International Conference on

Liver and Lung Cancer: Current and Future Research

Organized by
Chulabhorn Research Institute

Program - Abstracts

January 8-10, 2019
Chulabhorn Convention Center, Bangkok, Thailand
International Conference on
Liver and Lung Cancer: Current and Future Research

organized to commemorate the birthday celebrations of
Professor Dr. HRH Princess Chulabhorn Mahidol
the President of Chulabhorn Research Institute

January 8 - 10, 2019
Chulabhorn Convention Center, Bangkok, Thailand
## ORGANIZING COMMITTEE

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## INVITED SPEAKERS

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<td>Andrew X. Zhu</td>
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Venue: The Chulabhorn Convention Center, Bangkok, Thailand

Registration:

Location: The Registration Counter is located on the ground floor of the convention center.

Hours: Tuesday, January 8, 2019 from 08:00 AM to 14:00 PM
       Wednesday, January 9, 2019 from 08:30 AM to 17:00 PM

Secretariat Office:

Location: The Secretariat Office is located adjacent to the Convention Hall on the second floor of the convention center.

Hours: Tuesday, January 8, 2019 from 08:30 AM to 17:00 PM
       Wednesday, January 9, 2019 from 08:30 AM to 17:00 PM
       Thursday, January 10, 2019 from 08:30 AM to 13:00 PM

Audio-Visual Center:

Location: The Audio-Visual Center is located between the Secretariat Office and the Convention Hall on the second floor.

Hours: Tuesday, January 8, 2019 from 08:30 AM to 17:00 PM
       Wednesday, January 9, 2019 from 08:30 AM to 17:00 PM
       Thursday, January 10, 2019 from 08:30 AM to 11:00 AM

Poster Session:

Location: The Poster Presentation area is located on the second floor lobby of the convention center.

Hours: Posters should be set up for display on the morning of Tuesday, January 8, 2019.
       Presenters should be in attendance during the lunch break on:
       - Tuesday, January 8, 2019 from 12:30 PM to 13:00 PM
       - Wednesday, January 9, 2019 from 12:50 PM to 13:20 PM
       The display must be removed by 11:00 AM on Thursday, January 10, 2019.
08:00 – 09:00  REGISTRATION

**Session 1: Population Studies of Liver and Lung Cancer**

**Session Chairs:** Curtis C. Harris, National Cancer Institute (NCI), USA  
Jisnuson Svasti, Chulabhorn Research Institute, Thailand

**Abstract No.**

09:00 – 09:35  “The Changing Etiology of Liver Cancer”  
*John D. Groopman,* Johns Hopkins Bloomberg School of Public Health, USA  

09:35 – 10:10  “Opisthorchiasis and Cholangiocarcinoma: Khon Kaen Experiences”  
*Vajarabhongsa Bhudhisawasdi,* Khon Kaen University, Thailand  

10:10 – 10:45  “Intraductal Papillary Neoplasm of the Bile Duct: IPNB”  
*Vor Luvira,* Khon Kaen University, Thailand  

10:45 – 11:00  Break

**Session 1: Population Studies of Liver and Lung Cancer (continued)**

**Session Chairs:** Christopher A. Loffredo, Georgetown University, USA  
Anon Chotirosniramit, Chiang Mai University, Thailand

**Abstract No.**

11:00 – 11:35  “The Repertoire of Mutational Signatures in Human Cancer”  
*Ludmil B. Alexandrov,* University of California, San Diego, USA  

11:35 – 12:10  “Lung Cancer in Non-smokers”  
*Herman N. Autrup,* University of Aarhus, Denmark  

12:10 – 13:00  Lunch

12:30 – 13:00  Poster Session

13:00 – 13:35  “Diversity in Lung Cancer Risk and Survival”  
*Bríd M. Ryan,* National Cancer Institute (NCI), USA  

13:35 – 14:10  “Unique Molecular Profile of Thai Lung Cancer Patients in Novel Therapy Era”  
*Thanyanan Reungwetwattana,* Mahidol University, Thailand  

14:10 – 14:30  Break
International Conference on
Liver and Lung Cancer: Current and Future Research
January 8-10, 2019
Chulabhorn Convention Center, Bangkok, Thailand

Tuesday, January 8, 2019

OPENING CEREMONY:

14:30 - Guests to be seated in the Convention Hall

15:00 - Arrival of Professor Dr. HRH Princess Chulabhorn, President, Chulabhorn Research Institute, Thailand

- Report by Professor Mathuros Ruchirawat, Vice President for Research and Academic Affairs, Chulabhorn Research Institute, Thailand

- Opening Remarks by Professor Dr. HRH Princess Chulabhorn

15:10 - A TIGER-LC Report to Professor Dr. HRH Princess Chulabhorn by Dr. Xin Wei Wang, National Cancer Institute (NCI), USA

16:00 - Opening Ceremony concludes
- Reception

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SCIENTIFIC PROGRAM

Wednesday, January 9, 2019

Keynote Chair: Curtis C. Harris, National Cancer Institute (NCI), USA

09:00 – 09:45  **Keynote Lecture:**  
“CAR-T Cell Therapy and Advances in Cancer Immunotherapy”  
**Abstract No. KL**  
Carl H. June, University of Pennsylvania, USA

09:45 – 10:00  Break

**Session 2:** Cancer Drivers, Biomarkers of Risk, Diagnosis, Prognosis and Response to Therapy

Session Chairs: Xin Wei Wang, National Cancer Institute (NCI), USA  
Mathuros Ruchirawat, Chulabhorn Research Institute, Thailand

10:00 – 10:35  “Restoring Tumor Suppression in Advanced Cancers”  
**Abstract No. S-8**  
Scott W. Lowe, Memorial Sloan Kettering Cancer Center, USA

10:35 – 11:10  “Modeling and Preclinical Study of Intrahepatic Cholangiocarcinoma”  
**Abstract No. S-9**  
Nabeel M. Bardeesy, Harvard Medical School, USA

11:10 – 11:45  “Drivers of HCC”  
**Abstract No. S-10**  
Lars Zender, University Hospital Tübingen, Germany

11:45 – 12:20  “Molecular Subtypes of Liver Cancer”  
**Abstract No. S-11**  
Jittiporn Chaisaingmongkol, Chulabhorn Research Institute, Thailand

12:20 – 13:20  Lunch

12:50 – 13:20  Poster Session
**Session 2:** Cancer Drivers, Biomarkers of Risk, Diagnosis, Prognosis and Response to Therapy (continued)

**Session Chairs:** Tim F. Greten, National Cancer Institute (NCI), USA  
Nirush Lertprasertsuke, Chiang Mai University, Thailand

**Abstract No.**

**Bin Tean Teh,** Duke-NUS Medical School, Singapore  
Abstract No. S-12

13:55 – 14:30 “Detection and Localization of Surgically Resectable Cancers with a Multi-analyte Blood Test”  
**Nickolas Papadopoulos,** Johns Hopkins University, USA  
Abstract No. S-13

14:30 – 15:05 “Advances in Improving Low Dose CT Diagnosis of Early Stage Lung Cancer”  
**Stephen Lam,** University of British Columbia, Canada  
Abstract No. S-14

15:05 – 15:20 Break

15:20 – 15:55 “Telomere Maintenance and the Proteomic Landscape of Cancer”  
**Roger R. Reddel,** Children’s Medical Research Institute and University of Sydney, Australia  
Abstract No. S-15

15:55 – 16:30 “Precision Medicine of Lung and Liver Cancer: Focus on Metabolome and Microbiome”  
**Curtis C. Harris,** National Cancer Institute (NCI), USA  
Abstract No. S-16

16:30 – 17:00 “Current and Potential Biomarkers in Lung Cancer, Implication for Treatment Decision and Outcome”  
**Chanida Vinayanuwattikun,** Chulalongkorn University, Thailand  
Abstract No. S-17
Thursday, January 10, 2019

Session 3: Translational Studies

Session Chairs: Herman N. Autrup, University of Aarhus, Denmark
Chirayu Auewarakul, Chulabhorn Royal Academy, Thailand

Abstract No.

09:00 – 09:35 “Cell Immunity and HCC”
Tim F. Greten, National Cancer Institute (NCI), USA

09:35 – 10:10 “Precision Oncology in Liver Cancer”
Xin Wei Wang, National Cancer Institute (NCI), USA

10:10 – 10:45 “GPC3 as a CAR T-Cell Therapy Target in Liver Cancer”
Mitchell Ho, National Cancer Institute (NCI), USA

10:45 – 11:05 Break

11:05 – 11:40 “The Landscape of Targeted Therapies in Cholangiocarcinoma: Current Status and Emerging Targets”
Andrew X. Zhu, Harvard Medical School, USA

11:40 – 12:15 “15 Years of Precision Medicine has led to 6 Different Treatment Approaches for half of the Patients with Advanced Non-Small Cell Lung”
Bruce E. Johnson, Dana-Farber Cancer Institute, USA

12:15 – 12:50 “Combination of Immuno and Targeted Therapies of Lung Cancer”
Rafael Rosell, Catalan Institute of Oncology, Spain

12:50 – 13:00 Closing Remarks by Mathuros Ruchirawat, Vice President for Research and Academic Affairs, Chulabhorn Research Institute, Thailand

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Invited Session
Carl H. June, M.D.

Richard W. Vague Professor in Immunotherapy
Department of Pathology and Laboratory Medicine
Director, Center for Cellular Immunotherapies
Director, Parker Institute for Cancer Immunotherapy
Perelman School of Medicine
University of Pennsylvania
Philadelphia, PA. 19104-5156
USA
E-mail: cjune@upenn.edu

Dr. Carl June is the Director of the Center for Cellular Immunotherapies at the Perelman School of Medicine, and Director of the Parker Institute for Cancer Immunotherapy at the University of Pennsylvania. He maintains a research laboratory that studies various mechanisms of lymphocyte activation that relate to immune tolerance and adoptive immunotherapy for cancer and chronic infection. In 2011, his research team published findings detailing a new therapy in which patients with refractory and relapsed chronic lymphocytic leukemia were treated with genetically engineered versions of their own T cells. The treatment has also now also been used with promising results to treat children with refractory acute lymphoblastic leukemia, and adults with refractory lymphoma. CTL019, the CAR T cell developed in the June laboratory was the first gene therapy to be approved by the US FDA in August 2017.

June has published more than 400 manuscripts and is the recipient of numerous prizes and honors, including election to the Institute of Medicine in 2012 and the American Academy of Arts and Sciences in 2014, the Paul Ehrlich and Ludwig Darmstaedter Prize (shared w J. Allison), the Novartis Prize in Immunology (shared w Z. Eshhar and S. Rosenberg), the Karl Landsteiner Memorial award, the Karnofsky Prize from the American Society of Clinical Oncology, the Albany Medical Prize and a lifetime achievement award from the Leukemia and Lymphoma Society.
Immunotherapy is the latest breakthrough in cancer therapy, thanks to the remarkable clinical results of checkpoint inhibitors (1) and chimeric antigen receptor (CAR) T cells (2). The US Food and Drug Administration (FDA) approval in 2017 of two CAR-T cell therapies for the treatment of B cell malignancies in pediatric and adult patients is a landmark for cancer immunotherapies. In 2018, these therapies were also approved in the European Union, the United Kingdom, and Canada.

The emergence of immune-oncology as the first broadly successful strategy for metastatic cancer will require clinicians to integrate this new pillar of medicine with the pillars of chemotherapy, radiation and targeted small molecule compounds. Chimeric antigen receptor (CAR) T cells have proven that engineered immune cells can serve as a powerful new class of cancer therapeutics. Adoptive immunotherapy retargeting T cells to CD19 via a chimeric antigen receptor (CAR) is an investigational treatment capable of inducing complete tumor regression of B-cell malignancies when there is sustained survival of infused cells. Clinical experience has helped to define the major challenges that must be met to make engineered T cells a reliable, safe, and effective platform that can be deployed against a broad range of tumors. The emergence of synthetic biology approaches for cellular engineering provides the field with a broadly expanded set of tools for programming immune cells (3). In this presentation, I will discuss how these tools could be used to design the next generation of smart T cell precision therapeutics.

In solid tumors, we have observed antitumor activity in patients with ovarian cancer, pancreatic ductal adenocarcinoma, pleural mesothelioma and glioblastoma following infusion of CAR T cells expressing scFv specific for mesothelin or EGFRvIII (4, 5). However, this approach has not yet resulted in complete tumor eradication. Using genome edited T cells, it may be possible to enhance and prolong the activity of T cells that have disrupted immune and metabolic checkpoints. In preclinical studies, we show that TCR-specific T cells have enhanced antitumor activity following disruption of TCR alpha and beta genes and the PD1 gene using CRISPR/Cas9. This approach is just entering a clinical trial. These findings provide insights into the immunobiology of effector T cells and demonstrate the potential of multiplexed CRISPR/Cas9 genome editing to synthetically enhance the efficacy of immunotherapy. Finally, advances in T cell engineering, genetic editing, the selection of optimal lymphocytes, and cell manufacturing have the potential to broaden T cell–based therapies and foster new applications beyond oncology, in infectious diseases, organ transplantation, and autoimmunity.
References:


John D. Groopman, Ph.D.

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Associate Director for Population Sciences
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Dr. John Groopman is the Edythe H. Schoenrich Professor of Preventive Medicine at the Johns Hopkins Bloomberg School of Public Health and the Associate Director for Population Sciences at the Sidney Kimmel Comprehensive Cancer Center in the School of Medicine. He received his Ph.D. degree from the Massachusetts Institute of Technology and was also a post-doctoral fellow at MIT. He received further training as a staff fellow at the National Cancer Institute in the Laboratory of Human Carcinogenesis. Prior to coming to Johns Hopkins in 1989, Dr. Groopman was the Associate Dean at the Boston University School of Public Health. Dr. Groopman's main research interests involve the development and application of molecular biomarkers of exposure, dose and effect from environmental carcinogens. The environmental carcinogens studied include agents that are naturally occurring in the diet. A major emphasis of the research has been in the elucidation of the role of aflatoxins, a common contaminant of the food supply, in the induction of liver cancer in high-risk populations living in Asia and Africa. This work has led to the identification of a very strong chemical-viral interaction between aflatoxin and the human hepatitis B virus in the induction of liver cancer. These biomarkers have also been used in many collaborative molecular epidemiology studies of liver cancer risk and recently employed to assess the efficacy of a number of chemopreventive agents in trials in high-risk aflatoxin-hepatitis B virus exposed populations. This research is now being extended to develop genetic biomarkers of p53 mutations in human samples as early detection of disease biomarkers using a novel mass spectroscopy based method for genotyping developed in the laboratory. The most cited research publication from this research was the finding from a prospective cohort of over 18,000 people in Shanghai that established for the first time a viral-chemical interaction essential to the etiology of liver cancer, a leading cause of cancer death in the world. This work has led to the collaborative chemoprevention trials in China. Collectively, Dr. Groopman's expertise involves the biological consequences of exposures to mycotoxins and other environmental contaminates on human health. Thus, the research in our laboratory, resulting in over 290 peer-reviewed publications and chapters, focuses on the translation of mechanistic research to public health based prevention strategies. Dr. Groopman also served as a member of the National Advisory Council for the NIEHS and numerous other committees at the national and international level. Thus, Dr. Groopman has a long-standing record of commitment to interdisciplinary and translational research in oncology and public health. Finally, in recognition of his contributions to cancer prevention efforts, Dr. Groopman was the recipient of the 2016 American Association for Cancer Research – Prevent Cancer Foundation Award for Excellence in Cancer Prevention Research and the gave the Ronald Herberman Memorial Lecture for National Cancer Prevention Day in 2016.
The Changing Etiology of Liver Cancer

John D. Groopman

Johns Hopkins Bloomberg School of Public Health, Baltimore, MD USA

Collectively liver cancer accounts for nearly 9% of all reported cancer deaths and is one of the most common causes of cancer mortality worldwide http://gco.iarc.fr/ (1). The incidence of liver cancer varies enormously globally and unfortunately the burden of this nearly always fatal disease is much greater in the less economically developed countries of Asia and sub-Saharan Africa (2). HCC is also the most rapidly rising solid tumor in the US and Central America and is overrepresented in minority communities, including African-Americans, Hispanic/Latino-Americans and Asian-Americans (1,3,4). Overall, there are more than 850,000 new cases each year and more than 300,000 deaths annually in the People’s Republic of China (P.R.C.) alone (2). In contrast with most common cancers in the economically developed world where over 90% of cases are diagnosed after the age of 45, in high-risk regions for liver cancer onset begins to occur in both men and women by 20 years of age and peaks between 40-49 years of age in men and between 50-59 years of age in women (5-7). This earlier onset of HCC might be attributable to exposures that are both substantial and persistent across the life span. Gender differences in liver cancer incidence have also been well described and worldwide the number of cases among men were 554,000 and 228,000 among women in 2012 (8). These epidemiologic findings are also reflected in experimental animal data for one potent liver carcinogen linked to human HCC, aflatoxin, where male rats have been found to have an earlier onset and higher incidence of cancer compared to female animals (9). Thus, the consistency of the experimental animal and human data points to the important role that environmental exposures play in gender differences in HCC risk.

