model. For the next step, we are formulating the two FDA-approved antibiotics in a suitable formulation for pulmonary inhalation delivery in mouse lung infection models.

PC-07

DEVELOPING A NOVEL CLASS OF ANTIBIOTIC BY TARGETING tRNA METHYLTRANSFERASE (TRMD)

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Bacterial RNA modification pathways are critical to cell survival under stress and are ideal mechanism-based targets for developing antimicrobials. TrmD, which is tRNA-(N1G37) methyltransferase, is an essential enzyme in many bacterial pathogens and is structurally distinct from its human homologue, making TrmD an attractive target. In our study, we used meta-analysis of published hits for drug-like parameters to curate commercially available compounds with specific scaffolds. Screening whole cell activity of these compounds resulted in hits with potent MICs against Acinetobacter baumannii with TrmD inhibition as a possible mechanism of action. We will perform medicinal chemistry as a next step to optimize the initial hits for TrmD, guided by traditional MedChem parameters and the new eNTRYway rules for drug design. Compounds that inhibit the TrmD enzyme and kill the bacteria would be a novel class of antibiotic with a novel antibacterial mechanism, providing new treatment options against multidrug-resistant bacterial infections.

PC-08

INVESTIGATION OF THE *AGROBACTERIUM TUMEFACIENS* NOVEL ZUR-REGULON MEMBERS IN ZINC MODULATION

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Sufficient allocation of quintessential trace elements for metal-indispensable proteins is critical for bacterial survival and pathogenesis. In the case of Zn, bacteria have evolved systems to alter Zn metabolism in response to prevailing Zn levels. The Zn-dependent repressor Zur regulates a number of genes involved in Zn metabolism in *Agrobacterium tumefaciens*. An RNA-seq transcriptome analysis was performed comparing a *zur*-inactivation mutant to wild-type (WT) to identify the differentially-regulated genes (DEGs) of the Zur regulon. Thirty-two DEGs were identified and validated by quantitative RT-PCR (qRT-PCR). In silico investigations demonstrated that two COG0523-family metal chaperones, *atu4502* and *atu3633*, possess putative *zur* boxes in their promoter regions and are localized in different sub-cellular compartments. Moreover, their putative consensus sequences were identified to overlap with their respective zur boxes which provided vital insights into the mode of regulation of Zur. qRT-PCR analyses in the zur-mutant and WT indicated that both genes are negatively regulated by Zur. Insertional inactivation mutants, *atu4502*::Km and *atu3633*::Gm were constructed for physiological phenotypic characterizations. *atu4502*::Km was sensitive to the cell-impermeable metal chelator, EDTA in AB minimal media suggesting that is was localized in the periplasm. By contrast, *atu3633*::GM was sensitive to the cell-permeable chelator, TPEN in the same media suggesting that it was localized in the cytoplasm. Translational promoter-fusion assays indicated that both genes are directly regulated by Zur and that the *zur* boxes are crucial for transcriptional repression of each gene.

PC-09

ROLES OF 16S rRNA METHYLTRANSFERASE KSGA IN OXIDATIVE STRESS RESPONSE AND ANTIBIOTIC RESISTANCE OF *PSEUDOMONAS AERUGINOSA*

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Ribosomal RNA (rRNA) modifications are crucial in ribosome biosynthesis. The 16S rRNA adenine dimethyltransferase KsgA, specifically methylates adenines at positions 1518 and 1519 (A1518/1519) near the decoding center of ribosome. These methylations are conserved and play important roles in ribosome biogenesis and protein synthesis. In this study, we identified a lack of A1518/1519 methylation in the 16S rRNA of the *Pseudomonas aeruginosa ksgA* mutant. Using Biolog phenotypic microarrays, we screened the ksgA mutant against several antimicrobial agents and found that the absence of *ksgA* increased sensitivity to menadione, a superoxide generator, due to reduced superoxide dismutase (SOD) activity. Interestingly, a reduction in SOD activity was associated with lower SodM protein levels, despite unaltered sodM mRNA levels. Additionally, the *ksgA* mutant exhibited increased sensitivity to hygromycin B and tylosin. Molecular docking showed that hygromycin B bonds more strongly to 16S rRNA lacking methylation at A1518/1519, consistent with the observed sensitivity. The increased sensitivity to tylosin was linked to decreased transcription of *tufA*, *tufB*, and *tsf*, which encode elongation factors. Taken together, these results suggest that KsgA-mediated 16S rRNA methylation influences the oxidative stress response and antibiotic susceptibility in *P. aeruginosa*.

PC-10

ANTIMICROBIAL RESISTANCE MECHANISMS: INVESTIGATION OF ROLE OF THE FERRITIN-LIKE GENE ON ANTIBIOTIC RESISTANCE IN STENOTROPHOMONAS MALTOPHILIA

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Antimicrobial resistance (AMR) represents a significant global health challenge, with a pressing need to understand its underlying mechanisms. In this study, we explored the role of the *ferritin-like (frl)* gene in *Stenotrophomonas maltophilia* K279a and its involvement in iron homeostasis, antibiotic susceptibility, oxidative stress responses, and bacteria reproduction in low temperatures. The results of antibiotic susceptibility tests using the disk diffusion assay demonstrated that the frl knockout strain exhibited increased resistance to various antibiotics, including quinolones, aminoglycosides, cephalosporins, and β-lactams. The *frl* knockout strain also showed increased tolerance to oxidative stress-generating compounds, such as hydrogen peroxide, tert-butyl peroxide, diamide, and menadione sodium bisulfite. The *frl* gene is also involved in iron homeostasis, as evidenced by the *frl* knockout strain's increased resistance to iron-chelating agents and toxic ferrous sulfate. Intriguingly, the frl knockout strain demonstrated its ability to grow at low temperatures (20ºC and 25ºC), highlighting the frl gene's role in environmental adaptability. Analysis of *S. maltophilia* genomes available in public databases revealed that the *frl* gene is mostly found at a high frequency in the clinical strains compared to the environmental strains. Using flow cytometry, the *S. maltophilia* the wild-type strain K279a showed higher levels of intracellular ferrous ion (Fe^{2+}) than the *frl* knockout strain. This study underscores the *frl* gene's multifaceted contribution to antimicrobial resistance, oxidative stress response, and environmental adaptation in *S. maltophilia*, emphasizing the need for a One Health perspective in tackling AMR.

PC-11

ENROFLOXACIN-INDUCED ANTIMICROBIAL RESISTANCE OF STENOTROPHOMONAS MALTOPHILIA

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Enrofloxacin is a quinolone antibiotic commonly used in livestock activities. Improper use can lead to a release of antibiotics into the environments through animal waste and other products. Residual antibiotics can induce antimicrobial resistance (AMR) of the environmental microorganisms. Stenotrophomonas maltophilia is a gram-negative bacterium that is ubiquitous in nature found in both soil and water. It is the one of most important AMR organisms, causing hospital-acquired infection, especially in immunocompromised patients, commonly through contamination of medical devices. Nowadays, there are limited antibiotics available for treatment of S. maltophilia infection due to the bacterium's intrinsic resistance to many antibiotics. The suggested antimicrobial agents approved by the US Food and Drug Administration for clinical use including levofloxacin, minocycline, and trimethoprim/sulfamethoxazole. In this study, the effect of residual enrofloxacin on the induction of AMR development was investigated using S. maltophilia K279a as a microbial model. The enrofloxacin-resistant mutants of S. maltophilia was selected using a sequential subculture technique in the presence of increasing concentrations of enrofloxacin. Most of the enrofloxacin-resistant strains showed levofloxacin resistance due to elevated expression of intrinsic antibiotic resistance genes. Moreover, some of them showed reduced susceptibility to trimethoprim/sulfamethoxazole and minocycline, which are the first-line antibiotic recommended for the treatment of infections caused by S. maltophilia. Whole genome sequencing (WGS) was performed to identify the mutations in the enrofloxacin-resistant strains. Mutations in genes associated with resistance and metabolism regulation were identified in enrofloxacin-resistant strains, such as pilW, kef, and wxocBC. These genes could be the promising new candidates for antimicrobial resistance.

PC-12

QUINOCLAMINE INHIBITS MITOMYCIN-C-INDUCED SOS RESPONSE IN *PSEUDOMONAS AERUGINOSA* PAO1

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An increase in antimicrobial-resistant bacteria has led to increased mortality rate, economic losses, and limited therapeutic options. The SOS response plays a vital role in response to genotoxic stress and is thought to involve in the evolution of antibiotic resistance. *Pseudomonas aeruginosa* is a major pathogen that can cause severe symptoms in cystic fibrosis and immunocompromised patients. This study aimed to investigate the inhibitory effects of quinoclamine (QCM) on the SOS response in *P. aeruginosa* PAO1. The recA deletion mutant and *recA*-GFP reporter strains were constructed for SOS response study. The inhibitory effect of QCM on the SOS response induced by mitomycin-C (MMC) was assessed by monitoring green fluorescence intensity and absorbance at 600 nm. Cell viability was confirmed through colony counts, and scanning electron microscopy was used to observe cell morphological changes and elongation. The antibacterial susceptibility profiles of both wild-type and mutant strains were determined by a minimum inhibitory concentration (MIC). The results showed that MMC significantly increased *recA* expression compared to the uninduced group, and QCM significantly inhibited the MMC-induced SOS response in *P. aeruginosa* PAO1 in a concentration-dependent manner. SEM images phenotypically showed that bacterial cells exposed to MMC exhibited longer cell morphology compared to uninduced control cells. This elongation was suppressed by QCM treatemnt, which was similar to those of MMC-free and recA knockout mutant cells. The MICs of MMC, quinoclamine, nalidixic acid, ceftazidime, and cefotaxime against *P. aeruginosa* PAO1 were 1, >1024, 128, 1, and 4 μg/ml, respectively. In conclusion, QCM inhibited the MMC-induced SOS response in *P. aeruginosa* PAO1 by suppressing RecA activation, in turn preventing LexA auto-proteolysis, and consequently no activation of SOS regulons. The toxicity and efficacy test of QCM alone or in combination with antibiotics should be further investigated.

PC-13

TETRACYCLINE INDUCES MINOCYCLINE RESISTANCE IN *STENOTROPHOMONAS MALTOPHILIA*

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The increasing misuse and overuse of tetracycline antibiotics, especially in the veterinary and livestock activities, is one of the important factors contributing to antimicrobial resistance (AMR). *Stenotrophomonas maltophilia* is a soil and aquatic gram-negative bacterium that can cause hospital acquired infections. In this study, we investigated the effect of exposure of increasing concentration of tetracycline on the induction of minocycline resistance in *S. maltophilia* strain SMBT, which was isolated from a clinical sample. Sequential passages with increasing concentrations (2 to 32 µg/mL) of tetracycline were performed to select tetracycline-resistant strains. These strains were then tested for antimicrobial susceptibility against minocycline, which is a drug recommended for *S. maltophilia* treatment, using the Kirby-Bauer disk diffusion method. Five candidate minocycline-resistant clones were analyzed for the mutated genes using whole genome sequencing. The results revealed mutations in the *smlt4073 (smeT)*ene, which encodes a transcriptional repressor of the RND antibiotic efflux pump SmeDEF. To elucidate the role of the *smeT* gene in minocycline resistance, we constructed smeT knockout mutant strains using insertional inactivation with a suicide vector (pKnock) technology. The *smeT* knockout mutant showed decreased susceptibility to minocycline. Conversely, complementation of the smeT gene from a plasmid vector (pBBR1MCS-5) into the *smeT* knockout mutant increased minocycline susceptibility, while overexpression of smeT in the SMBT wild-type strain also resulted in increased minocycline susceptibility. To our knowledge, the contribution of smeT gene mutations to minocycline resistance in *S. maltophilia* has not been previously reported. Further analysis is needed to fully understand the role of *smeT* in the minocycline resistance of *S. maltophilia.*

PC-14

KILLING EFFECT OF PHAGE-CEFTAZIDIME COMBINATION AGAINST CEFTAZIDIME-RESISTANT *BURKHOLDERIA PSEUDOMALLEI* ON PIG SKIN

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Burkholderia pseudomallei (Bp) is a gram-negative bacilli found in environment throughout tropical regions, causing melioidosis. *B. pseudomallei* can infect both humans and animals and inhalation, cutaneous infection via inoculation or skin abrasion are the major routes of infection. Melioidosis is a serious infectious disease with a high mortality rate and antibiotic-resistant *B. pseudomallei* have increased, leading to treatment failures. Bacteriophages (phages) are ubiquitous viruses which infect bacteria and are being extensively explored as alternatives to antibiotics. The alternative therapeutic use of phages and antibiotic combination has been more considered. This study aims to determine killing activity of phage and ceftazidime (CAZ) combination against ceftazidime-resistant *B. pseudomallei* on pig skin model. A killing of ceftazidime-resistant *B. pseudomallei* (Minimal Inhibitory Concentration, MIC 128 µg/mL) on pig skin was determined by compared between single phage (S4, 10^5 PFU), cocktail phages (CT, 10^5 PFU, mixed of 5 phages including ST99, ST88, W12, S4 and F4 phage), ceftazidime (1/2 and 1/4 MIC), and phage-ceftazidime combination including S4(10^5 PFU)/CAZ (1/2 MIC) and S4(10^5 PFU)/CAZ (1/4 MIC), CT(10^5 PFU)/CAZ (1/2 MIC) and CT(10^5 PFU)/CAZ (1/4 MIC). Viable bacterial cell count of each condition was determined at various time by spot test (Colony Forming Unit, CFU). The results illustrated that the killing activity of S4 (10^5 PFU)/CAZ(1/2MIC) was the most effective for treatment on pig skin model approximately 98.85%. In summary, phage S4/CAZ(1/2MIC) combination was suggested for further investigation on animal model.