To date, the significant etiological factors associated with development of HCC have been defined by biomarker studies and they are infection in early life with hepatitis B virus (HBV) and lifetime exposure to high levels of aflatoxin B1 (AFB1) in the diet (10,11). Over the past 25 years, an appreciation for the role of the hepatitis C virus (HCV) has also emerged. HCV is contributing to HCC being the most rapidly rising solid tumor in the US and Japan (12). Detailed knowledge of the etiology of HCC has spurred many mechanistic studies to understand the pathogenesis of this nearly always-fatal disease (10,13,14). Fortunately, the successful development and deployment of some highly effective new drugs that cure HCV infection is a major advance and will hopefully diminish the role of this virus in liver cancer (15,16).

Alcohol is a recognized human carcinogen and has been causally linked to HCC. Alcoholic cirrhosis and heavy alcohol use have been repeatedly associated with an increase in HCC risk (23). However, it is unclear if alcohol use in the absence of cirrhosis influences HCC development (24). Several studies have demonstrated an increased risk of HCC up to 5-fold with consumption of more than 80g of alcohol per day or approximately 6-7 drinks per day (23). The risk of HCC ranges from borderline significant to doubled with chronic alcohol consumption of less than 80g/day (23). A synergism between alcohol and HBV and HCV infections has also been described (23,25).
In addition to the association of alcohol and HCC, in economically developed countries the dramatic rise in overweight and nonalcoholic fatty liver disease has also been related to increased HCC (26-28). Of major concern for the future are the role that obesity, diabetes and general underlying fatty liver disease will play in the development of liver cancer (29-31). While the historic risk factors for liver cancer described above are addressed through a spectrum of prevention methods, these new etiologic factors portend an increasing trajectory in the incidence of this disease. Both therapeutic and pre-disease interventions will need to be deployed now to blunt the impact of these risk factors in the decades to come. Collectively, the development and validation of a new generation of biomarkers reflecting complex processes such as inflammation will need to be deployed.

References:


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Dr. Bhudhisawasdi’s special interests are in cholangiocarcinoma, hepatobiliary surgery, acute abdomen, trauma, and surgical education.
Opisthorchiasis and Cholangiocarcinoma: Khon Kaen Experiences

Vajarabhongsa Bhudhisawasdi
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*Opisthorchis viverrini* has a three-host life cycle. The adult worms of the *Opisthorchis viverrini* (Southeast Asian liver fluke), live in the bile duct or sometimes in the gallbladder of human (the definite host). One adult flukes passes fully developed eggs into the bile and passed to feces, and after the egg are ingested by a Bithynia snail (first intermediate host) within the snails the miracidia hatch and develop sporocysts, rediae, and cercariae. Cercariae are released from the snail and penetrate the muscle of susceptible scaled freshwater fishes (secondary intermediate host) to develop into metacercariae which encysting as metacercariae in the muscles or under the scales. The human become infected by ingesting food contained infested raw fish (such as Goi-Pla). After ingestion, the metacercariae excyst in the duodenum and ascend through the ampulla of Vater into the bile ducts, where they attach to the mucosa and develop into adult worm which lay eggs around 3,000 eggs per day after 3 to 4 week. The adult worms reside in the human bile ducts for more than 20 years. The adults *Opisthorchis* caused mechanical immunological inflammation of the bile ducts that created segmental strictures and peribiliary fibrosis that further cause bile stasis and secondary bacterial infection together with secretion form the worms in the long terms causes dysplasia and neoplasia of bile ducts. From these events the cholangiocarcinoma of the bile duct developed and this type of tumours have very poor prognosis and difficult to treated. Cholangiocarcinomas is the most commmom malignant tumour of the native people who lives in rural area of the northeastern part of Thailand because of the eating habit of these people who prefer to eating raw fish which made the northeastern part of Thailand the highest prevalence area of Opisthorchiasis and also the highest incidence of Cholangiocarcinoma Pilot projects have shown that after praziquantel treatment high re-infection rates occurred within a short period of time. Even though antihelmintic drug Praziquantel can cure nearly 100% of Ophisthorchiasis but the reinfestation rate is quite high.

Because the fatality rate and difficult to treat Cholangiocarcinoma, to control this type of tumours by tertiary prevention (Surgery) or secondary prevention (screening) are not hopeful and very expensive and effective control both Opishorchiasis and Cholangiocarcinomas could be primary prevention via education programs by persuading people to consume cooked fish.
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Significant contributions to science:

1. Cholangiocarcinoma

My research has focus on clinical aspects of bile duct tumors, including cholangiocarcinoma. I have contributed to epidemiological research and role of adjuvant treatment for cholangiocarcinoma. Now I am conducting Cochrane systematic review to determine efficacy of adjuvant chemotherapy for cholangiocarcinoma and contributing to the Gecicca Study (randomized controlled trial comparing efficacy between Gemcitabine-Cisplatin and Gemcitabine alone for adjuvant treatment of cholangiocarcinoma).

2. Intraductal papillary neoplasm of the bile duct

Intraductal papillary neoplasm of the bile duct (IPNB) is now considered as a specific entity of bile duct tumor which sometime called ‘good cholangiocarcinoma’. I have reported the largest case series of outcome after resection of IPNB and have proposed the morphologic classification for IPNB with survival correlation.
Intraductal Papillary Neoplasm of the Bile Duct: IPNB

Vor Luvira

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Intraductal papillary neoplasm of the bile duct (IPNB) is now considered as a specific form of bile duct tumor. By definition, it is intraductal neoplastic growth of bile duct epithelia with a fine fibrovascular core. According to the latest classification of bile duct tumors, benign IPNB are considered pre-invasive lesions of the intraductal growth-type of intrahepatic cholangiocarcinoma or papillary type of extrahepatic cholangiocarcinoma. IPNB has unique characteristics that differ from other types of bile duct tumor, usually presents as multiple lesions with various stage of invasion. The cell of origin of IPNB is believed to be the cell in peribiliary glands, which is distributed along the extrahepatic and intrahepatic bile ducts. IPNB develops through an adenomacarcinoma sequence. Consequently, it usually progresses slowly, and the patient appears to have better survival than conventional cholangiocarcinoma. Intraductal papillary neoplasm of the bile duct is usually treated in the same manner as cholangiocarcinoma: the mainstay for which is surgical resection to obtain R0 resection (no microscopic tumor left behind in the patient body). After surgery, IPNB had a substantial more favorable prognosis than conventional cholangiocarcinoma.

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Dr. Ludmil Alexandrov is an Assistant Professor of Cellular and Molecular Medicine and Bioengineering at the University of California, San Diego. He earned his Bachelor of Science degree in Computer Science from Neumont University and received his Master's of Philosophy in Computational Biology as well as his Ph.D. in Cancer Genetics from the University of Cambridge.

Ludmil's research has been focused on understanding the mutational processes in cancer. In 2013, he developed the first comprehensive map of the mutational signatures in human cancer. More recently, Ludmil mapped the signatures of clock-like mutational processes operative in normal somatic cells, demonstrated that mutational signatures have the potential to be used for targeted cancer therapy, and identified the mutational signatures associated with tobacco smoking.

Ludmil has 75 publications in peer-reviewed journals from which 18 publications in Nature, Science, or Cell and another 27 publications in Nature Genetics, Nature Medicine, Cancer Cell, Science Translational Medicine, PNAS, or Nature Communications. In 2014, Ludmil Alexandrov was recognized by Forbes magazine as one of the “30 brightest stars under the age of 30”. In 2015, he was awarded the Prize for Young Scientists in Genomics and Proteomics by Science magazine and SciLifeLab, and he also received a Harold M. Weintraub Award by the Fred Hutchinson Cancer Center. In 2016, Ludmil was awarded the Carcinogenesis Young Investigator Award by Oxford University Press. In 2018, Ludmil was awarded the Balfour Prize Lecture of the Genetics Society, an Alfred P. Sloan Research Fellowship in Computational & Evolutionary Molecular Biology, and an Early Career Award by The International Academy for Medical and Biological Engineering. Ludmil is currently one of six co-investigators leading the Mutographs of Cancer project, a £20 million initiative to identify the unknown cancer-causing factors.
Cancer is the most common human genetic disease. All cancers are caused by somatic mutations. These mutations may be the consequence of the intrinsic slight infidelity of the DNA replication machinery, exogenous or endogenous mutagen exposures, enzymatic modification of DNA, or defective DNA repair. In some cancer types, a substantial proportion of somatic mutations are known to be generated by exogenous carcinogens, for example, tobacco smoking in lung cancers and ultraviolet light in skin cancers, or by abnormalities of DNA maintenance, for example, defective DNA mismatch repair in some colorectal cancers.

Each biological process causing mutations leaves a characteristic imprint on the genome of a cancer cell, termed, mutational signature. In this talk, I will present mutational signatures analyses encompassing 30,874 cancer genomes across 91 distinct types of human cancer revealing more than 60 different signatures of mutational processes. Some signatures are present in many cancer types, notably a signature attributed to the APOBEC family of cytidine deaminases, whereas others are confined to a single cancer class. Certain signatures are associated with age of the patient at cancer diagnosis, known mutagenic exposures or defects in DNA maintenance, but many are of cryptic origin. The results reveal the diversity of mutational processes underlying the development of cancer, with potential implications for understanding of cancer etiology, prevention and therapy.
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Molecular epidemiology and the role of environmental factors in carcinogenesis focus on air pollution.

Recipient of Princess Chulabhorn’s Gold medal, Member of the Danish Academy of Technical Sciences, Society of Toxicology (US) Educational award, Eurotox Merit award and European Environmental Mutagen Society F. Sobel Award.
Lung cancer is generally associated with tobacco smoking, however WHO has estimates that the incidence of lung cancer in non-smokers accounts for 25% of all cases and 55% of all female cases. Lung cancer in never smokers differs both etiologically and clinically from lung cancer attributed to smoking. While small cell lung cancer (SCLC) is the major histological among smokers, lung adenocarcinoma is the predominant type of lung cancer in non-smokers. Tobacco smoke contains numerous carcinogenic compounds, e.g. polycyclic hydrocarbons, nitrosamine, that after metabolic transformation can form metabolites that damage DNA and results in mutations in genes relevant for the development of cancer e.g. p53. The mutation pattern in p53 differs between smokers and non-smokers. In addition to p53 mutations, mutations in the epidermal growth factor receptor (EGFR) occur more frequently in female lung adenocarcinoma with a non-smoking history.

There is a paucity of data on risk factors for the development of lung cancer in never smokers. The incidence of lung cancer in non-smokers is higher in female than in males. Initially the focus has been on the exposure to environmental tobacco smoke, where the non-smokers are exposed to the same group of chemical carcinogens, but at a lower dose than the smokers. A recent meta-analysis in an Asian population indicates that the risk of lung cancer in people exposed to environmental tobacco smoke was slightly increased, but the analysis was mostly based upon case-control studies, whereas the results in cohort studies were inconclusive. Both active smoking and exposure for passive smoking has been classified by WHO International Agency for Research on Cancer as group 1 human carcinogen.

Ambient air does contain the same group of compounds as tobacco smoke, and exposure has been shown to increase the risk of lung cancer, and has also been classified by IARC as a group 1 carcinogen. The pathology of lung cancers associated with ambient air pollution is lung adenoma similar to environmental tobacco smoke. The focus in air pollution studies has been on the PM2.5, and it has been shown that the risk (per 10 µg PM2.5/m³) in female non-smokers is higher than in men. Surprisingly, people with a low BMI have a higher risk than people with BMI>30. In addition to genotoxic effects, PM2.5 has been shown to have epigenetics effects, that can influence the expression of genes relevant for lung cancer induction. In a small study nitrogen oxide exposure has also been associated with epigenetic changes in non-smoker lung cancer.

Indoor combustion of coal and biomass for cooking produces many different carcinogens, the chemical composition and levels at the carcinogens depends on the source of energy and type of stove. Both indoor combustion of coal and emission from burning biomass have been classified by IARC as group 1 and 2A, respectively. The conclusion is upon studies in women living in rural area of China. A special area of concern in the Asia-Pacific region is combustion products from incense sticks in the temple and the homes. The chemical composition of the combustion products depends on the type of incense stick. The link between exposure to incense smoke and lung cancer has been difficult to establish and only few small studies have shown an
increased risk of lung cancer in temple workers with high level of exposure. A major risk factor is the PAH formed by combustion, and a Taiwanese study has shown that exposure for total amount of PAH is higher than the acceptable level for cancer risk, whereas regular temple goers do not have an unacceptable exposure. A study conducted by CRI in Thai temples using biomarkers of exposure and effects, has shown that the workers were exposed to several carcinogens, e.g., benzene, 1,3-butadiene and PAH, using biomarkers of exposure and effects.

Exposure for residential radon in houses has been linked to an increased risk of lung cancer, and it has been estimated that 30% of all lung cancer death among non-occupationally exposed never smokers are related to indoor radon exposure. However a subset analysis in a Danish population has only shown a slight but not significant risk for lung cancer in non-smoking female.

Genetic analysis of lung cancer in non-smokers have shown that a genetic polymorphism in EGFR has been associated with an increased risk. However environmental factors play an important role by inducing both genetic and epigenetic changes as well as inflammatory processes.
Bríd M. Ryan, Ph.D.

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Dr. Ryan completed her undergraduate training in biochemistry at University College Cork, Ireland, in 2001. She received her Ph.D. in Cancer Biology from University College Dublin and in 2006 was accepted into the NCI Cancer Prevention Fellowship Program. In 2007, she completed a Masters of Public Health at University College Dublin. She worked under the mentorship of Dr. Curtis Harris during her postdoctoral training at the National Cancer Institute. In 2013, she became an NCI Stadtman tenure track investigator at the NCI.