PC-15

CHARACTERIZATION OF 5-METHYLURIDINE IN tRNA IN *PSEUDOMONAS AERUGINOSA*

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Pseudomonas aeruginosa possesses multidrug-resistant mechanisms causing nosocomial infections, which increase mortality rates in immunocompromised patients. The tRNA (uracil-5-)-methyltransferase TrmA methylates uridine as m5U on T-loop of all tRNAs at position 54; however the physiological role of TrmA has not been well-studied. Here, we demonstrated that TrmA homolog in *P. aeruginosa* methylates uridine to m5U54 using SAM as a methyl donor in all tRNAs. To investigate the biological role of *trmA*, a phenotypic microarray was performed. Surprisingly, the trmA mutant was more resistant to the drug polymyxin B. Proteomics and AQRNA-seq analyses showed that TrmA regulates expression of proteins belonging to type III secretion system, which involves in pathogenesis, through codon-biased mechanism. The absence of TrmA upregulated the type III secretion system, leading to increased IL-1β level in macrophages infected with the trmA mutant. On the contrary, loss of *trmA* led to hydrogen peroxide hypersensitivity, possibly resulted from reduced heme biosynthesis. Conclusively, this study uncovered the biological roles of trmA in translationally regulating gene expression and in responding to polymyxin B and oxidative stress.

PC-16

ROLE OF HERBICIDES IN ANTIBIOTIC RESISTANCE IN *STENOTROPHOMONAS MALTOPHILIA*

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Antimicrobial resistance (AMR) is a global health concern caused by the misuse and overuse of antibiotics, contributing to 4.95 million deaths in 2019. Antibiotic residues can induce antibiotic resistance in bacteria, however, the role of herbicides in relation to antibiotic resistance mechanisms remains insufficiently investigated. Therefore, *Stenotrophomonas maltophilia*, a Gram-negative bacterium and a known human pathogen, was used to investigate the physiological roles associated with exposure to herbicides, including glyphosate, Roundup (a glyphosate-based-herbicide), and 2,4-Dichlorophenoxyacetic acid (2,4-D). The minimum inhibitory concentration (MIC) of glyphosate, Roundup, and 2,4-D was 12, 24, and 8 mM, respectively. Then *S. maltophilia* were exposed to sub-lethal concentration of MIC prior to testing antibiotic susceptibility. The results indicated that the susceptibility of *S. maltophilia* to aminoglycosides and quinolones decreased upon exposure to glyphosate and Roundup, while resistance to quinolones was mainly associated with 2,4-D. To explore the mechanisms underlying glyphosate-induced antibiotic resistance, aminoglycoside resistance genes were analyzed, and their gene expression was evaluated. A dose-dependent response to glyphosate was assessed using both lethal and sub-lethal concentrations. At a sub-lethal concentration of glyphosate (6 mM), *arg1* expression was significantly induced by 170-fold, whereas lethal concentrations did not induce *arg1*, indicating that glyphosate at 6 mM is effective for inducing arg1 and contributing to an aminoglycoside resistance phenotype.

PC-17

SOXR-REGULATED OXIDATIVE STRESS RESPONSE AND ITS IMPACT ON ANTIBIOTIC RESISTANCE IN *STENOTROPHOMONAS MALTOPHILIA*

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Antimicrobial resistance is one of top threats for global health, as declared by World Health Organization. Understanding of bacterial defense mechanisms is crucial for developing novel and effective treatments. This study focuses on *Stenotrophomonas maltophilia*, an intrinstic antibiotic-resistant opportunistic pathogen, and explores the role of SoxR, a MerR transcriptional regulator, in regulating oxidative stress response and antibiotic resistance. Our previous research revealed that *S. maltophilia* SoxR is crucial in responding to paraquat-induced oxidative stress. We observed that SoxR regulates *mfsA* gene expression and activates another efflux pump, SmeVWX. Upregulation of *smeVWX* was observed when cells were induced by paraquat, and absent in the *soxR* mutant strain. In addition, increased expression of *mfsA* and *smeVWX* efflux pumps resulted in expressing antibiotic resistance phenotype, particularly against quinolones. Additionally, the conformational change of the SoxR protein between its reduced and oxidized forms is crucial for its function in gene regulation. We investigate SoxR site-directed mutations and discovered that several single or multiple mutations upregulate *mfsA* and *smeVWX,* similar to the expression pattern observed under paraquat-induced conditions. These results suggest a link between the SoxR-mediated oxidative stress response and the development of antibiotic resistance in *S. maltophilia.*

PC-18

GAPS IN ANTIMICROBIAL RESISTANCE RESEARCH IN SOUTHEAST ASIAN WATER SOURCES: AN ENVIRONMENTAL ONE HEALTH PERSPECTIVE

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Antimicrobial resistance (AMR) has posed a growing and complex challenge for over a decade. While most research has focused on AMR in clinical and animal samples due to their direct relevance to treatment, AMR patterns in aquatic environments remain understudied and may differ significantly based on geographic location. This review aims to assess the current state of AMR research in freshwater, seawater, and wastewater systems across Southeast Asia, identifying key gaps in the literature. A systematic search was conducted in the PubMed, Scopus, and ScienceDirect databases for studies published between January 2013 and June 2023 that focused on antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) in water sources. After applying inclusion criteria, 41 studies were selected for final analysis, with strong inter-examiner agreement (Cohen's kappa = 0.866). Of these, 23 studies focused on freshwater environments, while research on seawater and wastewater was less prevalent. Across the studies, Escherichia coli emerged as a key indicator for AMR detection, analyzed through both phenotypic and genotypic methods. Commonly detected ARGs included bla_{TEM}, *sul1*, and *tetA*, found at high levels in wastewater, freshwater, and seawater systems. The findings underscore the need for improved wastewater management and continuous water monitoring to mitigate the spread of AMR. This review highlights the importance of expanding research to cover more diverse water systems, including drinking water and seawater, to generate region-specific data.

PC-19

AN AUTOMATED SNAKEMAKE-BASED PIPELINE FOR THE DETECTION OF ANTIMICROBIAL RESISTANCE GENES IN THE GENUS *BURKHOLDERIA*

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Bacteria in the genus Burkholderia are Gram-negative pathogens widely distributed in the environment, commonly found in soils and surface waters worldwide. While many Burkholderia species play beneficial roles. A few, such as *Burkholderia pseudomallei (B. pseudomallei)*, cause severe infections in humans and animals. *B. pseudomallei* is the causative agent of melioidosis, a disease widespread in tropical regions. The disease targets individuals with recognized risk factors, the most prevalent being diabetes mellitus. Up to 36% of cases in Thailand and 16% in Australia occur in individuals without any identifiable risk factors, highlighting the unpredictable nature of *B. pseudomallei* and the significant challenges it poses for public health efforts. The rising concern over antimicrobial resistance (AMR) in *B. pseudomallei* necessitates an urgent investigation into the genetic basis of resistance to enhance treatment outcomes. We hypothesize that specific *B. pseudomallei* isolates harbor resistance genes contributing to treatment failures. To address this, we developed an automated Snakemake pipeline for the rapid and accurate analysis of AMR genes derived from genomic sequencing data. In this study, we analyzed 51 isolates, including *B. pseudomallei* isolates from Thailand (n=19) and *Burkholderia cepacia complex (B. cepacia)* isolates from Thailand (n=10), Europe (n=6), Australia (n=10), and Africa (n=6). Genomic sequences in FASTA format using the NCBI datasets Command-line tools. The automated pipeline streamlines genome sequence downloading, AMR gene identification using AMRFinderplus, and sequence alignment, providing a high-throughput solution for efficient resistance gene detection. The final analysis also includes phylogenetic relationships of resistant strains, aiding in the tracing of the evolution and spread of AMR within *B. pseudomallei* and *B. cepacia* populations. Our analysis AMR genes were found in isolates of *B. pseudomallei* samples from Thailand exhibited resistance genes in Class A and Class D beta-lactamases, and aminoglycoside resistance (tobramycin subclass). In Thailand, B. cepacia showed resistance in Class A beta-lactamases. Europe isolates of B. cepacia presented resistance in Class A beta-lactamases, while Australia isolates displayed resistance in both Class A beta-lactamases and tetracycline. Africa isolates showed resistance to Class A and D beta-lactamases, sulfonamide, aminoglycoside, and quaternary ammonium. Notably, resistance genes found in Thailand showed significant similarity to those identified in Africa, indicating potential gene transmission. Constructing a phylogenetic tree will elucidate relationships among isolates from different regions, enhancing our understanding of AMR dynamics. These findings enhance our ability to study AMR, offering crucial insights for clinical decision-making and public health interventions focused on managing melioidosis in high-risk populations.

PC-20

ANTIBIOTIC RESISTANCE GENES AND MOBILE GENETIC ELEMENTS FROM THE MICROBIOME OF BARBOUR'S SEAHORSES

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This study aimed to determine the antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) associated with the cutaneous and intestinal mucus of Hippocampus barbouri collected from Cantiasay Island. Water and sediments were also collected and analyzed for comparative purposes. Using shotgun metagenomics, this study revealed that genes conferring resistance to aminoglycosides (acrD and kdpE), aminocoumarin (mdtA, mdtB, and mdtC), beta-lactams (CMY-83, HERA-1, OmpK35, pbp2, penA, and porin), fluoroquinolones (mfd, emrA, emrB, hmrM, mdtH, patA, and pmrA), macrolides (efmA, smpC, macA, and macB), sulfonamides (leuO) and tetracyclines (tet39, tetC, and tetM) are present in all samples. For MGEs, genes that showed high prevalence were associated with the Tn3-family transposase (Tn21, Tn3, Tn4401, and Tn501), IS5-family transposases (IS5), IS6-family transposases (IS26, IS1216, and IS1216E), ISCR elements (ISCR1 and ISCR2), IS4-family transposase (ISSba6), MOB relaxases (MOBF, MOVP, MOVQ, MOBT, and MOBV). The relative abundance of MGEs in this study establishes their influence on the mobilization of ARGs in this set of samples associated with H. barbouri . These findings could potentially lay the groundwork for future research into the mechanisms that promote the emergence, evolution, and spread of antibiotic resistance in bacteria associated with wild and farmed seahorses.

PC-21

INNOVATIVE STRATEGIES TO COMBAT ANTIMICROBIAL RESISTANCE: INTEGRATING BIOFILM DISRUPTION, NANOTECHNOLOGY, AND ONE HEALTH APPROACHES

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Antimicrobial resistance (AMR) constitutes a global health crisis caused by antibiotic overuse and the development of biofilms that shield bacterial communities from therapeutic interventions. Biofilm-associated infections present a considerable challenge to human and animal health, leading to chronic diseases and the continuation of resistance mechanisms. Combating AMR necessitates new solutions incorporating advancements in synthetic biology, nanotechnology, and metagenomics to improve treatment efficacy and mitigate biofilm formation. This review will examine contemporary strategies in nano-drug delivery systems aimed at biofilm-associated bacteria, enhancing antibiotic efficacy and mitigating resistance. The review will analyze biofilm disruption techniques that target adhesion pathways and impede extracellular polymeric substance formation, utilizing drugs such as quorum sensing, efflux pump, and antimicrobial peptides. Biological methods for biofilm destruction will be emphasized alongside chemical tactics, focusing on the significance of combinatorial medicines that integrate chemical and biological breakthroughs. Environmental surveillance utilizing genomic and metagenomic methodologies will be examined as an essential strategy for monitoring antimicrobial resistance (AMR) genes across ecosystems, emphasizing the significance of the One Health concept in tracking and reducing AMR transmission among humans, animals, and the environment. Notwithstanding considerable advancements in these domains, deficiencies persist in formulating viable substitutes for antibiotics in agriculture. Future research should concentrate on improving drug development pipelines, refining medication delivery methods, and bolstering AMR surveillance across ecosystems to tackle the multifaceted issues of AMR in clinical and agricultural contexts.

PC-22

BIOFILM STRUCTURE OF COLISTIN-RESISTANT PSEUDOMONAS AERUGINOSA ON FOLEY CATHETER

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Antibiotics are widely used in agriculture and medicine, causing a growing number of microbes to acquire resistance and spread over the world. *Pseudomonas aeruginosa* colonized a variety of surfaces, including medical materials and food industry equipment, and forms biofilms, leading to chronic infections due to increased resistance to antibiotics, various irradiation treatments, environmental conditions, disinfectants, and the immune system. The rise in antibiotic resistance in P. aeruginosa complicates infection therapy. The antibiotic susceptibility of the bacteria *P. aeruginosa TISTR 1287* was examined using modified Kirby-Bauer disk diffusion susceptibility test. The study found that P. aeruginosa TISTR1287 is more resistant to Colistin than Carbenicillin, with diameters of 11.72 ± 0.07 mm and 22.56 ± 0.38 mm respectively. This Colistin-resistant *P. aeruginosa* was studied for biofilm formation on a foley catheter. After 24 hours of incubation with *P. aeruginosa*, SEM images revealed biofilm structure on the outer and inner surfaces of the foley catheter. Furthermore, the number of microorganisms present in the Biofilm structure has been determined. The foley catheter sized 16 mm^2 housed for 2.28 x 10^6 ± 0.04 bacterium cells within the biofilm structure. Furthermore, treating the foley catheter with bacteriophage specific to P. aeruginosa reduced the quantity of bacterial cells by 40.20 ± 9.71%. The eradication of antibiotic-resistant bacterium biofilm is a new challenge.

PC-23

AGROCHEMICAL-INDUCED MULTIDRUG ANTIBACTERIAL RESISTANCE IN *STAPHYLOCOCCUS AUREUS* AND *ESCHERICHIA COLI*

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Agrochemicals such as herbicides, insecticides, and fungicides are vital for global agricultural production, yet improper use can lead to environmental issues, including antimicrobial resistance (AMR), a growing concern within the One Health framework that connects human, animal, and environmental health. This study investigates how imidacloprid (insecticide), profenofos (insecticide), thiamethoxam (insecticide), fipronil (insecticide), glyphosate (herbicide), 2-methyl-4-chlorophenoxyacetic acid (MCPA) (herbicide), and hexaconazole (fungicide) contribute to AMR in *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) against amoxicillin, ceftriaxone, doxycycline, ciprofloxacin, erythromycin, and gentamicin. Agrochemical solutions were prepared in LB broth as per label instructions, filtered through bacterial filters, and diluted in ratios of 1:1, 1:4, and 1:16. Bacterial suspensions (0.5 McFarland) were incubated with agrochemicals at 37°C for 72 hours, then streaked on Muller Hinton Agar and incubated for 24 hours to isolate single colonies. Antibiotic susceptibilities were tested using disk diffusion, with controls maintained without agrochemicals. Statistical analysis (one-way ANOVA, followed by Šídák's and Dunnett's tests) determined significance ($p < 0.05$). Results showed glyphosate induced resistance in S. aureus to gentamicin, ceftriaxone, ciprofloxacin, doxycycline, and amoxicillin, but not erythromycin; in *E. coli*, it induced resistance to gentamicin, doxycycline, amoxicillin, and erythromycin, but not ceftriaxone or ciprofloxacin. Profenofos induced resistance in *S. aureus* to all antibiotics except erythromycin, while *E. coli* . coli developed resistance to all antibiotics except gentamicin. MCPA induced resistance to ciprofloxacin and amoxicillin in *S. aureus* and to all antibiotics except gentamicin and ceftriaxone in *E. coli*. Imidacloprid inhibited *S. aureus* growth at 1:1 and 1:4 dilutions, while *E. coli* was inhibited at 1:1; however, at 1:16, *S. aureus* developed resistance to amoxicillin and erythromycin, and *E. coli* to doxycycline, amoxicillin, and erythromycin. Thiamethoxam inhibited *S. aureus* at 1:1 and 1:4 dilutions, with resistance developing at 1:16 only to amoxicillin and erythromycin, while *E. coli* survived at 1:16 and developed resistance to doxycycline. Fipronil inhibited *S. aureus* at all dilutions, but *E. coli* developed resistance to all antibiotics except gentamicin. Hexaconazole inhibited both species at 1:1 and 1:4 dilutions; *S. aureus* developed resistance to amoxicillin, but *E. coli* showed no resistance. Multidrug resistance was observed in both bacterial species after exposure to agrochemicals, further indicating the significant role agrochemical exposure plays in driving antibacterial resistance. This is the first study to compare AMR development across a variety of agrochemicals in a single investigation, providing novel insights into how these chemicals contribute to bacterial resistance. The investigation emphasizes the potential role of agrochemicals in the development of antibacterial resistance and recommends further genetic studies to elucidate the underlying mechanisms. Additionally, it is recommended to evaluate resistance development at more dilution levels to better understand the scope of agrochemical-induced resistance.

PC-24

FACTORS AFFECTING RESISTANCE TO MULTIPLE DRUGS OF PULMONARY TUBERCULOSIS TREATMENT IN THAI CHAROEN HOSPITAL, THAI CHAROEN DISTRICT, YASOTHON PROVINCE

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Multidrug resistant tuberculosis (multidrug-resistance tuberculosis: MDR-TB) is still a global health problem.and national level until now. This research is a retrospective study aimed at studying factors affecting drug resistance for pulmonary tuberculosis treatment from sputum samples of new patients at Thai Charoen Hospital. Methods: A retrospective study was conducted on tuberculosis drug receipt at Thai Charoen Hospital, Yasothon Province, between October 2022 - September 2024, for a period of 2 years. Clinical outcome data were studied from the results of the specimens (drug susceptibility test; DST). From the community hospital to do tuberculosis culture at Yasothon Hospital. There were 65 samples of drug susceptibility testing results: Isoniazid, Rifampicin, Streptomycin, Ethambutol. The study results found that the majority were male patients, 69.20%. Results of drug susceptibility testing revealed that tuberculosis is resistant to multiple drugs. Isoniazid-resistant TB (HR-TB) totaled 5 cases, accounting for 7.69%. The average age of patients found was 56 years. It was found that factors affecting drug resistance in TB patients It is smoking or cigarette smoke among family members and there were 3 cases of diabetes mellitus, accounting for 60%. It was found that having had tuberculosis as well as living with a patient who is resistant to Isoniazid has a 20% chance of developing drug-resistant bacteria, respectively. Conclusion: From the factors affecting resistance to tuberculosis drugs in the lungs. This makes it important for multidisciplinary professionals who follow drug treatment to provide knowledge to patients and families. To prevent drug-resistant infections starting with intensive treatment. Especially patients with tuberculosis and those with chronic non-communicable diseases in the home. The multidisciplinary network must coordinate with primary care units and village health volunteers to monitor medication intake and separate tuberculosis patients from patients with chronic diseases.

PC-25

GENETIC DIVERSITY AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA ENTERICA* ISOLATED FROM NILE TILAPIA IN RETAIL MARKETS: IMPLICATIONS FOR PUBLIC HEALTH AND AQUACULTURE

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Antimicrobial resistant (AMR) *Salmonella enterica* in aquaculture presents significant public health concerns, particularly due to its potential spread through the food chain (1). This study focuses on genetic diversity and AMR of S. *enterica* in Nile tilapia (*Oreochromis niloticus*). A total of 714 Nile tilapia samples (liver, meat, gills, mucus, lungs, and intestines) were collected from various retail markets in Thailand between May and October 2023. Detection and serotyping of *Salmonella* were performed following the bacteriological analytical manual (2) and a previous publication (3). Antimicrobial susceptibility testing (AST) using agar microdilution method and whole genome sequencing (WGS) were carried out for detection of phenotypic and genotypic profile of AMR isolates. The prevalence of *S. enterica* in Nile tilapia samples was 15.3%, with higher prevalence in mucus (40.9%) and gill (26.5%) samples, respectively. Notably, 35 serovars were identified with Escanaba, Kentucky, and Othmarschen frequently detected, reflecting the wide distribution of these serovars in aquatic environments (4). High levels of phenotypic resistance to commonly used antimicrobials, including oxytetracycline (33.6%), tetracycline (16.0%), and ampicillin (12.8%). Additionally, 19.2% of the isolates were classified as multidrug-resistant (MDR). Despite the high resistance, the multiple antibiotic resistance index remained below 0.2, indicating relatively low antimicrobial exposure in the aquaculture environment (5). Although the prevalence of extended-spectrum beta-lactamase (ESBL)-producing isolates was low at 2.4%, it remains a concern due to the global increase in ESBL-producing bacteria. WGS analysis of 14 MDR isolates identified over 20 resistance genes, including $bla_{\text{TEM-1}}$B, $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-14}}$, *qnr*S1, and *tet*(A), along with efflux pump systems such as *mdsABC* and *mdtK*, which are known to contribute to MDR. The 14 MDR isolates were classified into nine distinct sequence types (ST13, ST26, ST34, ST321, ST413, ST446, ST469, ST1541, and ST2390), demonstrating high genetic diversity. Several of these sequence types have been associated with human infections and are commonly found in poultry products (6). These findings address the need for stricter antimicrobial regulations and improved management practices in aquaculture to mitigate the risks of AMR transmission.

PC-26

PREVALENCE OF COLISTIN-RESISTANT AND PLASMID-MEDIATED COLISTIN RESISTANCE GENES IN *E. COLI* ISOLATES FROM THE CHAO PHRAYA RIVER

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Antimicrobial resistance (AMR) is a major global challenge affecting both health and economics. The overuse of antibiotics in animal husbandry contributes to resistance and environmental contamination. Colistin is predominantly used in veterinary activities. Currently, colistin has been reintroduced for treating multidrug-resistant bacterial infections in humans. The plasmid-mediated mobile colistin resistance (mcr) gene, which confers resistance to colistin, has emerged as a significant health concern. This study was conducted to evaluate 118 *Escherichia coli* strains from the Chao Phraya River, the largest river in Thailand. Out of the 118 *E. coli* isolates, 61 (51.7%) were resistant to colistin. However, only a small percentage of the isolates (1/61; 0.6%) carried mcr-1, and 1.2% (2/61) showed co-occurrences of mcr-1 and mcr-2. This indicated that there are other mcr gene or mechanisms involved in colistin resistance. In addition, the colistin resistance isolates showed decreased antibiotic susceptibility to ampicillin (23/61, 37.7%), tetracycline (15/61, 24.6%), and ciprofloxacin (11/61, 18%). This research highlights the extent of colistin resistance in environmental *E. coli* isolates, providing crucial data that can aid in monitoring and managing AMR. The findings are essential for developing targeted strategies to mitigate the spread of resistance and protect public health.

D.
CLIMATE CHANGE AND COMMUNICABLE DISEASES

PD-01

CLIMATE CHANGE AND HEALTH NEXUS: MEASURES TAKEN BY THE HEALTH SECTOR IN SRI LANKA FOR ADAPTATION AND MITIGATION

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The climate change crisis is already causing, and is anticipated to cause, increasingly severe direct and indirect effects on human health. It is widely regarded as the most significant health threat facing humanity today, requiring immediate action. As a tropical nation, Sri Lanka is particularly vulnerable to the harmful impacts of climate change. Personal expertise, desk reviews and key informant interviews were conducted to understand the activities conducted by the health sector in Sri Lanka on adaptation and mitigation to climate change. Sri Lanka's health sector has recognized the critical need to systematically address climate change-related health impacts, given the island's vulnerability to climate threats. A dedicated focal unit (Environmental Health Unit) within the health sector has been established to address this issue. The importance of adaptation as a key strategy to address the health risks posed by climate change has been acknowledged, leading to the development of the Health National Adaptation Plan (2016-2025), which has been integrated into the country's National Adaptation Plan for climate change. Currently the Health National Adaptation Plan is being revised based on the vulnerabilities faced by the health sector in Sri Lanka. Nationally Determined Contributions (NDCs) for the health sector (2022-2030) have also been formulated and being implemented. The health sector expertise was provided for the development of Climate Change Policy in 2022 and the health sector was instrumental in incorporating health related objectives into it. Further, the health sector is actively engaged in advocacy, capacity building for healthcare staff and non health staff such as teachers, and raising awareness about climate-related health threats. Numerous activities are underway to strengthen the resilience of health facilities in responding to climate-related events. Efforts to reduce greenhouse gas emissions include streamlining healthcare waste management, transitioning to non-incineration methods for infectious and sharps waste, and installing solar panels on the roofs of healthcare facilities at both national and subnational levels. Ministry of Health is in the process of developing guidelines on green, healthy and safe hospital guidelines focusing on climate adaptation and mitigation. Health sector in Sri Lanka has committed for development of a low carbon and climate resilient health system. Health sector should take a lead role in climate action and the health sector in Sri Lanka has taken many measures to address this this important public health problem.