Her research program addresses several unanswered questions in lung cancer using an approach that integrates epidemiological, experimental and translational research. Disparities in lung cancer incidence, especially amongst men, have been evident for several decades. However, the potential etiological, genetic, and biological reasons behind these differences are underexplored and not well understood. Dr. Ryan’s laboratory investigates the science behind these health disparities and is using a multidisciplinary approach to address this question. She is also interested in applying a biological framework to understanding the mechanism of interaction between genetics and environment with regard to lung carcinogenesis. Specifically, her lab has an interest in both early and adult life exposures and mechanistically understanding how they mediate lung cancer risk later in life.
Diversity in Lung Cancer Risk and Survival

Bríd M. Ryan

Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, MD, USA

Worldwide, lung cancer remains the most commonly diagnosed cancer and the leading cause of cancer-related death. According to the most recent GLOBOCAN report, it accounts for 12% of all cancers in men and women, and 1 in 5 of all cancer-related deaths. By sex, lung cancer is the most commonly diagnosed cancer and the leading cause of cancer death in men, while among women, it is the third most incident cancer and second leading cause of cancer-related death. Despite decades of evidence-based policy and cancer control strategies, the global burden of lung cancer remains significant, and in some countries, is rising.

Figure 1: Global differences in lung cancer incidence. Taken from Bray et al., CA CANCER J CLIN 2018; 68:394–424.

Indeed, there are clear geographic differences in both lung cancer incidence and mortality (Figure 1). Lung cancer rates vary more than 20-fold across geographic regions, something that largely reflects the historical temporality of the tobacco epidemic and differences in patterns of tobacco exposure, including intensity and duration of smoking, type of cigarettes used, and smoking topography. Germline genetic differences in genes involved in nicotine metabolism, including CYP2A6, also contribute to these trends. Today, almost half of all lung cancer cases occur in countries ranked as medium
to low on the Human Development Index (HDI), a composite measure encompassing population health, knowledge, and living standards that indicates the development of a country. This is relevant, as one of the key factors that determines lung cancer survival is access to quality medical care. In addition to these inter-country geographical trends, lung cancer incidence and survival rates can also vary significantly within countries. However, personal smoking habits do not explain all incidence trends. For example, the high lung cancer incidence rates in Chinese women, despite their low smoking prevalence, likely reflect increased exposure to smoke from the burning of charcoal, which is used for heating and cooking. Global declines in air quality and rising air pollution are also impacting lung cancer risk.

The etiology of lung cancer is closely linked with both its histological presentation and molecular features. In smokers, squamous cell carcinoma used to be the most commonly diagnosed subtype until the switch to filtered cigarettes led to a rise in adenocarcinoma. The latter is the most commonly diagnosed subtype in never smokers. EGFR mutations and ALK fusions are frequently detected in tumors from never smokers, key observations as these are both actionable somatic alterations and as such significantly impact survival. To date, there has been less success in the identification of common, actionable mutation in tumors from smokers, but in some cases, the high mutation burden attributable to smoking can be a predictor of response to immune checkpoint inhibitors.

The molecular classification of lung cancer is important for understanding both a patient’s prognosis and likelihood of response to targeted therapies, and as such, is tightly linked to both survival and mortality patterns. It is also, as outlined above, tightly linked to etiological exposures: The prevalence of EGFR mutations mirrors global trends in lung cancer among never smokers.

New patterns of lung cancer risk are also emerging, such as an increase in the prevalence of lung cancer in never smokers, and in the United States, the observation that lung cancer incidence rates are now higher among young women than among young men, a new trend that does not seem to be related to sex-specific differences in smoking behavior. In the past decade or so, many countries have implemented both local and national restrictions on smoking, especially in public places. The international variability in these policy measures is also likely to contribute to ongoing global diversity in lung cancer risk and mortality. Because of this, continued epidemiological analyses are needed to identify and monitor these new trends, while molecular-based studies will be required to understand the genomic nature of these cancers.
Dr. Thanyanan Reungwetwattana is a consultant at Division of Medical Oncology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Thailand. She received her medical degree, Thai Board of Internal Medicine, and Thai Subspecialty Board in Medical Oncology at Ramathibodi Hospital, Mahidol University, Thailand. After that, she spent 3 years from August 2009 to June 2012 as a clinical research fellow in thoracic malignancies and also obtained a Master's Degree of Biomedical Science (Clinical Research Training Program) at Mayo Clinic Cancer Center, Rochester, MN, USA. Subsequently, she extended her training as a Clinical Fellow in Advanced Medical Oncology focusing on Cancer Drug Development Program at Roswell Park Cancer Institute, NY, USA from July 2012 to July 2013.

Dr. Reungwetwattana’s research interests are in lung cancer and drug development which involve both in clinical and translational settings. She has more than 20 publications in the famous peer-reviewed medical journals. Furthermore, Dr. Reungwetwattana has served as an editorial board member and committee of the Journal of Thoracic Oncology from June 2013 to present. She is also joining the Communication Committee of the International Association for the Study of Lung Cancer (IASLC) from 2015 to present and she is also the WCLC 2019 program committee.
Unique Molecular Profile of Thai Lung Cancer Patients in Novel Therapy Era

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Precision medicine is currently applied for almost all cancer types, especially, in NSCLC which is the prototype of successful targeted therapy. EGFR-TKI is the first effective targeted drug found in NSCLC for treatment in EGFR-mutation positive patients. The prevalence of EGFR mutation in NSCLC patients is higher in Asian population compared to the other population (50% vs. <20%). With the dramatic response to the treatment of the ORR 60-70% and OS more than 2 years, make EGFR-TKI is the first-line treatment in EGFR-positive mutation patients. The acquired resistance could be occurred after 9-13 months of treatment. The majority of patients (50-60%) in this group develop T790M as the mechanism of acquired resistance which is now we have the third-generation EGFR-TKI for overcoming this resistance.

The second approved targeted drugs for lung cancer is ALK inhibitors, which the history of development is similar to EGFR TKIs. Nowadays we do have 5 ALK inhibitors in the market to treat NSCLC with ALK fusion gene (7-10%).

The third and the forth targeted drugs are ROS inhibitor and BRAF inhibitors which are already approved for NSCLC patients with ROS fusion gene and with BRAF V600E mutation.

Moreover, the other oncogenic driven mutation (MET amplification, HER2, PIK3CA mutation, RET, NTRK, FGFR fusion etc.) could be the target of treatment in NSCLC as well as EGFR gene. Currently, the potential targeted drugs for inhibiting these oncogenic driven mutations are developing in the early phase clinical studies.

Each region of the world has the different in prevalence of oncogenic driven mutations in lung cancer, for example, EGFR mutation as mentioned above. Our institute found the unique molecular profile in Thai lung cancer patients, which have the high prevalence of BRAF V600E mutation (10%) and METexon14 splice site (9%). In this study, we also found 68%(113/166) of EGFR mutation, 32.5% (54/166) of KRAS mutation, 4.8% (8/166) AKT mutation (E17K), 2.4% (4/166) of ROS1 mutation, 0.6% (1/166) of PIK3CA mutation (H1047R), and 0.6% (1/166) of PTEN mutation. Furthermore, 40 patients (24.1%) had more than one mutation in each person. We further validated the positive results by Real-Time PCR. Thirteen patients were obtained tissue from different organs and some with different period of time. T790M usually develop later in EGFR-positive patients who failed 1st or 2nd generation EGFR-TKI. Two patients who had lung surgery different lobe in same operation, had different mutation in tissues and one patient who obtained tissue from lung and pleural effusion cell block in different period of time had totally different mutation.

In summary, Thai populations have unique molecular alteration compared to the other ethnicities, especially, higher of BRAF V600E and MET exon14 splice site. Our population also has high co-mutation prevalence. Tumor heterogeneity is needed to explore in the larger cohort. Molecular profile in NSCLC patient is very important and very useful for developing the new targeted drugs. I do believe that there will be the other effective novel targeted treatments for NSCLC approved in the near future which would improve the long term survival and QOL for patients.
Scott W. Lowe, Ph.D.

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Scott W. Lowe is Chair of the Cancer Biology and Genetics Program at Memorial Sloan Kettering Cancer Center (MSKCC) in New York City and an Investigator for the Howard Hughes Medical Institute. Dr. Lowe received his Bachelor’s Degree from the University of Wisconsin-Madison and his Ph.D. from the Massachusetts Institute of Technology.

He initiated his independent research at Cold Spring Harbor Laboratory, where his group made important contributions to our understanding of the p53 tumor suppressor network, as well as the processes of multi-step carcinogenesis, cellular senescence, and tumor-cell drug resistance. At MSKCC, his laboratory applies mouse models, functional genomics and cancer genomics in a coordinated effort to identify cancer drivers and dependencies.

These efforts have revealed fundamental insights into cancer mechanisms and identified potential therapeutic targets. Dr. Lowe’s work has been recognized by several awards, including a Sidney Kimmel Scholar Award, a Rita Allen Scholar Award, the Outstanding Investigator Award and the G.H.A. Clowes Memorial Award from the American Association for Cancer Research, the Paul Marks Prize, and the Alfred G. Knudsen Award. He has also been elected to the American Academy of Arts and Sciences and the National Academy of Sciences.
Restoring Tumor Suppression in Advanced Cancers

Scott W. Lowe
Sloan-Kettering Institute, MSKCC; Howard Hughes Medical Institute, USA

Cancer arises through an evolutionary process whereby normal cells acquire mutations that erode growth controls, leading to the expansion of aberrantly proliferating cells. Such mutations activate oncogenes or disable tumor suppressors, each bestowing new capabilities to emerging tumors. While the resulting genotypes that arise must confer a selective advantage to cancer cells, they may also produce cellular “addictions” that are potential targets for novel cancer therapies. Accordingly, our laboratory interrogates the genes and processes that drive cancer, explores how they interact to produce distinct tumor phenotypes, and attempts to identify therapeutically actionable vulnerabilities linked to particular cancer genotypes.

Our laboratory has historically studied the action of tumor suppressor genes, with a particular emphasis on the p53 tumor suppressor. Initially, we linked p53 to apoptosis, and later senescence, revealing key cellular failsafe mechanisms that counter oncogene-induced transformation. Current efforts are guided by our long-standing view that TSGs coordinates key regulatory nodes in growth control processes and the strategies nature uses to combat cancer; accordingly, we take both hypothesis-driven and non-biased approaches to understand tumor suppressor action with the long-term goal of identifying strategies to mimic their activity or alternatively, to exploit consequences of their inactivation.

Mutations targeting the TP53 tumor suppressor gene on chromosome 17p13 are the most frequent events in human cancer. p53-/- mice are tumor prone, and p53 loss cooperates with various oncogenes to promote transformation in vitro and tumorigenesis in vivo. Conversely, re-expression of endogenous p53 in p53 deficient tumors potentially inhibits tumor growth, implying that p53 loss is required to maintain tumorigenesis and validating the pathway as a therapeutic target. In human tumors, p53 is typically disabled through a two-hit mechanism involving a point mutation in one allele and a large deletion event targeting the second. While these events clearly inactivate p53, the p53 missense mutations can have gain of function activities and the deletions target additional haploinsufficient tumors suppressors that contribute to tumor phenotypes beyond p53 loss. Thus, p53 mutations are not merely a binary “on” off switch, but instead the nature of which can produce substantial phenotypic heterogeneity.

Wild-type p53 accumulates in response to cellular stress and regulates the expression of genes that alter cell fate and constrain tumorigenesis. These effector processes can include biological programs such as a G1 checkpoint, apoptosis, cellular senescence, differentiation, autophagy, and ferroptosis, among others. Which of these processes are most important for tumor suppression remains controversial, and it is likely that multiple processes are important, with the specific output dependent on cellular context (REF). One series of biochemical processes p53 can affect involve changes to cellular metabolism, which can have both positive and negative effects on tumor cell proliferation and survival. Still, genetic studies support a key role for p53-regulated changes in cellular metabolism during tumor suppression, though the details of how this might work remain unclear.
We have been interested in relating p53 control of cellular metabolism under circumstances where p53 is actively promoting tumorigenesis. To these end, we produced and studied mouse models of liver and pancreas cancer p53 function in tumorigenesis and tumor maintenance could be tightly regulated using a tetracycline responsive p53 shRNA. In these models, suppression of p53 promotes tumorigenesis, whereas p53 restoration halts tumor growth. By studying p53 control of metabolism in response to p53 reactivation, we show that p53 can trigger a rewiring of carbon utilization in the cell leading to changes in key cell metabolites that, in turn, control enzymes that influence gene regulation and cell fate. Details will be presented during the presentation but demonstrate that p53 can drive tumor suppression through biochemical changes in cell metabolism that produce biological changes leading to less malignant cell fates. Conversely, p53 loss can disable these safeguards and leads to dedifferentiated cell fates and promotes tumorigenesis.

Mutations in the KRAS oncogene are the second most common event in human cancer, and frequently co-occur with p53 mutations in human tumors. One explanation for this association is that p53 loss override cellular senescence during the course of tumor evolution. In a series of experiments aimed at developing a combination strategy to target KRAS mutant cancer, we showed that combined use of MEK and CDK4/6 inhibitors can restore senescence to KRAS/ mutant lung cancer cells, leading to a potent cytostatic response. In turn, senescent cells activate a program known as the senescence associated secretory phenotype (SASP), that provokes immune surveillance of senescent tumor cells and ultimately tumor regressions. These studies identify a means and method of invoking a unique form of NK cell mediated immune surveillance in lung tumors through molecularly targeted therapies that induce senescence. A detailed mechanism for how this occurs will be described in the presentation.

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Dr. Nabeel Bardeesy completed his B.Sc. and Ph.D. in biochemistry at McGill University in Montreal. He then pursued postdoctoral training in cancer biology at the Dana-Farber Cancer Institute and Harvard Medical School. Since 2005, he has been on the faculty of the MGH Cancer Center and Harvard Medical School where he is presently Associate Professor of Medicine and holder of the Gallagher Endowed Chair in Gastrointestinal Cancer Research. His laboratory focuses on elucidating the molecular pathways underlying pancreatic cancer and cholangiocarcinoma (CCA). He has played a leading role in the development of genetically engineered mouse models for these cancer types and in using these models to understand the roles of key gene mutations (e.g. KRAS, LKB1, GNAS, IDH) in promoting tumor growth and deregulating tumor cell metabolism.

Dr. Bardeesy is co-Principal Investigator (PI) of the Harvard Specialized Program in Research Excellence in Gastrointestinal Cancer, and PI of a DOD Translational Team Science Award for CCA studies and as well multiple NIH R01 and U01 grants. He is Director of the Scientific Advisory Board of the Cholangiocarcinoma Foundation and member of the SAB of the Forbeck Research Foundation. He has received several honors including the NCI Howard Temin Award, Kimmel Foundation Scholar Award, and V Foundation for Cancer Research Translational Award. He has trained many cancer researchers who presently hold faculty positions around the country and internationally and is the recipient of the Harvard Medical School Young Mentor Award.
Intrahepatic cholangiocarcinoma (ICC) is a deadly cancer of the bile ducts. There are multiple mutated drivers in subsets of ICC patients, creating challenges in patient management. Two of the most common genomic alterations are activating fusions of the Fibroblast Growth Factor Receptor 2 (FGFR2) gene and hot-spot gain-of-function mutations of the Isocitrate Dehydrogenase 1 and 2 genes (IDH1, IDH2). At present clinical trials targeting both FGFR signaling and mutant IDH1 are showing promise in ICC patients. In this presentation we will discuss the preclinical and translational study of the FGFR and IDH pathways in ICC. The development of murine and patient-derived model systems, the oncogenic pathways controlled by these genomic alterations, and approaches to target these tumors will be addressed. We will also discuss modeling ongoing clinical trials and attempts to understand and overcome drug resistance.
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Lars Zender, M.D., is Medical Director and Chairman of the Department for Internal Medicine VIII (Clinical Tumor Biology and Phase I Clinic) at University Hospital Tübingen and the Department of Vegetative and Clinical Physiology at Eberhard Karls University Tübingen, Germany. Lars Zender’s work especially focuses on the identification of new cancer genes involved in liver cancer development. He developed novel mosaic (chimaeric) liver cancer mouse models, which allow for high throughput functional genomic analyses. Together with a limited number of other laboratories worldwide, Lars Zender’s group has the expertise to conduct stable RNA interference screens for the identification and validation of new cancer genes directly in vivo.