PD-02

RISK-BASED THRESHOLDS FOR ENTERIC PATHOGENS IN RECREATIONAL WATERS: IMPLICATIONS FOR PUBLIC HEALTH

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Contamination of recreational waters by pathogens from human and animal waste poses serious health risks, particularly gastrointestinal illnesses from accidental ingestion while swimming. Although traditional water quality monitoring relies on general fecal indicator bacteria, such as E. coli, these indicators do not always accurately reflect the presence of harmful pathogens. Current standards also lack pathogen-specific, risk-based thresholds, limiting their ability to precisely assess the health risks posed by various microorganisms. This study addressed these gaps by developing critical concentration benchmarks for key pathogens, including norovirus, *Cryptosporidium* spp., and *Campylobacter jejuni*, using reverse Quantitative Microbial Risk Assessment (reverse-QMRA). The approach determined the maximum allowable pathogen concentrations based on risk benchmarks for recreational water users, with a specific focus on children, the most vulnerable group. The study further identified the minimum water sample volumes required for effective detection of pathogens using both traditional culture methods and advanced molecular techniques, such as digital PCR and quantitative PCR. The results showed that norovirus, which presented the lowest critical concentration, required sampling volumes up to 179 L to ensure reliable detection in freshwater environments under a 10% recovery rate. This work not only established pathogen-specific thresholds but also provided practical guidance on the sampling strategies necessary for effective routine monitoring. By applying advanced risk assessment models and innovative pathogen detection technologies, this research could offer a more accurate and comprehensive approach to monitoring waterborne pathogens, ultimately improving public health protection in recreational waters.

PD-03

EVALUATING TOMATO BROWN RUGOSE FRUIT VIRUS AS A ONE HEALTH TOOL FOR DETECTING HUMAN-SPECIFIC CONTAMINATION

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Tomato brown rugose fruit virus (ToBRFV) is an emerging plant pathogen of global concern, primarily affecting crops like tomatoes and peppers. Given the widespread human consumption of these vegetables, ToBRFV is frequently introduced into wastewater systems, making it a potential human-specific microbial source tracking (MST) marker. While ToBRFV's spread in agriculture is well-documented, its role as a marker for human sewage contamination remains underexplored, particularly in Southeast Asia. This study investigates ToBRFV as a potential MST marker in Thailand, assessing its specificity and prevalence in human sewage. Sewage samples were collected from multiple locations in Bangkok, and non-human fecal samples were taken from various animal species. Using qPCR, ToBRFV was detected in human sewage at concentrations as high as 5 log10 copies/100 mL. Notably, there was no cross-detection in feces from cattle, swine, chickens, ducks, or goats, underscoring the virus's specificity to human sources. ToBRFV demonstrated reliability comparable to crAssphage, a well-established human MST marker. Water pollution from human sewage can serve as a reservoir for waterborne communicable diseases, posing a significant public health risk. This study highlights the importance of microbial source tracking as an essential tool in the One Health approach, which focuses on monitoring and controlling pathogens across human, animal, and environmental health. By pinpointing human-specific contamination, MST markers like ToBRFV can play a key role in preventing the spread of waterborne diseases and improving global health surveillance.

PD-04

DISCOVERY OF NOVEL ANELLOVIRUSES IN THAI HUMAN SEQUENCING DATA

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Anelloviruses, belonging to the family Anelloviridae, are regarded as part of the normal viral flora of humans. Although first reported in Thailand in 1999, knowledge about these viruses in the country still remains very limited after 25 years. In this study, we systematically and comprehensively analysed 1,175 human sequencing datasets from Thailand to mine for anellovirus sequences using Entourage, our recently developed bioinformatics pipeline designed for viral sequence detection and discovery. Our analysis revealed that 12.68% of the samples contained anellovirus sequences, encompassing 77 complete genomes of anelloviruses and numerous partial genomes. These anelloviruses were classified into seven genera, four of which had not been previously reported in Thailand. Phylogenetic analysis of ORF1 protein sequences showed that Thai anelloviruses do not have a single origin, forming multiple lineages with non-Thai anelloviruses. Furthermore, sequence similarity network analysis suggested that many anelloviruses discovered in this study are novel species. Our discovery greatly expands the knowledge of anellovirus diversity in Thailand and underscores the potential of human sequencing data as a “treasure trove” for discovery of both known and novel viruses.

PD-05

DEVELOPMENT OF A WASTEWATER ASSAY FOR SENSITIVE IMMUNOSURVEILLANCE OF PATHOGEN EXPOSURE

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Infection surveillance has greatly benefited from direct detection of nucleic acids in wastewater. Wastewater detection goes beyond the limitations of testing individuals seeking medical care, thus detecting asymptomatic as well as symptomatic infections. Similarly, antibodies act as nature's disease surveillance system, providing a record of previous infectious exposures regardless of symptoms or overt disease. As a result, they serve as reliable markers for tracking infections circulating within a population. The average human excretes 2-3g of antibodies a day into feces and urine. Here we explore the possibility of detecting antibodies in wastewater as a novel biomarker for population health and develop workflows for quantitative detection of antigen-specific antibodies from wastewater as an unbiased measurement of the immunological history of a population. Using protein analysis techniques, we detect heavy and light chains of antibodies of various isotypes in wastewater. To overcome the chemical complexity and substantial interference in wastewater, we developed small binding proteins to sensitively detect antigen-specific antibody fragments. These detection reagents were incorporated into a high sensitivity assay to enable detection of antigen-specific antibodies and fragments directly from wastewater. We found that conventional ELISAs and workflows are ill suited for quantitative detection of antigen-specific antibodies in wastewater due to: 1) antibodies in wastewater exist predominantly as fragments, 2) a proportion of these antibodies are antigen-bound or non-specifically adsorbed onto biomass in wastewater, 3) detergents and other chemicals hamper sensitive and reproducible detection of antigen-specific antibodies in wastewater, and 4) 30-100 kDa substances block antigen binding. We developed small binding proteins (7kDa) that despite the chemical complexity in wastewater, bind to antibody fragments with high affinity. These binding proteins were incorporated into a modified MOSAIC (Molecule On-bead Signal Amplification for Individual Counting) assay to detect antigen-specific antibodies and fragments. Using this improved workflow, as a proof of concept we detect quantitatively SARS-CoV2 RBD and N protein specific antibodies in wastewater. The workflow and assay platform, demonstrated for SARS CoV2, is easily adaptable to survey other viruses or antigens. As a complement to nucleic acid-based detection methods, this approach to population serology (antibody detection) through wastewater testing offers a unique opportunity to monitor pathogens that are actively interacting with a population. It also enables the assessment of a population's ability to develop antibodies against these pathogens, as a readout of potential protection against circulating pathogens.

PD-06

DEVELOPMENT OF A PROTOTYPE OF RAPID CRISPR/CAS12A BASED DIAGNOSIS FOR HUMAN PAPILLOMAVIRUS

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Cervical cancer is the fourth most common cancer in women worldwide, which is associated with human papillomavirus (HPV), especially the high risk types (HR-HPVs). Early detection and surveillance are essential to prevent and treat the disease progression. Current diagnosis method is real-time PCR, which can only be performed in specialized laboratories and requires complex instruments and skill personal. On the other hand, rapid CRISPR/Cas12a based diagnosis could be detected the nucleic of virus. This study aimed to design and select gRNA for specificity and high efficiency to HPV16-E6 gene. We employed a bioinformatics to design CRISPR/Cas12a gRNA and developed RPA-CRISPR/Cas12a assay. The results shown that integrating specific gRNA and RPA-CRISPRCas12a with lateral-flow strips could specifically and directly detect HPV16-E6 gene in the liquid sample. Low temperature operation could be achieved in 50 minutes and immediately reading results with naked eyes within 5 minutes. Therefore, specific gRNA and RPA-CRISPRCas12a conveniently detecting nucleic of HPV16 without requiring technical expertise and expensive instruments.

PD-07

REGULATION OF CURCUMIN REDUCTASE curA THROUGH NaOCl AND NEM SENSING BY CurR REPRESSOR IN PSEUDOMONAS AERUGINOSA

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Curcumin is a natural polyphenol derived compound and a major bioactive ingredient found in the rhizome of *Curcuma longa* (turmeric). It has long been used as traditional herbal medicine in Asia. One of its therapeutic applications is the treatment of bacterial infections. The curcumin converting enzyme CurA has been discovered and characterized. However, the expression response and regulation of *curA* have not been reported. *Pseudomonas aeruginosa* is a nosocomial pathogen causing severe infections and high mortality rates in patients with immunocompromising conditions. In this study, the deletion of *curR* transcriptional repressor, caused increased expression of the downstream gene *curA*, which encodes a NADPH-dependent curcumin/dihydrocurcumin reductase. The DNA footprinting assay demonstrated that CurR directly binds to *curA* promoter at an imperfect 15-bp inverted repeat, 5′-TAGTTGA-C-TGGTCTA-3′. A *curA* promoter-lacZ fusion assay and site-directed mutagenesis further showed that the identified CurR binding site plays a crucial role in curA repression by CurR. The curA transcription was inducible by sodium hypochlorite (NaOCl) and N-ethylmaleimide (NEM) but not by curcumin. The oxidation and alkylation of CurR by NaOCl and NEM, respectively, resulted in the inactivation of its DNA-binding activity. Under the tested conditions, the deletion of either *curR* or *curA* did not affect the survival of *P. aeruginosa* under NaOCl stress, regardless of the presence of curcumin.

PD-08

OPTIMIZING SALIVA MICROBIAL DNA EXTRACTION TO REDUCE HUMAN DNA CONTAMINATION

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Human DNA contamination in saliva samples impacts oral microbiome analysis by obscuring bacterial community detection. This study aimed to optimize microbial DNA extraction protocol to minimize human DNA contamination while maximizing bacterial DNA yield, specifically adapting the methodology to align with the characteristics of saliva samples collected in the TIGER-LC biorepository. We evaluated several extraction methods, including the HostZERO Microbial DNA Kit, ZymoBIOMICS™ DNA Miniprep Kit (with and without Propidium Monoazide (PMA) treatment), and the DNeasy PowerSoil Pro Kit with PMA treatment. Extracted DNA was assessed through PCR with primers targeting the human PTGER2 gene and bacterial 16S rRNA genes, followed by gel electrophoresis. Full-length 16S rRNA sequencing was performed on selected samples to identify microbial profiles. The HostZERO kit produced about 1 ng of DNA but exhibited high levels of human DNA contamination. The ZymoBIOMICS™ kit yielded approximately 600 ng of DNA, also with significant human DNA contamination. After PMA treatment, human DNA contamination was effectively reduced, with the ZymoBIOMICS™ kit yielding an estimated 30 ng of DNA and the DNeasy PowerSoil Pro Kit providing 10 ng. Due to low yields, the HostZERO and DNeasy PowerSoil Pro kits with PMA were excluded from sequencing. Full-length 16S rRNA sequencing of DNA samples from ZymoBIOMICS™ with and without PMA showed no significant differences in bacterial diversity ($p > 0.05$). These findings suggest that PMA treatment effectively reduced human DNA contamination without affecting bacterial diversity. Although 16S rRNA sequencing did not indicate significant differences, shotgun metagenomic sequencing may provide further insights into microbial community composition.

PD-09

CHARATERIZATION OF PA0242 IN SHIKIMATE PATHWAY AND ITS REGULATION IN *PSEUDOMONAS AERUGINOSA*

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The *Pseudomonas aeruginosa* genome contains an operon (*PA0242-PA0247*) involved in the shikimate pathway. *PA0242* gene encodes a putative dehydroshikimate dehydratase (DSD), which converts 3-dehydroshikimate into protocatechuate (PCA), a key intermediate in microbial metabolism. PCA can serve as a precursor for important compounds such as siderophores and acetyl-CoA, and also exhibits biological activities such as antioxidant and antimicrobial properties. To investigate the role of *PA0242*, we generated a Δ*PA0242* mutant. The mutant could not grow on shikimate/quinate (SA/QA) as the sole carbon source but grew well when PCA was provided. Additionally, the mutant exhibited reduced virulence in a macrophage killing assay, impaired swarming motility, and increased sensitivity to hydrogen peroxide. These phenotypes were rescued by complementation with the wild-type gene. We also found that *PA0242* expression is repressed by Pip transcription regulator encoded by the nearby *PA0243* gene, as Δ*pip* showed increased *PA0242* expression. DNA binding assays confirmed Pip's specific interaction with the *PA0242* promoter. This study provides new insights into PCA metabolism in *P. aeruginosa*

PD-10

ANALYSIS OF THE IMMUNE RESPONSE TO STRUCTURAL AND NON-STRUCTURAL PROTEINS DERIVED FROM *CHIKUNGUNYA VIRUS*

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The trifecta of mosquito-borne viruses in Southeast Asia are dengue, chikungunya, and zika. Given the lack of an approved vaccine, chikungunya virus infection remains medically unaddressed with significant disease burden. From past periodic outbreaks occurring in Thailand, many patients experienced excruciating lingering arthralgia long after recovery. To better understand the mechanism of chikungunya virus infection, we aimed to determine the breadth of immunity directed towards the viral proteins. Towards this, we constructed a number of mammalian expression vectors for both structural and non-structural protein genes derived from different lineages of chikungunya virus. Viral genes were cloned into plasmid vectors with and without epitope tags. We confirmed tissue culture-derived viral protein expressions by detection with protein-specific or epitope tag antibodies in immunoblot. Using sera from patients with laboratory-confirmed chikungunya virus infection, we attempted to determine whether patient antibody responses were primarily directed towards the viral structural proteins, or whether a subset of patients elicit immunity to non-structural proteins. In parallel, we established several clinical isolates of different chikungunya virus strains with varying genetic background derived from Thai patients in which we have whole-genome sequence information. Combinatorial mutations introduced into chikungunya envelope glycoproteins and in the context of infectious viral clones are expected to shed light on the role of specific amino acid residues important in driving viral tropism and evolution.