Another key aspect in the scientific work of Lars Zender is his work on cellular senescence. In particular the Zender laboratory is studying the senescence-associated secretory phenotype and how senescent tumor cells and pre-cancerous cells are recognized and cleared by the immune system. Recent work from Lars Zender’s laboratory showed that a continuous antigen specific immune clearance of premalignant senescent hepatocytes is crucial for tumor suppression in the liver. He received many awards, including the Gottfried Wilhelm Leibniz Prize of the German Research Foundation (DFG). Lars Zender is holding an ERC Consolidator Grand and is spokesperson of the DFG funded Excellence Cluster “Image-Guided and Functionally Instructed Tumor Therapies (iFIT)”. 

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In my talk I will give examples how non-genetically engineered cancer mouse models can be combined with stable in vivo RNAi or Crispr/Cas9 screening technology to identify new therapeutic targets. I will discuss the pivotal role of academic drug discovery infrastructures for rapidly translating validated therapeutic target structures into clinical applications and will give an example of a novel and promising drug for the treatment of liver cancer which went first into man less than two years after completion of preclinical testing. Furthermore, I will discuss current research activities on a functional identification of vulnerabilities in senescent cancer cells (therapy induced senescence) as a prerequisite for the development of novel senolytic drugs.

Finally, I will follow up on recently published data (Seehawer et al., Nature 2018) on a novel mechanism how lineage commitment in liver tumorigenesis is regulated. Primary liver cancer represents a major health problem. It comprises hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), which differ markedly with regards to their morphology, metastatic potential and responses to therapy. However, the regulatory molecules and tissue context that commit transformed hepatic cells towards HCC or ICC are largely unknown. We showed that the hepatic microenvironment epigenetically shapes lineage commitment in mosaic mouse models of liver tumorigenesis. Whereas a necroptosis-associated hepatic cytokine microenvironment determines ICC outgrowth from oncogenically transformed hepatocytes, hepatocytes containing identical oncogenic drivers give rise to HCC if they are surrounded by apoptotic hepatocytes. Epigenome and transcriptome profiling of mouse HCC and ICC singled out Tbx3 and Prdm5 as major microenvironment-dependent and epigenetically regulated lineage-commitment factors, a function that is conserved in humans. Together, our results provide insight into lineage commitment in liver tumorigenesis, and explain molecularly why common liver-damaging risk factors can lead to either HCC or ICC.
Jittiporn Chaisaingmongkol, Ph.D.

Dr. Jittiporn Chaisaingmongkol is currently working at Chulabhorn Research Institute. She completed her Ph.D. at The German Cancer Research Center in Heidelberg, Germany in 2011 and was a visiting fellow at The National Institute of Health, USA from 2013-2015.

As a part of the TIGER-LC consortium, her main area of research is in liver cancer with an emphasis on genomics, environmental and etiological factors of primary liver cancer in Thai patients.
Primary liver cancer is a major public health concern worldwide, accounting for 8.2% of all cancer deaths with around 841,000 new cases annually. Most liver cancer (72%) is diagnosed in Asian countries. Primary liver cancer includes hepatocellular carcinoma (HCC) comprising 75%-85% of cases, intrahepatic cholangiocarcinoma (ICC) comprising 10%-15% of cases, as well as other rare types. HCC and ICC were considered to be separate diseases that originate from different cell populations. The diagnosis depends on their baseline clinical and pathological features. But more recently, evidence have shown that some HCC and ICC cases might share some common molecular characteristics and could be considered as continuous spectrum of the disease.

Classification of cancer molecular subtypes based on transcriptomic and/or genetic information has been used for generating a treatment algorithm for several types of cancer, including lung and breast cancers. The development of subtype classification in liver cancer started in the early 2000s using gene expression microarrays. Mutation profiling using DNA sequencing and SNP microarrays have shed some light on molecular subtypes of liver cancer as well. Furthermore, study of epigenetic features such as DNA methylation, micro-RNA patterns and tissue metabolomes was added to the arsenal. Through these efforts two major subtypes of HCC were identified as proliferation and non-proliferation classes. The proliferation subtype is associated with cell signaling pathways activation, increased chromosomal instability and stem cell properties. The tumors in this subtype often contain TP53 mutations and have a characteristic DNA methylation pattern and micro-RNA signatures. Overall, this HCC subtype is associated with tumor recurrence and poor clinical outcome. The non-proliferation subtype contains less-aggressive, well differentiated tumors leading to better prognosis. The gene expression pattern of this subtype is associated with activation of inflammation pathways.

Next-generation sequencing technology has helped identify mutation signatures in HCC samples associated with environmental exposure, including viral integration, smoking, aflatoxin B1, and aristolochic acid. The most prevalent mutations in HCC have been confirmed through this new sequencing technology and new mutations have also been discovered. The most common aberrations in HCC were found in TERT promoter, CTNNB1, TP53, AXIN1 and ARID1A.

Since the recognition of ICC as distinct liver cancer type in the 7th edition of American Joint Committee on Cancer Staging Manual in 2010, studies attempting to classify ICC subtypes have emerged. Two main molecular subtypes have been identified as proliferation and inflammation subtypes. Similar to HCC, ICC proliferation subtype is characterized by activation of oncogene pathways, chromosomal instability, and stem cell features and is associated with poor prognosis. TP53, EGFR, KRAS, IDH1/2 are the most common mutations associated to the proliferation subtype. Whereas, common mutations in chromatin remodeling genes, e.g. ARID1A, BAP1, PBRM1 did not show clear association with any subtypes. The inflammation subtype showed activation of inflammatory pathways and cytokine overexpression. The tumors in this subtype are well differentiated and the patients have good prognosis.
In the recent years many comprehensive molecular analyses of large HCC and ICC cohorts have been reported\(^4\)\(^-\)\(^7\). Integrative multi-omics approach of these new studies provided in-depth information about cellular and molecular complexity of liver cancer. The molecular classes founded in new studies largely agreed with older ones and also agreed with one another. The striking similarity between subsets of HCC and ICC tumors support a model of “multiple cells of tumor origin in primary liver cancer”. In this model, HCC and ICC can be distinct tumors that originate from mature hepatocytes and cholangiocytes, respectively. But there is evidence showing that mature hepatocytes can trans-differentiate into a biliary-like cell that in turn give rise to ICC. On the other hand, hepatic progenitor cells may also give rise to progenitor-like HCC or ICC, resulting in common molecular profiles in a subset of HCC and ICC cases\(^3\).

Despite tremendous effort to understand liver cancer progression and its classification, the challenge still lies in clinical implications. There is still no consensus in using molecular subtypes for clinical staging, nor is there any established liver cancer biomarker for guided cancer therapy. This could be due to high degree of inter- and intra-tumoral heterogeneity in liver tumors, especially in ICC. Furthermore, the most frequent mutations in liver cancer are not easily targetable. Nevertheless, new potential targets have been tested in early clinical trials including \(FGFR2\) and \(IDH1/2\), as well as an immune checkpoint inhibition approach.

Our decade long knowledge of molecular characterization of HCC and ICC has yet to be transferred to the bedside. More effort has to be put into this task in order to improve clinical outcome and quality of life of all liver cancer patients.

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Dr. Teh obtained his MD (1992) from the University of Queensland, Australia and his PhD (1997) from the Karolinska Institute, Sweden. Following postdoctoral works at Karolinska Institute, he joined the Van Andel Research Institute (VARI), USA in 2000 as a Senior Scientific Investigator heading the Laboratory of Cancer Genetics. From 2003, he served as the Deputy Director (Research Operations) of VARI and from 2008, Director of VARI International. He established the National Cancer Centre Singapore (NCCS)-VARI laboratory, which serves as a bridge between translational research and clinical medicine. In 2010 he received the Singapore Translational Research Investigator Award and relocated to Singapore. He served as the SingHealth Group Director for Translational Research from 2010-2012. His laboratory focuses on Genomics of Asian-Prevalent Cancers and in the last 5 years have made seminal discoveries in the field including hepatobiliary cancer, herbal carcinogen-related cancer and fibroepithelial tumors of the breast. He holds Adjunct Professorships at several universities worldwide including Baylor College of Medicine, USA, Nanjing University and Sun Yat-Sen University, China and the Karolinska Institute, Sweden. Dr. Teh has published extensively, with over 350 publications in high impact scientific journals. He is a past and present member of numerous editorial boards for journals including Lancet Oncology, Cancer Research, Molecular Cancer Therapeutics. Dr. Teh is a recipient of the 2015 Singhealth Distinguished Researcher Award, and co-recipient of the 2015 Singapore President Science Award and the 2018 AACR (American Association of Cancer Research) Team Science Award.
Cholangiocarcinoma of Different Etiology: 
Genomic and Epigenomic Studies

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It is well-known that Asian prevalent cancers are often associated with a pathogen or carcinogen. For example, hepatocellular carcinoma (HCC) is associated with hepatitis B and C viruses, gastric cancer Helicobacter pylori, and nasopharyngeal cancer Epstein-Barr virus (Figure 1). In this presentation, we would like to focus on cholangiocarcinoma of different etiology which are associated with chronic carcinogenic exposures leading to distinct genomic and epigenomic changes – these changes may be manipulated not only for diagnostic detection but may potentially serve as targets for therapeutic intervention. Importantly, our studies demonstrate that the same cancer type in different populations may be attributed to distinct carcinogenic environmental factors which can potentially be detected by distinct genetic and/or epigenetic alterations/modifications. Similarly, it reflects potentially distinct molecular mechanisms that may require different therapeutic regimes for effective treatment which represent tremendous unmet clinical needs.

CCA is a malignant tumor of bile duct epithelial cells with very poor prognosis which accounted for 10-25% of all primary liver cancers diagnosed worldwide. Recent studies have also shown heterogeneity for cell of origin demonstrating that a subset of CCA, particularly the intrahepatic CCA, may arise from hepatocytes (Seehawer et al., 2018). Several established risk factors for CCA are related to geography and etiology. In the Western countries, primary sclerosing cholangitis (PSC) is the most common risk factor of CCA and others include hepatolithiasis, choledochal cysts, cirrhosis, diabetes, obesity, alcohol consumption and smoking (Tyson & El-Serag, 2011). In contrast infestation of liver flukes called Opisthorchis viverrini (OV) has been associated with the highest incidence of CCA in northeast region of Thailand, and neighbouring Laos and Cambodia (Jusakul, Kongpetch, & Teh, 2015; Kongpetch et al., 2015). Every year, it is estimated that approximately 30,000 CCA cases occur in this region with the vast majority of cases found at late stage disease. Unfortunately, there is no effective treatment, therefore leading to extremely high mortality. It is thought that the common thread of all these CCA-associated risk factors may be chronic inflammation in the cells of origin. OV is a foodborne trematode that encysts as a metacercaria in the cyprinoid fish which are consumed raw or uncooked by inhabitants of the endemic region frequently. Once ingested the metacercaria will form adult OV which can inhabit the biliary tract within the
human host for over 10 years (Young et al., 2014). To date, three main carcinogenic mechanisms have been proposed: 1) mechanical damage to the biliary epithelia caused by the feeding activities of the parasites, 2) immunopathology because of infection-related inflammation and 3) the toxic effects of parasite excretory/secretory molecules (Smout et al., 2011). In response to OV infection, inflammatory cells are activated by proinflammatory cytokines and nitric oxide generated by inducible nitric oxide synthase. Nitric oxide produced in infected and inflamed tissues has been postulated to contribute to cholangiocarcinogenesis by causing damage to DNA and proteins (Dechakhamphu, Pinlaor, Sitthithaworn, Bartsch, & Yongvanit, 2010; Pinlaor et al., 2004; Thanan et al., 2013), resulting in mutagenic changes. It can also stimulate cyclooxygenase-2 expression, which can promote growth of cholangiocytes via activation of growth factors such as epidermal growth factor receptor, mitogen-activated protein kinases and interleukin-6 (Sia, Tovar, Moeini, & Llovet, 2013). Thus, chronic OV infection and inflammation can lead to accumulation of genetic, epigenetic and transcriptional alterations in CCA.

With the goal of better understanding the underlying oncogenic mechanisms in CCA, we characterized and compared the genetic and epigenetic changes in OV-related vs non-OV CCA (Chan-On et al., 2013; Ong et al., 2012) (A. Jusakul et al., 2017). Our multi-omics analysis reflected the contrasting etiologies of OV-related vs non-OV CCA, strongly positing that the same disease manifestation can be in fact driven by vastly different molecular circuitries and that each patient should be treated with appropriately targeted therapy. We observed that OV-infected tumors are characterized by p53 mutations and CpG Island hypermethylation whereas non-OV tumors are characterized by mutations in epigenetic modifier genes including IDH1/2 and BAP1. These findings set the stage for more in-depth epigenomic characterization and mechanistic dissection of the tumorigenic process. Besides our data, the potential role of epigenetic drivers in OV-related CCA has also been supported through several lines of evidence. Fluke infection is associated with prolonged bouts of pre-malignant inflammation. Inflammatory signalling induces enhancer activity in various cell types (Betancur et al., 2017; Brown et al., 2014; Hah et al., 2015; Schmidt et al., 2015), and CCA cells respond readily to inflammatory signalling (Hogdall, Lewinska, & Andersen, 2018; Yoo, Lim, & Choi, 2016). Chronic inflammation can lead to aberrant DNA methylation (Chiba, Marusawa, & Ushijima, 2012; Maeda, Moro, & Ushijima, 2017). Furthermore, recent studies have demonstrated that the activation of de novo enhancers during inflammatory response can co-opt coactivators from existing enhancers, leading to their deactivation (Hah et al., 2015; Schmidt et al., 2015). Notably, enhancers that maintain cell identity are particularly vulnerable to deactivation in this process (Hah et al., 2015; Schmidt et al., 2015). Consequently, chronic inflammation associated with OV infection may play a key role in setting up divergent enhancer landscapes between OV-related and non-OV CCAs. By investigating if inflammatory response instigates specific enhancer activity in OV-related CCAs, as well as the accumulation of enhancer dysregulation during OV-associated chronic inflammation in in vivo hamster CCA model will clarify their mechanistic link. These findings will deepen our understanding of early transformation events in inflammation-associated cancers, and may lead to the development of epigenetic biomarkers for early detection of inflammatory tissues at risk of malignant transformation.
References:


Session 2: Cancer Drivers, Biomarkers of Risk, Diagnosis, Prognosis and Response to Therapy

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Nickolas Papadopoulos received his doctorate from the University of Texas at MD Anderson Cancer Center. He conducted postdoctoral training with Bert Vogelstein at Johns Hopkins University.

He is considered an expert in cancer genetics, genomics and diagnostics.

Notable accomplishments are the discovery of the genetic basis of the predisposition to Hereditary Nonpolyposis Colon Cancer, the determination of the genetic landscapes of numerous cancer types.