PD-11

ANALYSIS OF THE NON-STRUCTURAL PROTEINS DEDUCED FROM WHOLE-GENOME SEQUENCING OF SARS-COV-2 STRAINS IN BANGKOK

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The coronavirus disease 2019 (COVID-19) global pandemic affected world population in magnitude not seen since the influenza pandemic of 1918. This coronavirus (SARS-CoV-2) was readily disseminated as an airborne pathogen, and new strains quickly evolved due to accumulating mutations in its RNA genome. To enable the tracking of emerging coronavirus variants and the evaluation of the COVID-19 vaccine effectiveness, The World Health Organization had asked that countries around the world contribute viral genome sequences to a centralized database. Despite the relatively expensive cost, our laboratory was able to contribute 244 whole-genome sequences to this effort by utilizing a commercial next-generation sequencing service. The urgent task was focused on the analysis of the spike protein gene, which determined viral genotypic variants and antigenicity. However, less attention was given to the analysis of the 16 non-structural proteins (nsP). To maximize the extraction of the SARS-CoV-2 sequence information at-hand, we deduced the amino acid sequence residues from the nsP1 and nsP5 genes of the SARS-CoV-2 circulating in Bangkok at the height of the pandemic and identified patterns of residue variations compared to the global strains. Three-dimensional structural models of these proteins were derived from a web-based prediction algorithm and compared to the published protein crystal structures. Residue changes from the circulating Thai strains were mapped to the protein structures to identify their locations and potential effects on the protein functions. Visual maps of protein mutational hotspots may serve as a foundation for further understanding of how nsPs of coronavirus evolved and potential targeting by small molecule inhibitors.

E.
ENVIRONMENTAL HEALTH ISSUES

PE-01

ATTENUATING EFFECTS OF VITAMIN C ON LEAD (Pb)-INDUCED PHYSIOLOGICAL AND ENDOCRINE DISRUPTIVE RESPONSES IN MALE ALBINO RATS

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Lead, a prevalent metallic pollutant, is extensively distributed in the environment globally and exerts harmful effects on many physiological and biochemical functions and systems. In this study, we have investigated the effects of vitamin C on lead-induced physiological, oxidative stress, endocrine disruptive responses of male albino rats. Twenty-four matured male albino rats were divided in four groups consisting of six animals using the completely randomized design (CRD), and exposed to lead (Pb) at 60 mg/kg body weight (BW), vitamin C at 100mg/kg BW, singly and in combination. The control group received isotonic water solution. Treatment was administered orally and lasted for 65 days. Blood samples were used for oxidative stress enzyme and hormone assays. Liver tissues was stored in RNA later for gene expression analysis. We observed significant increases in the expression and activity of antioxidant enzymes (catalase: CAT, superoxide dismutase: SOD, glutathione peroxidase: GPx), malondialdehyde (MDA) and nitric oxide (NO) in the Pb exposed animals, compared to the control. On the other hand, significant decreases in the reproductive hormones (testosterone, luteinizing hormone: LH, follicle stimulating hormone: FSH) was observed in Pb exposed animals, compared to the control. However, the lead-induced oxidative stress response and activity of antioxidant enzymes were reduced in the combination group treated with both Pb and vit. C, while the reproductive hormones increased significantly, compared to Pb treated animals indicating attenuating effect of vit. C. In conclusion, our findings have revealed the attenuating potential of vit. C on Pb-induced physiological and endocrine disruptive effects of male albino rats.

PE-02

ADVERSE HEALTH EFFECTS OF $PM_{2.5}$ IN HO CHI MINH CITY, VIETNAM

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Studies on the impact of ambient fine particulate matter ($PM_{2.5}$) have remained sparse in Vietnam. This study assessed the short-term effects of $PM_{2.5}$ on hospital admissions for acute lower respiratory infection (ALRI) among children under 5 in Ho Chi Minh City (HCMC) and determined the associations between maternal $PM_{2.5}$ exposure and newborn health. The study collected $PM_{2.5}$ data from two stations, 50,778 ALRI records, and 163,868 pregnancy records from 2016-2019. Time-series regression analysis was conducted to examine the associations between the daily $PM_{2.5}$ concentration and the number of children's hospitalizations. Maternal $PM_{2.5}$ exposure's impact on newborn health was assessed across five exposure windows: first month, first trimester, second trimester, third trimester, and entire pregnancy. The annual $PM_{2.5}$ concentration in HCMC was 28.0 µg/m3. Each 10 µg/m^3 increase in daily $PM_{2.5}$ concentration had a significant excess risk (ER) of 1.86% (95% confident interval: 0.24%~3.52%) of ALRI admission at lag6, with higher risks for male children (ER=2.43%) and those aged 2-5 (ER=3.15%). Prenatal exposure to $PM_{2.5}$ decreased birth weight and increased the risk of preterm birth. Each 10 µg/m3 increase in $PM_{2.5}$ during the second trimester lowered birth weight by 11.8g (95% CI: 5.2–18.3) and increased preterm birth risk by 23.1% (OR=1.231, 95% CI: 1.136–1.336). HCMC's annual $PM2_{.5}$ concentration exceeded the Air Quality Guideline of the World Health Organization. $PM_{2.5}$ exposure increases children's respiratory hospital admissions and negatively affects birth outcomes. The city needs action plans to reduce $PM_{2.5}$ pollution.

PE-03

THE HEALTH EFFECTS OF HOUSEHOLD AIR POLLUTION ON CHILDREN UNDER FIVE IN ADDIS ABABA, ETHIOPIA

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Household air pollution, driven by biomass fuels, poses a severe health threat in Ethiopia, where 90% of households are affected. It contributes to 50% of pediatric diseases globally, with pneumonia causing 18% of deaths. This study investigates the impact of indoor air pollution on children under five in Addis Ababa. The study used a community-based unmatched case-control and cross-sectional design. A census established the sampling frame before data collection. The research involved 2,235 children (312 cases and 968 controls) aged 0-59 months paired with their mothers, with a 1:3 case-to-control ratio. Cases met WHO criteria for acute respiratory infection in the two weeks before the survey; controls did not. Six of eleven Kebeles were randomly selected, with sample sizes based on household size. A census of every child in the selected Kebeles established the sampling frame. To assess exposure, a subsample of 110 kitchens was used to measure carbon monoxide and particulate matter pollution from biomass fuel XIII, using HOBO CO data loggers and UCB Particle Monitors. 99.65% of the study respondents were surveyed. The children's mean age was 24.15 (SD=14.98) months, with 330 (28.86%) aged 12-23 months. Most children lived in families using biomass fuel (87.5%), a polluting cooking fuel. Only 143 (12.5%) households used mixed fuel sources like electricity and LPG. While 351 (30.68%) families had a kitchen inside the living room, 712 (62.23%) had a separate kitchen. Over 417 (58.5%) kitchens lacked a chimney, and 666 (93.54%) kept windows closed during cooking. Acute respiratory infections in children under five were 10.1%. Key risk factors included biomass fuel (AOR=2.08), poor ventilation (AOR=4.31), large families (AOR=1.85), and the mother's small-scale business (AOR=0.50). Acute respiratory infections, exacerbated by indoor air pollution, are linked to biomass fuel use, inadequate home ventilation, and socioeconomic factors. Children are at higher risk due to maternal unemployment, large families, short childbirth intervals, and poor ventilation. Elevated carbon monoxide and particle matter levels surpass WHO guidelines. Solutions include promoting improved stoves, better home designs, and health education on reducing biomass fuel risks, proper child-carrying during cooking, and ensuring ventilation. Urban electrification is key for addressing these health issues.

PE-04

AWARENESS OF DENTAL FLUOROSIS IN BAN HUY PHAK SCHOOL, SUAN PHUENG, RATCHABURI PROVINCE, THAILAND

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Dental fluorosis is a developmental disturbance of dental enamel caused by chronic and excessive ingestion of fluoride during tooth development. It is endemic in certain areas depending on geological conditions worldwide and is usually associated with high fluoride levels in drinking water. The incidence of dental fluorosis was observed among schoolchildren at Ban Huay Phak School, located in western Thailand. This study aimed to investigate the awareness and perceptions of dental fluorosis and fluoride among the parents of these schoolchildren. Each student participant was assessed using the Dental Fluorosis Index based on the Dean Fluorosis Index, which includes a 6-point scoring system: severe, moderate, mild, very mild, questionable, and non-dental fluorosis. A questionnaire was distributed to the parents to evaluate their awareness, perceptions, and knowledge about dental fluorosis and fluoride. Ninety-one pairs of schoolchildren and their parents were included in this study. The community fluorosis index (CFI) for the schoolchildren was calculated to be 1.39, indicating medium public health significance. The overall knowledge and awareness among the parents were as follows: 30.8% were aware of “dental fluorosis,” 24.2% knew its cause, 24.2% were aware that they lived in a fluoride-endemic area, and 22% knew how to prevent dental fluorosis. In the severe to moderate group, only 50% of the parents could recognize tooth deformities in their children. The mitigation of high fluoride in water such as water treatment by reverse osmosis (RO), the dilution with low fluoride water or finding new water source should be introduced to this community. Additionally, enhancing health literacy regarding dental fluorosis and fluoride in the environment should be prioritized in high-risk areas across the country.

PE-05

POTENTIALLY TOXIC TRACE ELEMENTS AND THEIR HEALTH RISK ASSESSMENT IN LAKE RARA, A RAMSAR SITE, OF WEST NEPAL

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This study was carried out in Lake Rara, a Ramsar site of the western Nepal to find out the concentration of potentially toxic elements and their risk assessment. The sampling was performed in pre-monsoon and post-monsoon seasons of 2019. Water samples were collected in replicates from 18 sites in Rara. The site-selection was done based on land use patterns, stressors and accessibility. The concentration of 12 trace elements (Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Cd, Pb, As) were determined using Inductively Coupled Plasma-Mass Spectrometry. Enrichment factor (EF) was calculated to estimate the impacts of anthropogenic activities in the lake. Metal index (MI), potential ecological risk assessment, and health risk assessment (non-carcinogenic and carcinogenic) were also calculated. The most dominant trace element was iron followed by aluminium in Lake Rara. However, the most enriched elements were As and Cd. The metal index (MI) values of all the trace elements (except Fe and Mn) are less than 1 the lake and there is no ecological risk of lake water. The study shows Hazard quotient (HQ) value below 1 for all selected elements, suggesting no health risk (non-carcinogenic). Nevertheless, the carcinogenic risk of As, Cd, Pb and Cr from dermal pathway is “very low” while carcinogenic risk from ingestion pathway varies from “very low to medium” in the lake.

PE-06

CHARACTERIZATION AND SOURCES OF ELEMENTAL CARBON, ORGANIC CARBON IN FINE PARTICULATE MATTER ($PM_{2.5}$) IN THE SOUTHERN KEY ECONOMIC REGION OF VIETNAM

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This is the first study to report the characterization and sources of elemental carbon and organic carbon in fine particulate matter ($PM_{2.5}$) at the Southern Key Economic Zone of Vietnam (SKER). A total of 40 $PM_{2.5}$ samples were collected in 8 provinces and cities in the SKER from January to February 2021. Organic carbon (OC) and elemental carbon (EC) in $PM_{2.5}$ were analyzed using the NIOSH870 thermal-optical transmittance (TOT) standard method (Sunset Model 5L, USA). $PM_{2.5}$ mass concentration during the sampling periods ranged from 9.43 to 98.42 μg/m^3 with an average of 43.77 ± 27.58 μg/m^3, exceeding the World Health Organization (WHO) guideline of 15 μg/m^3 for 24-hour $PM_{2.5}$. The results showed that the average concentration of OC and EC were 10.82 ± 4.80 μg/m^3 and 2.59 ± 1.84 μg/m^3, respectively. The total of OC and EC accounted for 23.1% to 53.8% (average 35.1%) of the $PM_{2.5}$ mass. OC and EC concentrations were high in some sites near industry areas. Based on OC/EC ratios (5.51 ± 2.68) and analysis of OC and EC fractions, it showed that the main sources of carbonaceous aerosol in the SKER during the sampling period came from vehicle exhaust emissions, coal combustion, barbecue cooking, and biomass combustion.