Currently, he is focused on translating the genetic information derived from cancer genome analyses to clinical applications in early detection, diagnosis and monitoring of cancer.

He has received the V Foundation award, the Stewart award for Cancer Research, three times the AACR -Team Science Award, among others.
Detection and Localization of Surgically Resectable Cancers with a Multi-analyte Blood Test

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Detection of cancer at early stages while it is treatable with localized strategies such as surgery provides the best opportunity to decrease cancer related mortality. This has been proven to be true for several cancers for which effective screening modalities exist. However, the full potential of early detection has not been achieved because most cancers lack viable screening strategies - this results in cancers being detected at more advanced stages when they have poorer clinical outcomes. In addition, cancer screening is made inefficient by having to screen for each tumor type separately and there is a need for a test that can detect multiple tumor types. Liquid biopsies have the potential to improve upon existing cancer screening methods by providing a way to test for cancers that have no current strategies or by having superior performance than existing technologies. Methods for analyzing small fragments of cell-free tumor DNA (ctDNA) have advantages in sensitivity and specificity, offering an attractive opportunity to “screen” for several mutations at once from an easily obtainable blood sample.

A plasma based approach for the early detection of multiple cancers would represent a significant medical advance. We recently developed a multi-analyte blood test, coined “CancerSEEK”, for the detection of resectable cancers. The assay includes the detection of DNA mutations and protein biomarkers. Because the ultimate goal is to develop a screening test, specificity was of great importance, and we designed an assay with this in mind. For the DNA mutations we developed a small panel of only 1,934 positions in the genome that cover the most prevalent mutations that are known to be pathogenic in 16 genes. Using publicly available sequencing data, we found that there was a fractional power law relationship between the number of genomic positions required and the sensitivity of detection, with a plateau at approximately 1,900 bases. Larger panels are associated with more false positives and increased costs. Using only circulating tumor DNA (ctDNA) as an analyte has its limitations. Previous studies have established that not every tumor “sheds” DNA in the circulation and that DNA sensitivity can be limited by stochastic events. To increase the sensitivity of the assay, we included protein biomarkers. These are proteins that are known to be elevated in cancer, but their use as diagnostic markers was limited by specificity. To alleviate this, we used thresholds for proteins that had sensitivities >10% in detecting one or more cancer types and specificities >99%.

We evaluated CancerSEEK in a retrospective study that included 1,005 individuals with resectable non-metastatic cancers of the colon, breast, lung, ovary, pancreas, esophagus, liver or stomach, and 812 healthy controls. CancerSEEK tests were positive in a median of 70% of the eight cancer types with specificity greater than 99%. The sensitivities ranged from 69 to 98% for the detection of five cancer types (ovary, pancreas, esophagus, liver and stomach) for which there are no screening tests available for average-risk individuals. One limitation of liquid biopsy based screening tests is their inability to determine the cancer type in patients who test positive, which poses challenges for clinical follow-up. To examine whether the CancerSEEK test can help
identify a cancer’s tissue of origin, we used supervised machine learning to predict the underlying cancer type in patients with positive CancerSEEK tests. The algorithm took into account the ctDNA and protein biomarker levels as well as the gender of the patient. We then used this algorithm to study 626 cancer patients with positive CancerSEEK tests. Without any clinical information about the patients, we were able to localize the source of the cancer to two anatomic sites in a median of 83% of these patients.

The nature of the analytes is such that they are not tumor-type specific. For example, KRAS mutations can be identified in a large number of tumor types. Although CancerSEEK was validated in eight tumor types, the test has the potential to be cancer agnostic. The test is currently evaluated in a two-phase study, called DETECT, that includes 10,000 average risk asymptomatic individuals for phase A and 40,000 for Phase B. Although, Phase A is still ongoing, evaluation of the results from the first 7,000 individuals, indicates that the specificity is what we expected and that tumor-types other than the eight included in the retrospective study can be detected, indicating the pan-cancer approach of the test. Our vision is to develop a test for the early detection of cancer that will be part of routine physical exams.

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Stephen Lam MD, FRCPC is Professor of Medicine at the University of British Columbia, Distinguished Scientist, the Leon Judah Blackmore Chair in lung cancer research and MDS-Rix endowed director of translation lung cancer research at the BC Cancer Research Center. He chairs the Pan-Canadian Lung Cancer Screening Network. He has published over 290 peer reviewed papers and book chapters. He was the recipient of the IASLC Joseph Cullen Award for life-time scientific achievements in lung cancer prevention research, the Friesen Rygiel Award for Outstanding Canadian Academic Discovery, the Gustav Killian Medal by the World Association of Bronchology for pioneering contributions to the field early lung cancer diagnosis, as well as the Killam Research Prize in Applied Sciences and the Distinguished Achievement Award from the University of British Columbia. Dr. Lam received his medical training at the University of Toronto. He joined the UBC Faculty of Medicine in 1979 and BC Cancer in 1984.
Globally, lung cancer is the leading cause of cancer mortality in both men and women. The worldwide burden of lung cancer is significant and projected to rise during the coming years\(^1\). Two large, sufficiently powered randomized trials with adequate follow-up showed a significant mortality reduction benefit of lung cancer screening using low dose computed tomography (LDCT). The National Lung Screening Trial (NLST) showed a 20% lung cancer mortality rate reduction with screening using LDCT compared to chest radiography\(^2\). Very recently, the Dutch-Belgium NELSON trial reported a 26% reduction in lung cancer mortality in men and up to 61% mortality reduction in women with LDCT screening versus no screening\(^3\).

The US Preventive Services Task Force (USPSTF)\(^4\) and the Canadian Task Force on Preventive Health Care\(^5\) support screening with LDCT of the chest to decrease lung cancer mortality, LDCT is being implemented at the population level in the US and Canada. However, key issues remain regarding implementation of LDCT screening at the population level such as the optimal selection criteria to identify individuals with sufficient risk for screening and management of screen detected lung nodules.

Emerging data suggests USPSTF age and pack-years selection criteria are suboptimal. Only 40% to 45% of lung cancer patients in Canada and US would meet the USPSTF screening criteria (55-80 years who had smoked at least 30 pack-years and not starting screening after 15 years of smoking abstinence) had screening were available prior to diagnosis.\(^6\) The sensitivity to identify individuals at high risk of lung cancer for screening can be improved to as high as 80% using risk prediction models such as the PLCOm2012 risk prediction tool.\(^7\) However, in a mixed Caucasian and non-Caucasian population, the sensitivity is lower around 65%. Currently, there is no validated risk prediction tool for Asians. Incorporation of other risk factors such as outdoor and household air pollution and genetic susceptibility may improve the accuracy of risk prediction among Asians.

Another concern with population screening is the large number of lung nodules that are identified by LDCT most of which are benign. A number of lung nodule management guidelines and lung nodule malignancy risk prediction tools are available to guide nodule management. The action thresholds for early recall CT imaging study, PET/CT or biopsy vary in different guidelines. To facilitate nodule management, the PanCan Nodule Malignancy Probability Model was developed to help more accurately identify which nodules are at high risk of being lung cancer and require close surveillance or biopsy.\(^8\) Prospective evaluation of the clinical utility of the model is being investigated in the International Lung Screen Trial. Radiomics and deep learning approaches hold promise to improve the accuracy of risk prediction further. Computer assisted diagnostic (CAD) tools facilitates measurement of nodule size and density. CAD reduces inter-observer variability but they may not be generally available. Volumetric measurement is particularly useful for comparison of serial scans for evidence of growth. To measure size accurately especially to determine changes in volume, it is necessary to address standardization of technical requirements related to the scanners and image acquisition protocols.\(^9\)
Over 75% of screening CT detected lung cancers are ≤20 mm.\textsuperscript{10} Because of the small size of these lesions, the diagnostic yield of bronchoscopic biopsies is modest. To improve the diagnostic accuracy, ultrathin bronchoscopes and flexible 21G edged TBNA needles are now available for transbronchial aspiration or core biopsy.\textsuperscript{11}

 Advances in risk prediction tools, imaging and nonsurgical biopsy techniques will optimize benefits from a LDCT lung cancer screening program.

References:
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Roger R. Reddel is the Director of Children's Medical Research Institute (CMRI) and the Lorimer Dods Professor, Faculty of Medicine and Health, University of Sydney. His training included medical degrees from the University of Sydney, board certification in Australia as a medical oncologist, a Ph.D. in cancer cell biology at the University of Sydney, and postdoctoral research in molecular carcinogenesis at the US National Cancer Institute. His research at CMRI investigates the immortalisation of cancer cells, and he has contributed to the understanding of telomere length maintenance in cancer, especially the Alternative Lengthening of Telomeres mechanism. He recently commenced a research program that aims to deliver relevant proteogenomic data to the cancer clinic. He is a Fellow of the Australian Academy of Science and of the Australian Academy of Health and Medical Sciences.
Advances in DNA sequencing technology have led to many new insights into the genomic and transcriptomic changes in cancer, and have underpinned efforts to tailor treatment to the molecular changes in individual cancers. Proteomic technology has developed more slowly: although there have been many studies of cancer proteomics, it has not yet been possible to systematically survey the proteomic landscape of cancer because high-throughput proteomic capability has been lacking.

SWATH mass spectrometry (MS) is a Data-Independent Acquisition (DIA) version of MS, in which data regarding all detected peptides are permanently recorded and can be interrogated later with a spectral reference library. When SWATH-MS is combined with sample preparation using Pressure Cycling Technology (PCT), reproducible proteomic data can be obtained from small tissue samples [T. Guo et al., Nat. Med. 21: 407-413, 2015]. The reproducibility of PCT-SWATH-MS has been assessed by a study in which cell samples and synthetic peptide dilution series were analysed in 11 laboratories worldwide [B.C. Collins et al., Nat. Commun. 8:291, 2017]. This showed that >4,000 proteins were consistently detected in HEK 293 cells and reproducibly quantified, indicating that this method may be suitable for large-scale protein quantification.

We have begun a pan-cancer proteomic study using PCT-SWATH-MS technology with many modifications to make it more suitable for scaled-up application. A custom-built facility (ProCan®) with equipment funded by the Australian Cancer Research Foundation, currently contains six HPLC-MS units and is designed for continuous operation, with the aim of generating 10,000 cancer proteomes in duplicate (20,000 proteomics files) per year, from fresh-frozen or formalin-fixed paraffin-embedded tumor samples. A large-scale study of data reproducibility across MS instruments and over time is currently underway. Two early cancer studies have each generated >1,000 proteomes, and a study that will be completed in mid-2019 is generating 6,000 proteome files from 1,000 cancer cell lines. We are establishing software pipelines required for efficient analysis of proteomic data on this scale.

The research program supported by the ProCan facility intends to survey the proteomic landscape of human cancer, including lung and liver cancer, adding information about proteins to the rapidly growing body of other cancer 'omic data. The main focus of the program is utilization of multi-omic data to support improved clinical decision-making regarding choice of treatment for individual cancer patients. To achieve these objectives, we are collaborating with many research groups who already have retrospective collections of well-annotated pre-treatment cancer samples for which the outcome of treatment is known and, preferably, where genomic and/or other 'omic data are available.

To find predictors of treatment outcome, we aim to integrate clinical and histopathology data with 'omics data. Histopathology is a critically important tool for interpreting the proteomes of the complex mixtures of cells and extracellular material that constitute tumors. Our approach, therefore, is to cut two sections from tumors: a thin section for
histopathology and an immediately adjacent thicker section for proteomic analysis. The histopathology image is digitized and stored with the other data for analysis by machine learning techniques.

An additional approach to interrogation of the proteomic data relies will be the identification of molecular signatures of cancer phenotypes known to contribute to clinical outcome. An example of this is cancer telomere biology. The ability of cancer cells to undergo unlimited proliferation, referred to as cancer cell immortality, is one of the hallmarks of cancer. Cancer cells are known to escape the normal limits on proliferative capacity by activating one of two known telomere lengthening mechanisms – either the ribonucleoprotein reverse transcriptase enzyme, telomerase [E.H. Blackburn & K. Collins, Cold Spring Harbor Perspect. Biol. 3: a003558, 2011], or an homologous recombination-dependent DNA replication process, Alternative Lengthening of Telomeres (ALT) [H.A. Pickett & R.R. Reddel, Nat. Struct. Mol. Biol. 22: 875-880, 2015]. For many tumor types, the presence of telomerase or ALT correlates with clinical outcome: in glioblastoma, ALT is associated with significantly longer survival [V. Hakin-Smith et al., Lancet 361: 836-838, 2003], but for many tumor types ALT is associated with worse outcome [J.D. Henson & R.R. Reddel, FEBS Lett. 584: 3800-3811, 2011]. In a preliminary study [Y. Wu et al., unpublished data], fifty-five cell lines were classified according to their telomere length maintenance mechanism and analysed by PCT-SWATH-MS. Machine learning identified a proteomic signature that distinguishes cell lines that utilize telomerase or ALT, and the protein at the top of the list is a novel component of the ALT mechanism. The ability of this proteomic classifier to correctly identify telomere length maintenance mechanism is currently being validated in independent tumor sets.

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Dr. Curtis Harris received his M.D. from Kansas University School of Medicine. He completed his clinical training at the University of California-Los Angeles, and at the NCI. He has held positions of increasing responsibility at the NCI, and is also an Adjunct Professor of Oncology at Georgetown University School of Medicine. Harris has received numerous honors throughout his distinguished career including the Alton Ochsner Award relating Smoking and Health (American College of Physicians), Deichmann Award (International Union of Toxicology), Charles Heidelberger Award (International Society of Gastroenterological Carcinogenesis), the Distinguished Service Medal (the highest honor of the U.S. Public Health Service), NCI Outstanding Mentor Award in 2007 and 2013, Ph.D. (Honorary) Nippon University School of Medicine, the AACR-Princess Takamatsu Award, and most recently in 2014 the ILCA Nelson Fausto Award and AACR-American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention and in 2017 the Alumnus of the Year, School of Medicine, University of Kansas. He serves as an honorary member for the Japanese Cancer Association and is a Fellow at the American Society of Clinical Investigation and the AAAS. Harris has published more than 700 journal articles, 100 book chapters, and edited 10 books, and holds more than 30 patents owned by the U.S. Government. He also serves as Editor-in-Chief for the journal, Carcinogenesis, and has held or currently holds elected offices in scholarly societies and non-profit foundations including the American Association of Cancer Research, the International Society of Differentiation, the Keystone Symposia on Molecular and Cellular Biology, International Liver Cancer Association and the Aspen Cancer Conference. Harris has a wide range of scientific interests and accomplishments spanning molecular genetics and epigenetics of human cancer to molecular epidemiology and precision medicine of human cancer risk and mechanistic biomarkers of cancer diagnosis, prognosis and therapeutic outcome. Harris has also co-authored an espionage novel, High Hand by Curtis J. James.
Liver Cancer Metabolome

Liver cancer is the second leading cause of cancer-related deaths worldwide. A tremendous future burden of this disease is evident from the predicted 2.4-fold rise in liver cancer incidence, indicating an urgent need for early diagnostic biomarkers that are inexpensive to deploy in the clinic. We initially conducted a pilot study in the NCI-MD cohort comprising of 98 hepatocellular (HCC) cases, 101 high risk subjects and 95 controls, to evaluate whether previously identified urinary metabolite biomarkers of lung cancer could aid in the diagnosis of liver cancer. Ultra-performance liquid chromatography tandem mass-spectrometry (UPLC-MS/MS) was employed for quantitation of creatine riboside (CR), N-acetylneuraminic acid (NANA), cortisol sulfate, and a lipid molecule designated as 561+. Validation of the findings from the NCI-MD cohort was carried out in the TIGER-LC cohort (n=370 cased, 471 high risk subjects, 251 controls), where intrahepatic cholangiocarcinoma (ICC), the second most common primary hepatic malignancy, is highly prevalent. Metabolite quantitation was also conducted in TIGER-LC tissue samples (n=48 ICC, n=52 HCC). All four cancer metabolites were found to be significantly increased in liver cancer when compared to high risk subjects and controls in the NCI-MD study, results of which were validated in the TIGER-LC cohort. The four-metabolite profile performed better in classifying ICC than a clinically utilized ICC tumor marker, CA19-9, and their combination led to a significantly improved model (AUC=0.88, P=9.4E-7). Metabolites CR and NANA were significantly elevated in ICC when compared to HCC cases in both urine and tissue samples.

Lung Cancer Metabolome

Lung cancer is a major health burden causing 160,000 and 1.6 million deaths annually in the United States and worldwide, respectively. While seeking to identify stable and reproducible biomarkers in noninvasively collected biofluids, we assessed whether previously identified metabolite urinary lung cancer biomarkers, creatine riboside (CR), N-acetylneuraminic acid (NANA), cortisol sulfate, and indeterminate metabolite 561+, were elevated in the urines of subjects prior to lung cancer diagnosis in a well-characterized prospective Southern Community Cohort Study (SCCS). Urine was examined from 178 patients and 351 non-diseased controls, confirming that one of four
metabolites was associated with lung cancer risk in the overall case-control set, whereas two metabolites were associated with lung cancer risk in European-Americans. OR of lung cancer associated with elevated CR levels, and adjusted for smoking and other potential confounders, was 2.0 [95% confidence interval (CI), 1.2-3.4; P= 0.01]. In European-Americans, both CR and NANA were significantly associated with lung cancer risk (OR = 5.3; 95% CI, 1.6-17.6; P= 0.006 and OR=3.5; 95% CI, 1.5-8.4; P= 0.004, respectively). However, race itself did not significantly modify the associations. ROC analysis showed that adding CR and NANA to a model containing previously established lung cancer risk factors led to a significantly improved classifier (P= 0.01). Increasing urinary levels of CR and NANA displayed a positive association with increasing tumor size, strengthening a previously established link to altered tumor metabolism. These replicated results provide evidence that identified urinary metabolite biomarkers have a potential utility as noninvasive, clinical screening tools for early diagnosis of lung cancer.

Lung Cancer Microbiome

Lung cancer is the leading cancer diagnosis worldwide and the number one cause of cancer deaths. Exposure to cigarette smoke, the primary risk factor in lung cancer, reduces epithelial barrier integrity and increases susceptibility to infections. Herein, we hypothesize that somatic mutations together with cigarette smoke generate a dysbiotic microbiota that is associated with lung carcinogenesis. Using lung tissue from 33 controls and 143 cancer cases, we conduct 16S ribosomal RNA (rRNA) bacterial gene sequencing, with RNA-sequencing data from lung cancer cases in The Cancer Genome Atlas serving as the validation cohort. Overall, we demonstrate a lower alpha diversity in normal lung as compared to non-tumor adjacent or tumor tissue. In squamous cell carcinoma specifically, a separate group of taxa are identified, in which Acidovorax is enriched in smokers. Acidovorax temporans is identified within tumor sections by fluorescent in situ hybridization and confirmed by two separate 16S rRNA strategies. Further, these taxa, including Acidovorax, exhibit higher abundance among the subset of squamous cell carcinoma cases with TP53 mutations, an association not seen in adenocarcinomas. The results of this comprehensive study show both microbiome-gene and microbiome-exposure interactions in squamous cell carcinoma lung cancer tissue. Specifically, tumors harboring TP53 mutations, which can impair epithelial function, have a unique bacterial consortium that is higher in relative abundance in smoking-associated tumors of this type. Given the significant need for clinical diagnostic tools in lung cancer, this study may provide novel biomarkers for early detection.

References:


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Chanida Vinayanuwattikun, MD., Ph.D. graduated from Mahidol University with first-class honors in 2000. She performed her internal medicine residency and fellowship in oncology at Faculty of Medicine, Mahidol University and Ph.D. in Biomedical Science at Chulalongkorn University. She is board certified in internal medicine (2006), medical oncology (2008) and also Education Commission for Foreign Medical Graduates (ECFMG; 2008). Therefore, she joined the medical oncology division, faculty of medicine, Chulalongkorn University and has been involved with many translational researches in lung cancer by incorporating advanced molecular techniques to construct advanced knowledge in cancer biology. She has been awarded from Royal Golden Jubilee Ph.D. Scholarship from Thailand Research Fund, Thailand 2008, International Development Education Award from American Society of Clinical Oncology, USA, in 2010, Merck Millipore Young Scientific Award, Thailand, in 2011, and Fellowship Award from International Agency for Research on Cancer (IARC), France; the specialized cancer agency of the World Health Organization, in 2014. She received 2-year post-doctoral training in molecular genetics at genetic epidemiology group, IARC, Lyon, France. Currently, she focused on lung cancer, head and neck cancer, and sarcoma research.
Current and Potential Biomarkers in Lung Cancer, Implication for Treatment Decision and Outcome

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Lung cancer biomarkers research had been extensive explored, related to clinical outcome as part of translational research. Type of cancer biomarkers could define as diagnostic biomarker, prognostic biomarker and predictive biomarker. Early detection of lung cancer before clinically detection is an important aspect of diagnostic biomarker which included risk assessment of having disease. Low-dose computerized tomography of thorax for smoking patient, age more than 55 years was the only evidence to detect early stage of lung cancer and led to improve disease outcome. Several prognostic markers in lung cancer also had been discovery however still could not apply in clinical practice. The update revised staging system AJCC8th edition was the only evidence that had been tested in large retrospective and prospective cohort, correlated with prognosis.

Predictive biomarkers had important role to decide patient treatment and current adopted in standard clinical practice. Using predictive biomarkers guided-treatment was defined as precision medicine in cancer to strengthen benefit of treatment according to biology of cancer. The most important predictive biomarkers up to date are EGFR mutation and ALK rearrangement. These mutations are significant alteration that led to abnormal cell proliferation and metastasis. Presence of EGFR mutations commonly exon 19 deletion, exon 21 L858R substitution could define treatment response from EGFR tyrosine kinase inhibitors such as gefitinib, erlotinib, afatinib and osimertinib. While presence of ALK rearrangement also define treatment response from ALK TKI such as crizotinib, ceritinib, alectinib and brigatinib. Several clinical trials had been conducted and shown significant improvement of disease control compare to standard chemotherapy. Furthermore with progression of cancer immunology knowledge in checkpoint inhibitor, PD-L1 gene level of expression could predict response from anti-PD-(L)1 blockage, despite diversity of PD-L1 antibody testing and cut-off value. Currently, four anti-PD-(L)1 antibodies, nivolumab, pembrolizumab, atezolizumab and durvalumab had been approved for treatment lung cancer either monotherapy or combination with other agents.
Dr. Greten is an expert in gastrointestinal (GI) oncology and tumor immunology. His specific research focus is hepatocellular carcinoma and tumor immunology. He is trying to better understand how tumors in the liver interact with the immune system. Dr. Greten received his training in medical oncology, gastroenterology and hepatology in Germany and he has been performing basic and translational research studies in tumor immunology for more than 20 years. He is currently studying novel immune-based approaches to treat patients with hepatocellular carcinoma, cholangiocarcinoma and tumors of the GI tract metastasizing into the liver. Dr. Greten combines his medical expertise in gastroenterology, hepatology and medical oncology with his research expertise in tumor immunology. His research can be best described by the three terms “liver”, “cancer” and “immunology”. Dr. Greten and his team try to better understand how tumors in the liver interact with the immune system and he utilized this knowledge to develop better treatment options for patients with tumors of the GI tract. Dr. Greten is an expert on immune suppressor mechanisms occurring in patients with liver cancer (and murine models of liver cancer). In his recent work published in Nature in Science he studied how fatty liver disease and the gut microbiome control anti-tumor immunity in the liver. Dr. Greten is also principle investigator of a number of immunotherapy trials in patients with GI cancer and pioneered the combination of locoregional and immune checkpoint blockade in HCC.
Cell Immunity and HCC

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Immunotherapy has gained a lot of interest in the context of HCC. The basic idea of currently employed immune based treatment approaches is to activate tumor specific immune responses using immune checkpoint inhibitors. However, this approach dismisses multiple mechanism, which tumors have developed to escape immune-surveillance and to suppress anti-tumor immunity. We study how local factors in the tumor microenvironment such as stress dependent mechanism, diet induced mechanisms (NASH) and the microbiome control anti-tumor immunity in the liver.

We have recently started looking at the effect of the gut microbiome on anti-tumor immunity in the liver. It is known that dysbiosis impairs the efficacy of immunotherapy including the immune checkpoint blockade anti-PD1 treatment in melanoma patients. However, the role of gut bacteria in anti-tumor surveillance in the liver is still poorly understood. Using a primary liver model, we found that altering commensal gut bacteria induced a liver-selective anti-tumor effect. A selective increase of hepatic CXCR6+ NKT cells was observed, independent of mouse strain, gender or presence of liver tumor. In vivo studies using both antibody-mediated cell depletion and NKT-deficient mice confirmed that NKT cells mediated inhibition of tumor growth in the liver. Further investigation showed that NKT cell accumulation was regulated by the expression of CXCL16, the solo ligand for CXCR6, on liver sinusoidal endothelial cells, which form the lining of liver capillaries. Primary bile acids increased CXCL16 expression, while secondary bile acids showed the opposite effect. Removing gram-positive bacteria, which contains the bacteria mediating primary-to-secondary bile acid conversion, by vancomycin was sufficient to induce hepatic NKT cell accumulation and decrease liver tumor growth. Feeding secondary bile acids or colonization of bile acid-metabolizing bacteria reversed both NKT cell accumulation and inhibition of liver tumor growth in mice with altered gut commensal bacteria. In non-tumor liver tissue from patients with primary liver cancer, primary bile acid CDCA levels correlated with CXCL16 expression, the opposite was found with secondary bile acid GLCA indicating the finding also applies to humans.

More recently we started to look at the role of gut microbiome in cholangiocarcinoma and more specifically in colitis and cholangiocarcinoma. Colitis is a well-known risk factor for intrahepatic cholangiocarcinoma (ICC), but the mechanism is unknown. Using several mouse models, we recapitulated the tumor-promoting effect of colitis on ICC. Colitis increases bacterial translocation to liver, and neomycin-sensitive bacteria mediate the accelerated ICC, accompanied by a robust increase of polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC). Depleting PMN-MDSC abolished the colitis-enhanced ICC. Accumulation of PMN-MDSC was controlled by gut microbiome. Increased liver PMN-MDSC can also be found in patients with colitis. Our study provides novel knowledge of how gut microbiome affect liver antitumor immunity and contribute to colitis-promoted ICC progression.
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Dr. Wang received his PhD degree from New York University School of Medicine. He currently serves as a Senior Investigator, Deputy Chief of the Laboratory of Human Carcinogenesis, and co-Director of the Liver Cancer Program at the Center for Cancer Research, National Cancer Institute of the United States. He is a world-renowned cancer biologist with a special focus on functional genomics of liver cancer using genome-scale technologies paired with several international collaborative initiatives and clinical studies. He oversees a basic/translational research program emphasizing new molecular approaches to define tumor subtypes and cancer drivers, and currently serves as co-PI of the NCI Liver Moonshot Initiative and the international TIGER-LC consortium. He has served on the editorial board of Hepatology, Cell & Bioscience, and as Associate editor of Molecular Carcinogenesis, as well as Executive Editor of Journal of International Biological Sciences, among others. In addition, he served on the International Liver Cancer Association (ILCA) Governing Board and as Chair of the ILCA SIG on Molecular Classification and Signaling Pathways. He is recipients of the NIH Director’s Award, NCI Director’s Award and the NCI Mentor of Merit Award. He is also a recipient of an Honorary Professorship from Fudan University, the Blue Faery Award from the Adrienne Wilson Liver Cancer Association, among others. He has co-authored over 170 scientific papers in peer-reviewed journals, such as Cancer Cell, Cell, Nature, Nature Genetics, Nature Medicine, New England Journal of Medicine, Science, etc, with h-index of 65 by Web of Science and 73 (i10-index: 140) by Google Scholar.
Primary liver cancer (PLC) consists of two main histologically distinct subtypes, i.e., hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA), and their diagnoses and treatment decisions are uniquely based on their baseline clinical features. However, each type is genetically, clinically and biologically heterogeneous, and lacks a dominant actionable molecular driver. Patient ethnicity, sex, underlying liver diseases due to various etiological factors such as viral hepatitis resulting from hepatitis B, C or D virus infection, alcoholic liver disease, parasitic infection, or dietary-related obesity, etc., are known causative factors for PLC. Different etiological factors may elicit different molecular mechanisms to initiate carcinogenesis, which lead to different molecular distinct subtypes and complex tumor microenvironment and molecular and biological heterogeneity. Consistently, genomic analyses reveal a complex mutational landscape with vast inter-tumor heterogeneity (sample by sample) and intra-tumor (within each tumor) heterogeneity in PLC. Each histological tumor type and tumor cells within each tumor type display striking molecular and biological variations. Consequently, tumor molecular heterogeneity poses a major challenge to define key oncogenic drivers and signaling pathways responsible for early stage carcinogenesis and develop diagnostic tools and effective treatment modalities. It contributes to drug resistance and tumor relapse following therapy, which poses a substantial challenge to improving outcomes of patients with PLC. The establishment of patient populations with associated well-annotated biobanks and well-characterized molecular features is essential to better define unique tumor molecular subtypes with actionable drivers. Molecular-based technologies such as integrated genomics, transcriptomics and metabolomics provide a superior resolution to distinguish tumor subtypes, which allow for stratification of patients with greater homogeneity and can assist in molecular re-staging.

It is thought that PLC initiation and progression are the consequence of an accumulation of multiple somatic alterations of drivers that contribute to a set of common cancer hallmarks and are ideal druggable targets for targeted therapies. However, in addition to a vast number of passengers, potential PLC drivers identified by whole genome sequencing technologies can include both functional drivers and histological drivers that are no longer needed for the maintenance of a tumor being detected and thus not suitable for therapeutic targeting. A major challenge is to effectively and efficiently identify drivers and signaling pathways that serve as an PLC specific vulnerability. Using integrated genomics, transcriptomics and metabolomics approaches, we have recently shown that HCC and iCCA share common molecular subtypes and driver genes (1). An Asian-specific common subtype is linked to unique metabolic processes and gut microbiome-mediated bile acid metabolism regulates liver cancer (1,2). Consistently, genetic models have revealed the roles of microenvironments induced by different damaging risk factors on directing lineage commitment to either HCC or iCCA (3). We further show that oncogenic activation of RNA binding protein NELFE and MYC signaling contributes to an aggressive HCC subtype (4). We also demonstrate that characterization
of molecular features of tumor cells at the single cell level may provide a better understanding of tumor cell communities and help define key drivers responsible for tumor initiation and progression (5). These new strategies offer promise to better delineate critical gatekeepers of cancer initiation and progression, which can be further honed by integrated genomics to identify key drivers and functionally linked networks in PLC (4-11). Encouragingly, we have recently launched a Liver Moonshot initiative with a goal to build an NCI IRP-based collaborative National Translational Science Network of precision-based immunotherapy for liver cancer (Figure 1). With its knowledge, we may be able to identify biomarkers and actionable targets for early PLC intervention.