PE-07

CONTENTS OF ARSENIC IN FREE-RANGE CHICKEN IN SUBURBAN AREA OF KUI BURI, PRACHUAP KHIRI KHAN, THAILAND

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The investigation of total arsenic (As) content was conducted in free-range chicken raised in the suburban area of Kui Buri district, Prachuap Khiri Khan province, Thailand. Chicken samples including meat, liver, and intestine were collected from five locations. The surrounding water and soil in these locations were also collected. All samples were prepared for total As analysis using a wet digestion method with nitric acid. The arsenic levels were determined using the Inductively Coupled Plasma-Mass Spectrometry (ICP-MS). The mean concentrations of total arsenic ranged from 46.37 - 97.84, 33.92 - 48.23, and 52.75 - 147.53 µg/kg in meat, liver, and intestine, respectively. The human health risks were assessed by the determinations of Estimated Daily Intake (EDI) and Target Hazard Quotient (THQ). Both values were within the FAO/WHO safe limits. This indicates that the exposure to As via these free-range chicken consumption would not pose health risks to Thai adults. The mean of soil and water total arsenic concentrations ranged from 1.87 - 3.38 mg/kg, and 0.25 to 3.69 µg/L, correspondingly. These values were also well below the permissible limits set by the Soil Quality Standard and Surface Water Quality Standard of Thailand.

PE-08

SCREENING OF MICROPLASTIC DEGRADABLE BACTERIA FROM SAMUT SONGKHRAM MANGROVE AREA, THAILAND

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Microplastics (MPs) are widely distributed throughout the environment including mangrove areas. They can harm both biota and human health. Due to their physicochemical properties, MPs are quite resistant to environmental degradation. This study aimed to identify bacteria capable of degrading microplastics from mangrove sediment in Samut Songkharm province, Thailand. The research focused on five microplastic types: polyethylene (PE), polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and polyvinyl chloride (PVC). Twenty-five bacterial strains were isolated from microplastic-contaminated sites. They were from the genera Bacillus, Fictibacillus, and Pseudoalteromonas which have been known as primary organisms involving in biodegradation. The results demonstrated that these bacteria could reduce microplastic quantities by 0.01 – 26% under laboratory conditions. This reveals the potential of bacterial biodegradation as a promising approach to mitigate environmental impacts of microplastics. Identification of effective bacteria is, thus, so crucial for large-scale applications. The combination of bacterial activity and mangrove plants could eventually further enhance microplastic reduction.

PE-09

GENOTOXICITY AND FIBROSIS IN HUMAN HEPATOCYTES *IN VITRO* FROM EXPOSURE TO LOW DOSES OF PBDE-47, ARSENIC, OR BOTH CHEMICALS

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Improper disposal and recycling of electronic waste (e-waste) has been shown to cause extensive environmental pollution and human health effects. Among the pollutants, 2,2',4,4' Tetrabromodiphenyl Ether (PBDE-47) and arsenic are highly prevalent. This study aimed to investigate genotoxic and fibrosis effects, and their mechanistic relationships from exposure to PBDE-47, arsenic, or both chemicals in a human hepatocyte epithelial cell line (THLE-2). Non-cytotoxic concentrations of 5 μM PBDE-47 (2848 ppb), 0.5 μM arsenite (37.46 ppb), or co-exposure to both were selected and cells were exposed for 7 days. The co-exposure increased the effect of lipid peroxidation (MDA and 4-HNE) and the expression of inflammatory genes (*CXCL6, CXCL8*, and *TGF-β1*) over that of PBDE-47 or arsenite alone. Furthermore, the co-exposure significantly increased the level of mutagenic DNA adducts including MDA-derived DNA adducts (Pyrimido[1,2-a]purin-10(3H)-one, M1dG), 8-hydroxydeoxyguanosine (8-OHdG) and 8-nitroguanine; but decreased mRNA expression of an antioxidant defense regulator (*NFE2L2*) and DNA repair genes (*hOGG1* and *XRCC1*). Regarding biological effects, the co-exposure increased cell migration, a hallmark of epithelial-mesenchymal transition (EMT); down-regulated the epithelial expression (*E-cadherin*); up-regulated mesenchymal expression (Vimentin); and promoted fibrosis expression (up-regulated *ACTA2, FSP-1,* and *COL1A1*). Collectively, these findings indicate that the co-exposure significantly induced a cascade of toxicological effects of overexposure to individual chemicals. The observed genotoxicity, abnormal gene expression, and fibrosis in hepatocytes indicate mechanisms and potentially further increase of health hazards than currently recognized in populations exposed to e-waste chemicals.

PE-10

EFFICIENT REMOVAL OF BISPHENOL A USING A MAGNETIC CARBON NANOFIBER ADSORBENT DERIVED FROM BACTERIAL CELLULOSE

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Bisphenol A (BPA) contamination in water is a significant environmental concern due to its widespread use in plastic products, which can leach into water sources and pose serious health risks to humans. To address this issue, this study developed a magnetic carbon nanofiber adsorbent as a promising solution for the efficient removal of BPA from contaminated water. The adsorbent was synthesized from bio-based bacterial cellulose and $FeCl_3$ through impregnation, freeze-drying, and pyrolysis at 700°C. This process resulted in a material with a naturally fibrous and porous 3D structure and strong magnetization properties. Adsorption studies indicated that the adsorbent demonstrated a high adsorption capacity for BPA, reaching 618 mg/g, notably outperforming other adsorbents. Furthermore, the adsorbent maintained 96% of its initial adsorption efficiency after 10 consecutive recycling cycles, while still retaining its magnetic properties. The use of bacterial cellulose as a renewable carbon nanofiber precursor and FeCl3 as both a source of magnetic particles and a green pore-forming agent resulted in the creation of a superior magnetic carbon nanofiber adsorbent with sustainable characteristics. This offers a highly efficient and environmentally friendly method for mitigating the impact of BPA contamination in water.

PE-11

DIESEL EXHAUST NANOPARTICLE EXPOSURE DISRUPTS CD34⁺ HEMATOPOIETIC STEM AND PROGENITOR CELL DIFFERENTIATION IN EXPERIMENTAL STEM CELL NICHE MODELS

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Recent reports confirm the presence of traffic-related ultrafine particles (UFPs) in perinatal and fetal tissues, though their effects, particularly through transplacental exposure, are not well understood. While transplacental UFP exposure may be linked to childhood hematological and immune disorders, including leukemia, the mechanisms and associated risks remain unclear and inconsistent across studies. One potential site of UFP deposition is the bone marrow (BM), which contains hematopoietic stem cells (HSCs) and stromal cells, including mesenchymal stem cells (MSCs). This study aimed to investigate the toxicity of diesel exhaust particles (DEPs), a major component of urban air particulates with a black carbon core and bound toxicants such as polycyclic aromatic hydrocarbons (PAHs) and heavy metals, on BM stem cells using umbilical cord-derived CD34⁺ hematopoietic stem and progenitor cells (HSPCs) and UC-MSCs as exposure models. In our experimental stem cell niche models, we demonstrated that direct DEP exposure reduced CD34⁺ HSPC differentiation into myelomonocytes, with aberrant expression of genes related to monocyte function. Indirect toxicity, via DEP-induced senescence in UC-MSCs, also reduced CD34⁺ HSPC differentiation, highlighting the role of the senescence-associated secretory phenotype (SASP). The combination of direct DEP exposure and indirect toxicity through UC-MSC SASP factors, including altered small extracellular vesicle (sEV)-microRNAs and inflammatory cytokines, led to more pronounced suppression of CD34⁺ HSPC differentiation. Our study underscores the potential adverse effects of transplacental UFP exposure on fetal BM stem cells, which may increase the risk of developing hematopoietic and immune disorders.

PE-12

NATURAL RUBBER FOAM: UTILIZING CLEAN ENERGY FOR WATER PURIFICATION AND REMEDIATION

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Water scarcity is a critical global concern that threatens food security, economic stability, and ecosystem health, affecting billions of people. Population growth, climate change, pollution, and inadequate water management practices frequently exacerbate the issue when demand exceeds supply. Additionally, the world faces the concurrent challenge of declining fossil fuel availability. While technologies exist to address the clean water shortage, these solutions often involve high costs, significant infrastructure demands, and substantial energy consumption. These challenges are particularly acute in rural or remote areas, where access to such technologies can be limited and implementation more complex. To address this challenge, solar steam generation (SSG) offers a sustainable approach to freshwater production. This study presents an innovative dual-functional composite foam made from natural rubber and carbon black. This foam is designed for a cost-effective SSG system that not only produces fresh water but also removes heavy metals from polluted sources. Produced using the Dunlop process, the foam absorbs over 96% of sunlight, has a thermal conductivity of 0.052 W/m·K, and achieves a water evaporation rate of 1.40 kg/m^2/h. It efficiently converts 83.38% of sunlight into heat and maintains excellent stability. The technology for large-scale production of this foam is already available, and the raw materials are plentiful. Due to its performance, this natural rubber foam holds great potential for use in solar-driven water purification systems, making it a viable option for both commercial and community-based water treatment applications.

PE-13

EFFECTS OF PARTICULATE AIR POLLUTION ($PM_{2.5}$, ULTRAFINE PARTICLES, AND PAHs) ON THE FORMATION OF BPDE-DNA ADDUCTS, TELOMERE LENGTH, AND MITOCHONDRIAL DNA COPY NUMBER IN HUMAN EXHALED BREATH CONDENSATE AND HUMAN BRONCHIAL EPITHELIAL CELL LINE (BEAS-2B)

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Traffic-related particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs) have been linked to respiratory diseases and cancer risk in humans. Genetic damage, including benzo[a]pyrene diolepoxide (BPDE)-DNA adducts as well as alterations in telomere length (TL) and mitochondrial DNA copy number (mtDNA-CN) are associated with respiratory diseases. This study aimed to investigate the association between exposure to traffic-related particulate pollutants and genetic damage in exhaled breath condensate (EBC) in human subjects and a bronchial epithelial cell line (BEAS-2B). Among the 60 healthy recruited subjects, residents living in high-traffic-congested areas were exposed to higher concentrations of PM2.5 (1.66-fold, $p < 0.01$), UFPs (1.79-fold, $p < 0.01$), PAHs-$PM_{2.5}$ (1.50-fold, $p < 0.01$), and PAHs-UFPs (1.35-fold, $p < 0.05$), than those in low-traffic-congested areas. In line with increased exposure to particulate air pollution, the high-traffic-exposed group had significantly increased BPDE-DNA adducts (1.40-fold, $p < 0.05$), TL shortening (1.24-fold, $p < 0.05$), and lower mtDNA-CN (1.38-fold, $p < 0.05$) in EBC. The observations in the human study linking exposure to $PM_{2.5}$, UFPs, and PAHs bound to either $PM_{2.5}$ or UFPs with the aforementioned biological effects were confirmed by an *in vitro* cell-based study, in which BEAS-2B cells were treated with diesel exhaust particulate matter (DEP) containing fine and ultrafine PM and PAHs. Increased BPDE-DNA adducts levels, shortened TL, and decreased mtDNA-CN were also found in treated BEAS-2B cells. The shortened TL and decreased mtDNA-CN were in part mediated by decreased transcript levels of h*TERT*, and *SIRT1*, which are involved in telomerase activity and mitochondrial biogenesis, respectively. These results suggest that exposure to traffic-related particulate pollutants can cause genetic instability in respiratory cells, which may increase the health risk of respiratory diseases and the development of cancer.

PE-14

COMPARISONS OF MEASUREMENTS AND MODELLING OF POLLUTANTS AT AN URBAN AND PERI-URBAN SITE IN BANGKOK

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Air pollution is a global concern due to its potential to cause harm. Exposure of carcinogenic pollutants to pregnant mothers can potentially elevate risk of development of cancer in the child. The effects of pollution are being investigated in a human volunteer study based in a high pollutant urban location and lower exposure peri-urban area in Bangkok. A measurement campaign was used to validate pollutant concentrations at both sites. A community building was hired in a residential area near to Ratchaphiphat hospital, and a government building was hired near to Taksin hospital. Between 30th June and 20th July 2023, four comparison measurements were made using the Dekati ELPI classic (Ratchaphiphat) and Dekati ELPI+ (Taksin). Air was sampled onto size separated stages and collected for analysis. In total, there were two weekday and two weekend measurements, lasting 72 hours. CO and O_3 was measured at the Ratchaphiphat site, there are two Pollution Control Department sites near to Taksin hospital. To support the field results, computational modelling of pollutants in Thailand has been used. A mesoscale non-hydrostatic 3-D meteorological model, WRF-Chem-CRI, an online fully coupled chemistry transport model is used to produce the pollutant maps. Having previously investigated a large domain covering Thailand, Vietnam, Laos and Cambodia, the model was refined to investigate two Bangkok sites. Models of the recruitment sites showed good agreement of $PM_{2.5}$ with measurements and showed lower benzene levels at the peri-urban site compared to the high pollutant site.