Selected publications


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Dr. Ho is a Senior Investigator in the Laboratory of Molecular Biology at the National Cancer Institute (NCI). He is also the Chair of the Department of Biochemistry for the FAES Graduate School at the NIH. Dr. Ho received a B.S. in biology from East China Normal University in 1990. He received a M.A. in cell and molecular biology from San Francisco State University. After working at DNAX Research Institute and Protein Design Labs as a research associate, he moved to the University of Illinois at Urbana-Champaign where he won a NIH/NIDA NRSA predoctoral fellowship and received a Ph.D. in immunology in 2002. In his PhD thesis research, Dr. Ho designed antagonist antibodies that can inhibit cocaine binding on the dopamine transporter. Dr. Ho completed a postdoctoral fellowship with Ira Pastan at the NCI where he developed the ‘mammalian cell display’ method for directed evolution of antibodies. Dr. Ho was recruited to the NCI in 2008 as a Tenure-Track Investigator and was promoted as a tenured Senior Investigator in 2015. Dr. Ho has contributed through his career to understanding of cell surface receptor-ligand interactions, including the mesothelin-MUC16/CA125 interaction in ovarian cancer, mesothelin expression in lung adenocarcinoma and cholangiocarcinoma, the GPC3/Wnt signaling complex in hepatocellular carcinoma, and GPC2 expression in neuroblastoma. Dr. Ho has pioneered the methods involving single domain antibodies that have the ability to bind buried regions in signaling complexes and production of inhibitory antibodies that target tumor-specific glypicans. Several antibody therapeutics, including immunotoxins and CAR T cells, have been developed in his lab for treating liver cancer and childhood cancers. He has received many honors including APAO Scientific Achievement Award, NIH Merit Award, NIH DDIR Innovation Award, NCI Director's Innovation Award, Mesothelioma Foundation Award, and Ovarian Cancer Research Fund Award. Dr. Ho is the Editor-in-Chief of Antibody Therapeutics.
GPC3 as a CAR T-Cell Therapy Target in Liver Cancer

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Background: Glypicans are cell surface proteoglycans with heparan sulfate chains that are linked to the cell surface via a glycosylphosphatidylinositol anchor. Glypicans have the ability to regulate cellular processes including survival and differentiation. The abnormal expression of glypicans regulates tumor progression by modulating Wnt, hedgehog (Hh) and other signaling pathways. The N-, M-, and C-lobes are named according to their relative structural position in the glypican protein. Our work (and that of other labs) have demonstrated that glypican-3 (GPC3) is highly expressed in over 70% of hepatocellular carcinoma (HCC) and that GPC3 is a promising therapeutic target for treating liver cancer.

Methods and Results: In the most recent study, we analyzed two chimeric antigen receptors (CARs) targeting GPC3, CAR (HN3) recognizes the N-lobe of GPC3 while CAR (hYP7) targets the C-lobe that is close to the cell surface. In a real-time cell cytolytic assay, we found that CAR (hYP7) T cells showed higher cytolytic activity than CAR(HN3) T cells. The Luminex analysis showed that CAR (hYP7) T cells produced significantly more Th1/Th2 cytokines and chemokines than CAR (HN3) T cells following exposure to liver cancer cells. In the HCC peritoneal dissemination xenograft mouse model, a single intraperitoneal infusion of CAR (hYP7) cells exhibited sustainable antitumor efficacy, and all of the mice survived without recurrence 10 weeks post treatment (Figure 1). Interestingly, we demonstrated that single injection of CAR (hYP7) T cells derived from either healthy donors or HCC patients significantly regressed tumor growth in the orthotopic HCC mouse model. Using a highly sensitive droplet digital PCR (ddPCR) method, we detected high frequencies of CAR T cells in the spleen and liver tumor microenvironment of mice in the CAR (hYP7) treatment group. Furthermore, we found that CD8+ CAR (hYP7) T cells recovered from mouse spleens exhibited higher lytic activity against HCC cells than CD4+ CAR (hYP7) T cells. Finally, we found that the GPC3-targeted CAR T cells downregulated the Wnt signaling in HCC cells during the CAR T-cell treatment.

Conclusion: We found that the hYP7 antibody-derived GPC3-specific CAR is a promising therapeutic that should be tested for the treatment of advanced stage liver cancer.

Figure 1. Schematic of the CAR (hYP7) T cell production and evaluation in mouse models. Flow chart of the production of CAR (hYP7) T cells and determination of their efficacy in NSG mice bearing human HCC xenografts. The figure was adapted from Li and Ho, Methods in Molecular Biology (In Press).
Dr. Andrew X. Zhu is Professor of Medicine at Harvard Medical School and Director of Liver Cancer Research at Massachusetts General Hospital Cancer Center. The major focus of his research is to develop more effective therapies for hepatocellular carcinoma (HCC) and cholangiocarcinoma. The second area is to develop novel circulating and imaging biomarkers for targeted therapeutics that have prognostic and/or predictive significance. The third area is to define and characterize known or novel genetic mutations in HCC and cholangiocarcinoma, assess their potential correlation with clinical outcomes and as therapeutic targets, and dissect the molecular mechanisms of drug resistance to targeted therapy.

As a widely published author, Dr. Zhu has served as a principle investigator in many clinical trials in HCC, cholangiocarcinoma and other gastrointestinal cancers. An internationally recognized leader in HCC and cholangiocarcinoma, he led early efforts of developing several molecularly targeted and immunotherapy agents in liver cancers and studying the predictive and surrogate circulating and imaging biomarkers. He and his colleagues initially identified the presence of IDH mutations and the resistance mechanism to FGFR inhibitor in cholangiocarcinoma. He is a founding board member of the International Liver Cancer Association, Fellow of American College of Physicians, and a member of ASCO and AACR. Dr. Zhu serves on the Hepatobiliary Cancer committee of the National Comprehensive Cancer Network, the Grants Selection Committee of ASCO, the Hepatobiliary Cancer Task Force of The NCI Gastrointestinal Cancer Steering Committee, the American Joint Committee on Cancer Hepatobiliary Task Force, the Hepatocellular Carcinoma Practice Guidelines Committee of the American Association for the Study of Liver Diseases, and the Clinical Advisory Board of The Cholangiocarcinoma Foundation. He has received several awards for his work, including V Foundation Translational Research Award, Lorenzo Cappussotti Award, and Jonathan Kraft Translational Award.
The Landscape of Targeted Therapies in Cholangiocarcinoma: Current Status and Emerging Targets

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Cholangiocarcinoma is a relatively rare malignancy that arises from the epithelial cells of the intrahepatic, perihilar and distal biliary tree. The incidence of intrahepatic cholangiocarcinoma (ICC) has been rising steadily for decades and molecular profiling studies suggest that it is likely greatly under-diagnosed. Majority of the patients present with locally advanced or metastatic disease. Despite treatment with the standard regimen of gemcitabine and cisplatin, prognosis remains dismal with a median survival of less than one year. The anatomical, pathological and molecular heterogeneity of cholangiocarcinoma presents with unique challenge for drug development. Recently, targeted and whole exome sequencing efforts have defined the landscape of mutations underlying these tumors and revealed ICC as having a completely unique genetic profile among all other epithelial malignancies. Importantly, a significant percentage of ICC harbors oncogenic driver mutations that confer sensitivity to specific targeted therapies already in clinical development. Novel genetic signatures including IDH mutations and FGFR2 fusions have been identified in ICC and clinical trials targeting these unique signatures are actively ongoing. The author will discuss the current status of targeted therapy in cholangiocarcinoma.
Bruce E. Johnson, M.D., is the Chief Clinical Research Officer and Director of the Center for Cancer Precision Medicine at Dana-Farber Cancer Institute. He is an Institute Physician at the Dana-Farber Cancer Institute and Brigham and Women’s Hospital, as well as Professor of Medicine at Harvard Medical School. Dr. Johnson was one of the scientists who discovered the association between epidermal growth factor receptor mutations and response to epidermal growth factor receptor-tyrosine kinase inhibitors. His research is devoted to testing novel therapeutic agents for their efficacy against lung cancer and other thoracic malignancies.

Dr. Johnson is a member of the American Association of Physicians. He served on the American Society of Clinical Oncology (ASCO) Board of Directors from 2008-2011; received the ASCO Cancer Foundation’s Translational Research Professorship in 2008; was selected as an ASCO Fellow in 2012; was elected President of ASCO for the 2017-2018 term, and is currently serving as the Immediate Past President. Dr. Johnson was selected as the 2018 Giant of Cancer Care® in Lung Cancer, which recognizes physicians “who have made significant contributions to the cure and treatment of those living with cancer”. He was also awarded the International Association for the Study of Lung Cancer (IASLC) 2010 Scientific Award for his “life-time scientific contribution in thoracic malignancy research.” Dr. Johnson was one of the leaders of the team awarded the American Association for Cancer Research (AACR) 2010 Team Science Award, recognizing “an outstanding interdisciplinary research team for its innovative and meritorious science that has advanced or likely will advance our fundamental knowledge of cancer.”

Dr. Johnson received his Doctor of Medicine from the University of Minnesota, did postgraduate training at the University of Chicago and the National Cancer Institute, and came to the Lowe Center at Dana-Farber in 1998 after serving for six years as the head of the Lung Cancer Biology section of the National Cancer Institute’s Medicine Branch.
15 Years of Precision Medicine has led to 6 Different Treatment Approaches for half of the Patients with Advanced Non-Small Cell Lung Cancer

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The initial treatment of patients with advanced non-small cell lung cancer (NSCLC) has changed dramatically since the early 2000s when all were treated with platinum-based doublet chemotherapy. Fifteen years have now passed since three independent research groups discovered the association between epidermal growth factor receptor (EGFR) mutations and sensitivity to EGFR-tyrosine kinase inhibitors (TKIs) in approximately 15% of patients with NSCLC in the United States and Europe, and 30% in these patients in East Asia. The prospective randomized trials for previously untreated patients with EGFR mutations treated with either platinum-based chemotherapy or EGFR-TKIs were reported from 2009 to 2014. The two-fold increase in progression-free survival (PFS) of these EGFR-TKIs over combination chemotherapy led to the regulatory approval of erlotinib, gefitinib, afatinib, and dacomitinib around the world as initial therapy for these patients in the past decade. The past 2 years have seen another advance with the third-generation EGFR-TKI, osimertinib, which prolongs PFS approximately 2-fold compared to other EGFR-TKIs (19 months) in previously untreated NSCLC patients with sensitizing mutations of EGFR, a dramatic advance which has led to a change in the standard of care.

Patients with ALK rearrangements make up approximately 5% of patients with lung cancer. The prospective randomized trials for previously untreated patients with ALK rearrangements treated with either pemetrexed platinum chemotherapy or ALK inhibitors were reported from 2014 to 2017. The two-fold increase in progression-free survival of the patients treated with the ALK inhibitors, as reported from 2014-2018 led to the regulatory approval for crizotinib, ceritinib, and now alectinib as initial therapy for patients with ALK rearrangements. Patients with ALK rearrangements from around the world treated with alectinib have more than a two-fold increase in PFS (approximately 3 years) compared to those treated with the first generation ALK inhibitor crizotinib.

The reach of precision medicine has now led to FDA approvals for rarer events in lung cancer including ROS1 rearrangements, BRAF V600E mutations, and NTRK rearrangements, each making up approximately 1% of lung cancer patients. The approvals by regulatory agencies include crizotinib for lung cancer patients with ROS1 rearrangements who achieve a response rate of 70% and a PFS of approximately a year and a half. The combination of dabrafenib plus trametinib have been approved by regulatory agencies for lung cancer patients with V600E BRAF mutations respectively in both the US and Europe in 2017, with a response rate of approximately 60% and PFS of 10 months—which led to their regulatory approval in this rare subset on the basis of a single arm study. Larotrectinib has been approved by the US FDA for treating a wide variety of cancers including lung cancer with NTRK rearrangements yielding a response rate of approximately 70% and PFS in excess of 9 months. The list of known genomic abnormalities that can be effectively treated with targeted agents now make up approximately 23-38% of NSCLC patients and is likely to continue expanding with the reports of effective inhibitors for RET rearranged lung cancers.
Immunotherapy with checkpoint inhibitors has also revolutionized the initial treatment for some patients with advanced NSCLC. The assessment of Programmed Death-Ligand 1 (PD-L1) by immunohistochemistry has identified another subset of patients who can originally be treated with immunotherapy rather than platinum-based doublet chemotherapy. Patients’ tumors assessed by immunohistochemistry with >50% of their NSCLC cells staining for PD-L1 have a 40% improvement in survival compared to NSCLC patients treated with chemotherapy. The proportion of NSCLC patients with more than 50% of their tumor staining for PD-L1 make up approximately 25% of patients with lung cancer and these patients do not typically overlap with those with genomic changes that can be effectively treated with targeted agents. Therefore, 48-63% of patients with advanced NSCLC can initially be treated with targeted agents or immunotherapy rather than platinum-based chemotherapy, a dramatic change for patients with this deadly disease in the past 15 years.
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Dr. Rosell is involved in the application of translational genetic research to the clinical setting and has implemented large-scale screening for EGFR mutations in lung cancer to select patients for treatment with EGFR inhibitors instead of chemotherapy.

Rafael Rosell’s expertise in translational medicine has earned him several international awards, including recognition by The Lancet as the highest European authority in lung cancer, the Int'l Anti-Cancer Treatment Congress's Raymond Bourgine Award and the European Society for Medical Oncology’s Hamilton Fairley Award for lifetime achievements in science and clinical/laboratory research, among others.

Dr. Rosell is member of the Int'l Association for the Study of Lung Cancer and was Scientific Chair of their 2005 World Conference on Lung Cancer. He has served on the Scientific Program Sub-Committee for the annual congress of the European Society for Medical Oncology and was Scientific Chair of the 1st & 2nd European Lung Cancer Congress sponsored by IASLC/ESMO. He also participated in the ASCO Scientific Programs Committee from 2002-2004.

Dr. Rosell has authored over 600 articles in scientific journals, and has given more than 800 presentations at international conferences. He is Editor-in-Chief of *Translational Lung Cancer Research* and is on the Editorial Board of several professional journals, including *Annals of Oncology, Clinical Cancer Research* and *Future Oncology*.
Combination of Immuno and Targeted Therapies of Lung Cancer

Rafael Rosell

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Abstract: The therapeutic possibilities for patients with metastatic melanoma have changed due to the development of targeted therapies that inhibit oncogenic signaling pathways, as well as immune modulating therapies, that unleash the patient’s antitumor immunity. These therapeutic changes have impressively increased the median overall survival of patients. Considering the dramatic, but transient, responses that occur with targeted therapies for a subgroup of patients and the durable responses that can be achieved with immunotherapy in a subset of patients, a lot of effort is ongoing for the clinical development of combinations for these two therapeutic approaches. Herein, we discuss the existing preclinical and clinical data for the combination of targeted therapies and immunotherapy, focusing mainly on melanoma and non-small cell lung cancer (NSCLC).