PE-15

DISTRIBUTION AND ACCUMULATION OF PER- AND POLYFLUOROALKYL SUBSTANCES IN HUMAN TISSUE WITH THYROID CANCER

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Per- and polyfluoroalkyl substances (PFASs) have attracted worldwide attention due to their high stability and bioaccumulation. Studies have shown that PFASs may be thyrotoxicity in animals or human. Thyroid hormones (FT4, FT3, TSH, TT3, TT4) play a crucial role in regulating various physiological functions and serve as important indicators for assessing thyroid diseases. Abnormal levels of thyroid hormones can lead to thyroid disorders, such as hypothyroidism or hyperthyroidism, which can profoundly impact health. However, evidence about the association between PFASs exposure and thyroid cancer is still limited. In this study, we examined the distribution of PFASs in thyroid pathological changes tissue (PT) and thyroid normal tissue (NT) from the patients with thyroid cancer (n = 144) and non-cancerous thyroid diseases (n = 66). Among 21 PFASs, 6 PFASs (PFBA, PFHpA, PFOA, PFNA, PFUdA, PFOS) were frequently detected in PT (median: 0.0019–278.6268 ng/g) and NT (median: 0.0017–30.2482 ng/mL) samples. PFHpA concentrations in thyroid were positively correlated with age, with higher levels of PFHpA in the older. The highest concentrations in 6 PFASs compounds is PFOA in thyroid tissues (278.6268 ng/g). Male had significantly higher concentrations of PFNA and PFUdA than female ($P<0.05$). The negatively correlated were observed between TT3 with PFHpA, PFNA and PFUdA ($P<0.05$), and FT4 also had negatively correlated with PFOS and PFUdA ($P<0.01$). The logistic regression model indicated a significant positive correlation between PFHpA and PFOA levels and thyroid cancerous risk, suggesting that for each unit increase in PFHpA, PFUdA and PFOA concentration, the likelihood of developing cancerous tissue is higher compared to non-cancerous thyroid diseases tissue. Interestingly, the analysis also revealed a significant negative association between thyroid cancerous risk and the concentrations of PFOS. Our research has indicated significantly difference PFASs concentration between human PT and NT, suggested a link between PFASs exposure and thyroid cancerous. PFOA exhibited the highest concentration in this study and is widely recognized as one of the most frequently detected PFAS in scientific research with extensive industrial production. males exhibited higher concentrations of PFNA and PFUdA than female, suggested that the different PFAS absorption mechanism between males and females. The negative association between thyroid hormones and PFAS exposure may reflect the potential inhibitory effects of these compounds on thyroid hormone synthesis, secretion, or metabolism. PFHpA, PFUdA and PFOA exposure was associated with an increased risk of thyroid cancer, while higher levels of PFOS appeared to be linked to a reduced likelihood of thyroid cancerous tissue development. The mediation model suggested that PFHpA and PFUdA decreased TT3, FT4 levels so that increased the risk of thyroid cancer. This study employed multiple statistical methods to analyze the relationship between PFAS exposure and thyroid disease. Our results provide epidemiological support for the future study on the potential clinical thyrotoxicity of PFAS.

PE-16

MICROBIAL REGROWTH AND BIOSTABILITY CONTROL IN BANGKOK WATER PURIFICATION PROCESSES

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The chlorine-resistant bacteria can regrow in the water distribution system causing problems with water consumption and utilization. This study would like to investigate strategies to control the regrowth microorganisms. Our results revealed that the tap water produced from the Bang Khen Water Purification Plant contained higher organic and nitrogen substances than that from the Mahasawat Water Purification Plant. However, Mahasawat Plant has a slightly higher phosphorus concentration. The average assimilable organic carbon (AOC) in Bang Khen tap water was 192.3 µg acetate-C, whereas the AOC of Mahasawat /L was 78.4 µg acetate-C/L. The AOC level in Bang Khen was higher than the biostability level which the microbes will have limited growth. The total organic carbon (TOC) concentration was high in the dry season, while the AOC was high in dry and wet seasons. Nitrogen and total dissolved solid (TDS) were elevated in dry seasons; although, phosphorus and iron increased in the wet season. The solid contact coagulation tank could remove phosphorus higher than the pulsator (29.5% vs 8.6%), while the pulsator better removed TOC (67.6% vs 54.9%) and AOC (66.1% vs 52.6%). In addition, polyaluminium chloride (PACl) had better removed TOC (61.8% vs 57.3%) and AOC (62% vs 53.5%) than alum, while alum reduced phosphorus (25.5 % vs 18.1%) and nitrogen (13.2% vs 2.6%) better than PACl. The laboratory experiment showed that the significant growth limiting factors for both purification plants are chlorine, microorganism number, and microbial nutrients, respectively. The most limited nutrient in the Mahasawat purification plant was iron; although, the one in the Bang Khen purification plant was different in each sampling season either phosphorus, carbon, or iron. The microbial community of raw water in the two purification plants was partly different. Algae increased significantly in the water production process and was replaced by bacteria after entering the distribution system. Opportunistic pathogens including *Legionella, Mycobacterium*, and *Pseudomonas* aeruginosa presented less than 5.0% of the total microbial community. The numbers of *Pseudomonas* and *Mycobacterium* were positively correlated with the organic matter concentrations. The residual chlorine in the distribution system should be controlled with the total residual chlorine higher than 1.1 mg/L and the free residual chlorine higher than 0.7 mg/L as limited cell numbers were observed. The Total cell count (TCC) was positively correlated to iron, AOC, and phosphorus. Therefore, the water purification processes should control microbial nutrients in water purification processes and ensure sufficient levels of residual chlorine in the distribution system.

PE-17

ASSESSMENT OF IMMUNOMODULATORY EFFECTS OF FIVE COMMONLY USED PARABENS ON HUMAN THP-1 DERIVED MACROPHAGES: IMPLICATIONS FOR ECOLOGICAL AND HUMAN HEALTH IMPACTS

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Parabens are widely used as broad-spectrum anti-microbials and preservatives in food, cosmetics, pharmaceuticals, and personal care products. Studies suggest that the utilization of parabens has substantially increased over the past years, particularly during the global pandemic of coronavirus disease 2019 (COVID-19). Although parabens are generally recognized as safe by the U.S. FDA, some concerns have been raised regarding the potential health effects of parabens associated with immunotoxicity. Herein, we comprehensively investigated several key characteristics of immunotoxicants of five commonly used parabens (methyl-, ethyl-, propyl-, butyl-, and benzyl parabens) in human THP-1 derived macrophages, which are effector cells serving as a first line of host defense against pathogens and tumor immunosurveillance. The results indicate parabens, at concentrations found in humans and biota, significantly dampened macrophage chemotaxis and secretion of major proinflammatory cytokines (TNF-α and IL-6) and anti-inflammatory cytokine (IL-10), corroborating the mRNA expression profile. Furthermore, some parabens were found to markedly alter macrophage adhesion and cell surface expression of costimulatory molecules, CD80+ and CD86+, and significantly increase macrophage phagocytosis. Collectively, these findings heighten awareness of potential immunotoxicity posed by paraben exposure at biologically relevant concentrations, providing implications for human health and ecological risks associated with immune dysfunctions

PE-18

EFFECT OF THE TIDAL REGIME AND SEASON ON BACTERIAL COMMUNITY COMPOSITION IN A TROPICAL ESTUARY

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Estuaries are ecosystems typically subjected to a high spatial and temporal variation in physicochemical parameters and are also affected by human activities. In those aquatic ecosystems, tides can rapidly change not only the physicochemical conditions in a river, but also the abundance and composition of the biota. However, whether tides also affect microbial communities within the diurnal tidal regime is unclear. Here we assessed the bacterioplankton community composition and diversity at four tidal water levels in a tropical estuary with mixed semidiurnal tide regimes (i.e., two high and two low tides with different amplitudes) during the dry and wet seasons. The most abundant phyla in both seasons were Proteobacteria (33.8 - 49.6%), Actinobacteriota (9.25 - 27.6%), Bacteroidota (10 - 15.7%), and Cyanobacteria (2.8 - 8.8%). Bacterial community composition significantly differed among the four different tidal levels, but only during the dry season. During this season, the presence of putative pathogens (up to 84% of the whole bacterial community) suggests that human activities in the basin largely affect water quality in this river. Overall, our study illustrates the strong coupling between river hydrodynamics and variability in bacterial composition

PE-19

EXPOSURE TO DICHLOROMETHANE OR GLYPHOSATE STIMULATED THE TRANSFORMATION OF CHOLANGIOCYTES AND INCREASED THE INVASIVENESS OF CHOLANGIOCARCINOMA CELL LINES

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There are growing concerns about agrochemicals and their potential link to biliary tract disease in humans. Thailand, an agricultural nation with widespread use of dichloromethane (DCM) and glyphosate (GLY), has the highest incidence of cholangiocarcinoma (CCA). However, there is a significant knowledge gap regarding whether DCM or GLY can affect the risks of CCA. This study aimed to investigate the potential effects of DCM or GLY exposure on CCA development using in vitro models. The effects of DCM or GLY on DNA damage, gene expression, and cell transformation were investigated in the normal human cholangiocyte cell line, MMNK-1 cells. MMNK-1 cells were treated with DCM or GLY at concentrations relevant to environmental and food contamination among the local population in Northern Thailand. Interestingly, a significant increase in the levels of two different mutagenic DNA adducts, 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 8-nitroguanine was observed after exposure to DCM (25 μM, 24 h) or GLY (0.1-1 ppm, 10 days). Furthermore, DCM or GLY treatments stimulated the cell transformation and altered the expression of cancer-related genes (*CXCL8* and *MMP9*), suggesting the induction of an inflammatory microenvironment associated with cell transformation. Additionally, DCM treatment increased the invasion and migration capabilities of two different cancer cell lines (HuCCA-1 and RMCCA-1), consistent with the increase in the expression of CXCL8 and MMP9 genes. This study provides evidence to support the potential risk of DCM and GLY, in CCA development, highlighting the need for further research to understand their health impacts, especially in high-incidence regions of Thailand.

PE-20

THE RELATIONSHIP BETWEEN DNA DAMAGE AND LEAD EXPOSURE IN CHILDREN

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In recent years, lead pollution as well as its effects on public health has been becoming important issues. Lead poisoning causes the dysfunctions about physiological, biochemical and behavioral of human. The aims of this study was to evaluate the relationship between DNA damage and lead exposure in children at contamination areas. The DNA damage in blood was analyzed by comet assay. The results showed that there was a significant difference in DNA damage between two groups. The mean of tail length (3.49 ± 1.27 μm compared with 1.75 ± 0.68 μm, $p<0.001$), the percentage of ADN in the tail (3.97 ± 0.92 compared with 1.71 ± 0.42, $p< 0.001$) and olive tail moment (0.32 ± 0.12 compared with 0.12 ± 0.05, $p< 0.001$) were significantly higher in exposed group than control group. The DNA damage index were increased at higher level of PbB ($p< 0.001$). There was clearly positive relationship among tail length, olive tail moment, percentage of DNA and lead concentration in blood ($p< 0.001$). This study is the first research to evaluate the relationship between DNA damage and lead exposure in Vietnamese children.

PE-21

EXPOSURE TO DICHLOROMETHANE OR GLYPHOSATE STIMULATED THE TRANSFORMATION OF CHOLANGIOCYTES AND INCREASED THE INVASIVENESS OF CHOLANGIOCARCINOMA CELL LINES

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Exposure to airborne particulate PAHs is associated with respiratory diseases and lung cancer in humans. Pregnant women are particularly susceptible to PAH exposure because they can easily penetrate the placental barrier and potentially cause developmental abnormalities in the fetus. This study aimed to assess the effects of PAH exposure during pregnancy on DNA damage in pregnant women and newborns. Analysis of particulate PAHs showed that the traffic-congested area had significantly higher concentrations ($p<0.01$) of PAHs bound to $PM_{2.5}$ (PAHs-$PM_{2.5}$) and ultrafine particles (PAHs-UFPs) than those in the low-traffic area by 1.9- and 1.3-fold, respectively. PAH exposure in pregnant women assessed by urinary 1-hydroxypyrene (1-OHP), a metabolite of PAHs, was significantly higher ($p<0.05$) in high-traffic exposed subjects than in low-traffic exposed subjects. High-exposed pregnant subjects had higher levels of mutagenic DNA damage in urine including benzo[a]pyrene diol epoxide (BPDE) -DNA adducts (2.36-fold), and Ethenodeoxyadenosine (1,1N^6εdA; 2-fold). In addition, newborns from high PAHs exposed mothers have higher BPDE-DNA adducts and 1,1N^6εdA in placental tissues. Maternal levels of 1-OHP were significantly associated with DNA adducts in maternal urine and placental tissues. Exposure to PAHs during pregnancy has the potential to increase DNA damage in pregnant women and placental tissue which may affect fetal development. Therefore, this study highlights the importance of being aware of in utero exposure to particulate PAHs which may lead to an increased health risk of disease development in newborns.

PE-22

ASSESSMENT OF PRENATAL EXPOSURE TO AIRBORN MICROPLASTICS AND POTENTIAL HEALTH EFFECTS IN MOTHER AND NEWBORNS

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Growing evidence indicates that maternal exposure to toxic substances during pregnancy can profoundly impact fetal development and childhood disorders. Airborne microplastics (MPs) contribute to pollution that can be directly inhaled posing health risks to humans. This study aimed to evaluate prenatal exposure to airborne MPs and their potential health effects on pregnant women and newborns. The total concentrations of MPs in ambient air at high-traffic location were approximately 1.5-fold significantly higher than those at low-traffic location (4.32 μg/m^3 vs 2.88 μg/m^3, $p<0.05$). The concentrations of total MPs in maternal urine were also higher in the high-traffic exposed group, compared to the low-traffic exposed group with detected MPs including N6, N66, PE, PS, PVC and SBR. Urinary biomarker of oxidative DNA damage (8-hydroxydeoxyguanosine, 8-OHdG) and lipid peroxidation (8-iso-prostaglandin F2α; 8-isoPGF2 and malondialdehyde; MDA) were higher in the high-exposed mothers. The concentration of MPs in placental tissue was significantly higher in high-exposed mothers (19.64 vs 16.37 μg/mg; $p<0.05$). In addition, DNA damage including 8-OHdG and ethenodeoxyadenosine (1*N*^{6}εdA), a lipid peroxidation-derived DNA adduct, were significantly higher ($p<0.05$) in placental tissue from high-exposed mothers by 4.31- and 3.20-fold, respectively. The concentration of MPs in the placenta was significantly correlated with placental 8-OHdG ($p<0.05$) and 1*N*^{6}εdA ($p<0.01$). These results suggest that traffic emissions contribute to the air pollution of MPs. Exposure to MPs during pregnancy can increase DNA damage in pregnant women. The presence of MPs and DNA damage in placental tissue indicates transplacental fetal exposure to MPs, potentially increasing the risk of adverse birth outcomes and disease development in newborns.

F.
FOOD SAFETY AND SECURITY

PF-01

SAFETY ASSESSMENT OF LOW PIPERINE *PIPER NIGRUM* EXTRACT

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This study, we evaluated the genotoxicity of dichloromethane extract obtained from black pepper dried fruits, namely low piperine *Piper nigrum* extract (PFPE)), using an *in vivo* model. Male and female Sprague-Dawley rats (11 weeks old) were orally treated with PFPE at doses of 125, 250, 500 and 1,000 mg/kg BW for 3 days (n = 6 animals/group). The results showed that PFPE had no significant changed in body weight, general behavior, hematology and blood parameters, organ weight and liver histopathological analysis to male and female rats. Sperm concentrations were reduced in all PFPE treatment groups compared to the normal group but not showed significant different to vehicle group. Interestingly, PFPE at a dose of 500 mg/kg BW presented a percentage of abnormal sperm morphology similar to the normal group. For micronucleus test, PFPE did not significantly decrease the percentage of the ratio of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) (P/N) in male and female rats compared to vehicle and normal groups. These results indicate that PFPE did not cause bone marrow toxicity. Taken together, the results herein obtained indicate that PFPE at doses of 125, 250, 500 and 1,000 mg/kg BW did not present significant cytotoxicity and spermatogenesis when administered to female and male rats.

PF-02

MICROBIOME PROFILING OF HYDROPONIC GREENHOUSES FOR DEVELOPMENT OF RAPID PATHOGEN DIAGNOSTICS

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In the face of land and water constraints in Singapore, hydroponics-based vegetable farming is an environmentally and socio-economically sustainable method for vegetable production. Using 90% less land and water to generate the same amount of crop, hydroponic-based vertical urban farming will play an important role in Singapore's "30 by 30" drive towards local food production while contributing to the Singapore Green Plan 2030. Despite its potential to supplement food production sustainably with minimal environmental impact, hydroponics is highly susceptible to the rapid spread of plant diseases through its recirculating nutrient-rich media. To address this challenge, we conducted longitudinal analysis of the microbiome present in the circulating media of five operational hydroponic greenhouses cultivating a variety of produce using next-generation sequencing. Our analysis of the microbiome profiles revealed that the composition of microbial communities in the circulating media is influenced by the types of crops being grown. During fungal disease outbreaks, significant perturbations were observed in the microbiome of affected greenhouses, disrupting bacterial, fungal, and protist communities without affecting alpha diversity. Armed with insights into the microbiome, we developed molecular tests for identifying the fungal pathogens responsible and established methods for their quantitative tracking. Subsequently, we constructed an assay prototype that enables farmers to detect and manage plant pathogens on-site, thereby enhancing the agricultural robustness and productivity of hydroponic farming in Singapore. As hydroponics is anticipated to gain traction globally in the pursuit of sustainable food production amidst challenges posed by climate change, water scarcity, population growth, and land degradation, our work holds promise for advancing the resilience and efficiency of hydroponic systems worldwide.

PF-03

TUSLOB: RAPID DIPSTICK DNA EXTRACTION SYSTEM FOR AFRICAN SWINE FEVER VIRUS DETECTION

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Early detection of veterinary disease outbreaks through effective surveillance protocols is crucial for initiating timely control responses, thereby preventing severe economic losses in the livestock industry. For molecular diagnostic methods to be effectively used in field or resource-limited settings, simple and cost-efficient sample preparation systems are essential. In this study, TUSLOB (a regional dialect in the Philippines, meaning “to dip”) is a simple but effective DNA extraction technology that was designed for Point of Need (PON) PCR-based detection of African Swine Fever Virus (ASFV). Using a simple dipstick format, it facilitates DNA extraction from various sources such as farms, slaughterhouses, animal quarantine checkpoints, and other non-laboratory settings. The extraction process is completed in less than 10 minutes where the genomic DNA is captured and released by dipping the dipsticks to four sequential buffer solutions (lysis, washing, binding, and elution), commonly included in the DNA extraction process. TUSLOB’s sensitivity in extracting ASFV was evaluated by comparing it with an automated DNA extraction system. With the resulting PCR efficiency at par with the automated system, results showed that TUSLOB extraction system can potentially offer fast and reliable nucleic acid extraction from ASFV infected blood samples, The simplicity, speed, and cost-effectiveness of this extraction system is suitable in resource-limited areas, proving its potential use in field diagnostics, veterinary services, and academic institutions alleviating the effect of this veterinary disease in food security of the country.

PF-04

ONE HEALTH CHALLENGES FROM HEAVY METAL EXPOSURE RISK IN A COASTAL AREA OF NORTHEN VIETNAM

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The concept of One Health has been developed as the appreciation that human health is intricately connected to those of other animals and the environment that they inhabit. The United Nations (UN) Sustainable Development Goals are relevant to One Health as they include targets for health and wellbeing, clean water and sanitation, and climate action, as well as sustainability in marine and terrestrial ecosystems. Heavy metal contamination of water sources has emerged as a major global environmental concern, threatening both aquatic ecosystems, vegetables and human health. Chromium (Cr VI), Cadmium (Cd), Lead (Pb), and Arsenic (As) have been recognized as environment contaminants in many parts of the world. A cross-sectional study was carried out to estimate the risk of health effects from heavy metal-contaminated water and food consumption among residents of a northern coastal area of Vietnam. Cr, Cd, Pb, and As concentrations in water from 222 wells, 40 seafood and 135 vegetable samples were examined. A human health risk assessment was then conducted by following the guidelines from the US Environmental Protection Agency. The results showed that the average concentrations of the metals in the well water were in the order of Cr > Pb > As > Cd. 70%-90% of seafood and vegetable samples had Pb, Cd and Cr concentrations higher than permitted standards. 50%-60% of the water samples exceeded the TDI (Tolerable Daily Intake) for As and Pb. The estimated average cancer risk levels for adults consuming well water for drinking purposes and food were determined to be higher than acceptable for As, Cr and Cd. The estimated cancer risk due to consumption of food contaminated with HMs in the population decreases gradually from As > Cr > Pb > Cd. These results indicate that there is an urgent need to develop surveillance and interventions to improve drinking water quality and foodstuff security to minimize the health risks to the local population thus contributing to a One Health vision for a better future and sustainable development.

PF-05

EFFECT OF DIFFERENT STORAGE TEMPERATURES ON AFLATOXIN CONTAMINATION OF PEANUT (*ARACHIS HYPOGAEA* L.) IN MYANMAR

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Peanut is the most important oil seed crop in Myanmar. Peanut production and utilization have been important due to its high oil content and nutritive values. Peanuts are contaminated with aflatoxins, which are poisonous, mutagenic, carcinogenic substances, and the greatest concern to food safety. The objective of this study was to assess the effect of different storage temperatures on chemical and aflatoxin contamination of peanut seed quality. Pin Pyant 6 Month peanut variety from Department of Agricultural Research (DAR) was evaluated for moisture content, crude oil contents, water activity content, free fatty acid content, peroxide value, and aflatoxin content of B1, B2, G1, and G2 at every one-month intervals for three months. Crude peanut oil was obtained by extracting from shelled and crushed peanuts with screw press extraction method to analyze the free fatty acids and peroxide value. Aflatoxin content was determined by using Ultra-High Performance Liquid Chromatography (Hitachi) at Advanced Center for Agricultural Research and Education (ACARE), Yezin Agricultural University, Nay Pyi Taw. In this experiment, the peanut seed of Pin Pyant 6 Month was analyzed the chemical properties and aflatoxin contents at different storage temperatures (ambient temperature of 27- 36 °C, the cold room at 15°C, and 20°C) for the different storage durations (1, 2 and 3 months). The experimental design was (3 x 3) factorial arrangement in Completely Randomized Design (CRD) with three replications. Storage temperature at 20°C in the cold room was the best suitable storage conditions for peanut seeds because of lower level of moisture (4.55 %), water activity (0.58 aw), free fatty acid (0.38%) and peroxide value (2.42 mEq/kg). In addition, the lowest aflatoxin content of B1, B2, G1, and G2 (1.39 ppb, 3.66 ppb, 0.69 ppb, and 0.79 ppb) were observed at the storage temperature of 20°C after three months of storage conditions. The highest aflatoxin content of B1, B2, G1, and G2 (2.36 ppb, 4.22 ppb, 0.99 ppb, and 1.67 ppb) were found at the ambient temperature of (27- 36 °C) over the three months of storage. Moreover, crude oil content was slightly decreased (44.56% to 43. 65%) after the three months of storage duration. The storage temperature at ambient condition (27-36 °C) gave the highest chemical properties and aflatoxin content during the three months of storage period.

PF-06

IMPACT OF VACCINATION AND WATER MANAGEMENT ON TILAPIA LAKE VIRUS CONTROL IN HATCHERY SYSTEMS

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Tilapia Lake Virus (TiLV) has become a serious concern for the global tilapia farming industry, particularly affecting Nile tilapia (*Oreochromis niloticus*) and red hybrid tilapia. While tilapia is a major source of affordable protein, especially in countries like Thailand, the outbreaks of TiLV can lead to significant losses, with young fish being especially vulnerable to the infection. Currently, there is no vaccine or treatment for TiLV, which leaves farmers to rely on farm management and biosecurity practices to mitigate its spread. In this study, we investigated the effectiveness of vaccination and water management, including recirculating water systems and UV disinfection, in controlling TiLV outbreaks in tilapia hatcheries. Our results indicated that TiLV levels were significantly higher in the water from non-vaccinated ponds, which also experienced greater fish mortality. In contrast, ponds with vaccinated fish showed lower mortality rates, suggesting that vaccination reduces the viral load in water and mortality during outbreaks. We also detected baseline levels of TiLV RNA circulating in the farm's water and found that the current UV disinfection system was ineffective in reducing TiLV RNA levels at any stage of treatment. Additionally, TiLV RNA was identified in the water brought in with nursery fish, indicating a possible route of virus introduction. These findings highlight the importance of vaccination in managing TiLV outbreaks. However, improvements in water treatment systems, such as UV disinfection, are necessary to better control TiLV in hatchery environments. A combined approach of effective vaccination and improved water management could offer a more robust defense against TiLV in commercial tilapia farming.

PF-07

COMPARISON OF ALLERGENS IN COMMON BEANS (*PHASEOLUS VULGARIS*) AND SOYBEANS (*GLYCINE MAX*)

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Over the past decade, demand for plant-based dietary products, including legumes, has increased significantly. However, some plant-based products from legumes may have unlisted allergens due to the low number of reported allergic reactions. Common beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) are legumes from the Fabaceae family, both rich in proteins, essential nutrients and minerals. However, soybeans are considered one of the major food allergens. In this study, proteomic and in-silico analysis were performed to identify and compare allergens between mature seeds of common beans and soybeans. The samples were digested with trypsin and analyzed with the nanoLC/timsTOF Pro 2 and PEAKS® studio Xpro software. Using the Viridiplantae database, we could identify 654 proteins in common beans and 651 proteins in soybeans. Of these, 340 proteins were common to both samples, while 314 proteins were unique to common beans and 311 were unique to soybeans. Allergens were predicted by the Allergen Database for Food Safety with FAO/WHO method. The results revealed 80 and 83 allergens in common beans and soybeans, respectively. Endochitinases (Pers a 1.0101) and serpins (Tri a 33.0101) were identified only in common bean seeds. In contrast, hydrophobic seed protein (Gly m 1.0101), major pollen allergen (Lol p 5.0101), non-specific lipid-transfer protein 1 (Pis s 3), oleosin Ara h 10.0101 (Ara h 10.0101) and profilins (Pyr c 4.0101) were identified only in soybean seeds. Forty-five allergens were identified in both samples, indicating common allergens in both legumes. This work could provide researchers with information on plant-based food safety.

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