Keywords: Immune checkpoint blockade (ICB); targeted therapies; melanoma; lung cancer; BRAF, epidermal growth factor receptor (EGFR)

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Poster Session
Andrographolide (AP1) Inhibits Cholangiocarcinoma Cell Invasion In Vitro Model

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Cholangiocarcinoma (CCA) arises from the transformation of cholangiocytes of the epithelial bile duct and it is a prevalent disease in South-East Asia including Thailand. Local invasion and metastasis is one of the main reasons for treatment failure. New treatment options that block invasion at diagnosis are urgently needed. In Thailand, there has been an intensive pursuit for new treatment derived from herbal medicine. Andrographolide (AP1) is one of the major diterpene lactones that considered being the most active and important constituent of Andrographis paniculata, a medicinal plant that is included in “the National List of Essential Drugs: List of Herbal Medicinal Products”. AP1 has several therapeutic effects including hepatoprotective effects, promotes bile flow, potent anti-angiogenic, anti-inflammatory, and anti-cancer properties in xenograft tumour model. Treatment with AP1 blocks numerous signaling pathways as well as promoting apoptosis and inhibiting cell cycle proteins. However, AP1 and its relation to CCA invasion is poorly understood. Proline-Rich Homeodomain protein (PRH/HHEX) is a transcription factor that panels liver development. PRH is a tumour suppressor in hepatocytes and in breast and prostate tissues. In contrast PRH can function as oncoprotein in some cancers such as leukemia. Our current data suggested that PRH is a potential oncoprotein in CCA. High PRH expression is associated with highly invasive cholangiocarcinoma cell lines. Low dose AP1 inhibits the invasive properties of CCA cells. Interestingly treatment of CCA cells with AP1 decreases the Proline-Rich Homeodomain protein (PRH/HHEX) protein levels. The association between AP1 and PRH in CCA and the mechanism in which AP1 blocks invasion is in progress.
CX-4945 Induces Methuosis, a Caspase 3 Independent Cell Death Mechanism, in Cholangiocarcinoma Cell Lines

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Cholangiocarcinoma is a disease with a poor prognosis and increasing incidence and hence there is a pressing unmet clinical need for new adjuvant treatments. Protein kinase CK2 (previously casein kinase II) is a ubiquitously expressed protein kinase that is up-regulated in multiple cancer cell types. The inhibition of CK2 activity using CX-4945 (Silmitasertib) has been proposed as a novel treatment in multiple disease settings including cholangiocarcinoma. In our study, it is interesting to detect methuosis at the dose range that inhibits CCA cell line proliferation. Methuosis was characterized by the formation of cytosolic vacuoles. The vacuoles contained extracellular fluid and had neutral pH. Moreover, methuosis was found not only in CCA cell lines but also in other cancer cell lines. This effect of CX-4945 was found not to be related to its protein kinase CK2 inhibitory effect. Our data suggest that CX-4945 inhibits cell proliferation and induces cell death via CK2-independent pathways.
Roles of PRH and NF-κB Signalings on Cholangiocarcinoma Cell Invasion

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Cholangiocarcinoma (CCA) is a lethal cancer of bile ducts. The CCA incidences were found relatively higher in the population of China, Korean, Thailand, and other Southeast Asia countries than the western countries. Potential risk factors of CCA are related to a chronic inflammation of biliary track or liver damage. The involvement of NF-κB and Proline-rich homeodomain (PRH) protein in CCA disease progression is of great interest as therapeutic targets. Cells invasion was studied by using transwell assay and several molecular approaches and techniques were used, including quantitative real-time PCR and Western blot analysis. We demonstrated that activation of NF-κB p65 by LPS and IL-6, which are inflammatory inducers, increased CCA cell invasion but has little or no effect on CCA cell proliferation. Furthermore, activity of NF-κB was required for PRH transcription. Further studies on mechanisms regulating crosstalk between PRH and NF-κB signaling are undergoing.
Poster Session

P-04

Adenosine Inhibits Cholangiocarcinoma Cells in a Receptor-independent Mechanism

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Cholangiocarcinoma (CCA) is an aggressive cancer of the bile ducts. Novel second line treatment is in need for this type of cancer since the current therapeutic options available at the moment are not effective. Inhibitory effects of extracellular nucleotides have been investigated in many types of cancers. Inhibitory effects of adenosine was reported on cholangiocarcinoma cell lines recently. Two CCA and one immortalized cholangiocyte cell line were used. The effects of adenosine on cell proliferation and motility were examined by MTT and wound healing assay, respectively. Protein level was examined by Western blot analysis. Adenosine inhibited cell proliferation and cell motility all cell lines tested. However, immortalized cholangiocytes showed resistance in proliferation inhibition. cAMP production in CCA cell lines was not altered by adenosine treatment while that of immortalized cholangiocytes was increased. Furthermore, inhibition of adenosine receptors did not change the inhibition from adenosine, while inhibition of adenosine transporter and adenosine kinase, an enzyme that converts adenosine into AMP, reduced inhibitory effect from adenosine. Further studies on mechanism of receptor-independent inhibition of adenosine on CCA cell lines are undergoing.
p53 Represses the Mevalonate Pathway to Mediate Tumor Suppression


There are still gaps in our understanding of the complex processes by which p53 suppresses tumorigenesis. Here we describe a novel role for p53 in suppressing the mevalonate pathway, which is responsible for biosynthesis of cholesterol and nonsterol isoprenoids. p53 blocks activation of SREBP-2, the master transcriptional regulator of this pathway, by transcriptionally inducing the ABCA1 cholesterol transporter gene. A mouse model of liver cancer reveals that downregulation of mevalonate pathway gene expression by p53 occurs in premalignant hepatocytes, when p53 is needed to actively suppress tumorigenesis. Furthermore, pharmacological or RNAi inhibition of the mevalonate pathway restricts the development of murine hepatocellular carcinomas driven by p53 loss. Like p53 loss, ablation of ABCA1 promotes murine liver tumorigenesis and is associated with increased SREBP-2 maturation. Our findings demonstrate that repression of the mevalonate pathway is a crucial component of p53-mediated liver tumor suppression and outline the mechanism by which this occurs.

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DNA Damage Response in Cholangiocarcinoma Cells upon Treatments of Genotoxic Agents

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Cholangiocarcinoma (CCA), a lethal cancer of the epithelial cells lining the biliary tract, is the second most common type of primary liver cancer. The major risk factors of this cancer associate with chronic inflammatory of the bile duct, primary sclerosing cholangitis, hepatolithiasis and liver fluke (\textit{Opisthorchis viverrini}) infection. This type of cancer is associated with poor prognosis and limited treatment options due to the late clinical presentation of the advanced disease. Although surgery resection is the major method to cure CCA, not all patients are qualified for complete surgical resection due to the stage of the disease. Therefore, improvement of chemotherapeutic strategy is necessary to provide the better treatment outcome for the patients. There are several types of genotoxic agents commonly used to treat cancer patients. The effects of these drugs cause high levels of damaged DNA leading to cell cycle arrest or cell death.

This study aims to understand the DNA damage response pathway in cholangiocarcinoma cell lines established from Thai patients upon treatment with genotoxic drugs. Four different chemotherapeutic drugs including gemcitabine (nucleotide analogue), 5-fluorouracil (thymidylate synthase inhibitor), cisplatin (platinum-based alkylating agent) and doxorubicin (topoisomerase II inhibitor) were used to treat three cholangiocarcinoma cell lines (KKU100, HuCCA1, and RMCCA1) and an immortalized cholangiocyte cell line (MMNK1). The degree of growth inhibition of each genotoxic drug among CCA cell lines varied depending on cell type and origin. KKU100 is the most resistant and MMNK1 is the most sensitive cells. To further investigate the DNA damage response signaling pathway (ATM and ATR) in CCA cell lines upon genotoxic drug treatments, ATM inhibitor (KU-55933) and ATR inhibitor (VE-821) were used to treat CCA and immortalized cell lines in combination with chemotherapeutic drugs. Although, the co-treatment of both KU-55933 and VE-821 with each drug shows the most effective inhibitory effect on cell growth, the combination of VE-821 and genotoxic drugs exhibits more inhibitory effects than KU-55933. In addition, analysis of protein levels in CCA cells treated with drugs for 48h revealed that p53 phosphorylations at serine 15 were not notable in three of CCA cell lines but MMNK1 immortalized cells and RmCCA1 also has truncated p53 protein. However, p21 protein was upregulated in CCA and MMNK1 cell lines, independent of their p53 (Ser15) phosphorylation status, upon genotoxic drugs treatments. Moreover, phosphorylation of H2AX, a marker for DNA damage, was increased in treated cells and the levels were more pronounced in the combination of genotoxic drugs and ATM/ATR inhibitors. In conclusion, this study demonstrated that inactivation of ATR signaling pathway sensitizes CCA cells to genotoxic drugs treatments. Understanding the DNA damage response pathways in CCA cells may provide the better knowledge on the development of chemotherapeutic treatment in the future.
Arsenite Exposure Potentiates Apoptosis-inducing Effects of Tumor Necrosis Factor-alpha through Reactive Oxygen Species

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Tumor necrosis factor-alpha (TNF-\(\alpha\)) is a proinflammatory cytokine released by immune cells during inflammation process. Sodium arsenite (NaAsO\(_2\)) is an environmental toxic metal. The effects of excess NaAsO\(_2\) on TNF-\(\alpha\) response and its intracellular signaling are not well understood. We hypothesized that NaAsO\(_2\) exposure might affect cellular response to TNF-\(\alpha\). Using HeLa cell model, we found that the combination of NaAsO\(_2\) and TNF-\(\alpha\) clearly decreased cell viability and mitochondrial membrane potential, but increased percentage of early and late apoptotic cells and cleaved-poly (ADP-ribose) polymerase (PARP). Moreover, the combination prolonged the phosphorylation of mitogen-activated protein kinase (MAPK) members including c-Jun-N-terminal kinase (JNK), p38, and extracellular signal related kinases (ERK), and increased intracellular reactive oxygen species (ROS), in comparison to treatment of NaAsO\(_2\) or TNF-\(\alpha\) alone. We further investigated the role of ROS and MAPK signaling on this event by inhibiting ROS production and MAPK. An antioxidant N-acetylcysteine pretreatment diminished the apoptosis-inducing effect of NaAsO\(_2\) and TNF-\(\alpha\) combination and also inhibited MAPK signaling. Using specific inhibitor of p38 (SB203580) and siRNA-p38 surprisingly increased cell apoptosis and this effect was not observed by JNK and ERK inhibition. This study suggests that p38 may possibly be a survival mediator in response to environmental toxicant-related inflammation. In conclusion, NaAsO\(_2\) exposure might amplify inflammation-related tissue injury by potentiating the apoptosis-inducing effect of TNF-\(\alpha\) through ROS-dependent mechanism.

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Insulin Stimulates Growth of Insulin Receptor Positive Cholangiocarcinoma RMCCA-1 Cells

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Diabetes mellitus type 2 (DM-2) is a chronic disease characterized by high glucose level and insulin resistance. Hyperinsulinemia was found in prediagnostic and early phase of DM-2. It has been reported that DM-2 patients have a higher cancer incidence than non-diabetic control populations. Previous studies pointed out hyperinsulinemia as a potential link between diabetes mellitus and the increased cancer risk. The present study was carried out to determine the effect of insulin on the growth of cholangiocarcinoma (CCA) cells. Western blot analysis showed that insulin receptor (IR) was highly expressed in RMCCA-1 and KKU-100 cells but this receptor was not detected in HuCCA-1 cells and normal immortalized cholangiocyte MMNK-1 cells. By conducting MTT assay in serum free condition, insulin treatment (5-1000 nM) for 24 and 48 h significantly increased cell viability of both RMCCA-1 and KKU-100 cell lines but this growth promoting effect was not evidenced in HuCCA-1 and MMNK-1 cell lines. By using bromodeoxyuridine (BrdU) incorporation assay, insulin treatment for 48 h cause cell proliferation of RMCCA-1 not HuCCA-1 cells. Cell cycle analysis also showed that insulin produced an induction of S-phase in RMCCA-1 but not in HuCCA-1 cells. Pretreatment with insulin receptor antagonist, S961 peptide (10-100 nM), partially mitigated the growth promoting effect of insulin in RMCCA-1 cells. Altogether, the results suggest that insulin can stimulate growth of CCA cells in part through insulin receptor activation.
Pathway Analysis on Metabolic Genes and Serum Metabolites in HCC Reveal Aberrations Associated with Fatty Acid and Lipid Metabolism

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Hepatocellular carcinoma (HCC) is among the deadliest cancer in Thailand. The Thailand Initiative in Genomics and Expression Research for Liver Cancer (TIGER-LC) cohort was established to discover molecular markers that can be utilized for early detection, diagnosis, and possible molecular mechanisms underlying the disease. Previous work on the discovery cohort of 199 cancer patients found 491 metabolic genes correlated with 40 tumor-specific tissue metabolites and 75 serum metabolites, which also associated with patient outcome. In this study, we analyzed metabolic genes identified previously at the pathway level to determine possible molecular mechanisms of HCC in this cohort. We performed pathway analysis of 491 metabolic genes from HCC samples in TIGER-LC cohort by using Gene-Set Enrichment Analysis (GSEA) and Ingenuity Pathway Analysis (IPA). Two-class comparison were used in both analyses, where the classes are the matched tumor and adjacent non-tumor samples from the cohort. From GSEA, we found that fatty acid metabolic process, lipid metabolic process, and small molecule metabolic process were dysregulated. The analysis from IPA revealed similar trends as GSEA, with the top deregulated molecular and cellular functions were lipid metabolism, molecular transport, and small molecule biochemistry. Further scrutiny of IPA results found several transcription factors that might explain the connection between previous results with the molecular function identified in this study. In conclusion, these pathways and associated transcription factors in their networks might be important in the future of early detection and possible intervention of HCC for the Thai population.
Phosphoproteome Profiling of Isogenic Cholangiocarcinoma Extracellular Vesicles Reveals a Discovery Strategy for Novel Cancer Biomarker

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The Northeastern region of Thailand is well known to have a high incidence and mortality of cholangiocarcinoma (CCA). Protein phosphorylation status has been reported to reflect a key determinant of cellular physiology, but identification of phosphoproteins can be a problem due to the presence of phosphatase. Extracellular vesicles are stable towards circulating proteases and other enzymes in human blood and can be recognized before the onset of cancer progression. Here an in vitro metastatic model of isogenic CCA cells was used to provide insight into the phosphorylation levels of EV proteins derived from highly invasive cells. Gel-based and gel-free proteomics approaches were used to reveal the proteins differentially phosphorylated in relation to tumor cell phenotypes. Twenty-six phosphoproteins were identified with a significant change in phosphorylation level. Phos-tag western blotting was then employed to validate the candidate phosphoproteins. Importantly, the aberrant phosphorylation of EV proteins might serve as a promising tool for the development of a biomarker for metastatic CCA.
Prevalence and Factors Associated with Hepatitis B Infection among the Hill Tribe Youths, Northern Thailand

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Hepatitis B virus (HBV) infection is a major viral infection cause of liver cancer. People live in Western Pacific Region are the highest vulnerability for its infection including some marginalized populations living northern Thailand who migrated from south China such as the hill tribe people. Hill tribe youths are become the second and third generations in Thailand but still practice their original cultural related practices including sexual behaviors. The study aimed to estimate the prevalence of hepatitis B virus (HBV) infection and to detect the factors associated with hepatitis B infection among the second and third generation of hill tribe youths in Thailand.

A cross-sectional study was conducted to estimate the prevalence of HBsAg and anti-HBs, and to determine factors associated with combined HBsAg and anti-HBs among the hill tribe youths living in northernmost Thailand. A validated questionnaire and 5 ml blood specimen were used as research instruments. The Wondfo Diagnostic Kit® and the Wondfo One Step HBsAg Serum/Plasma Test® were used for detection of anti-HBsAg and HBsAg respectively. Logistic regression was used to detect the association between variables at the significant level $\alpha=0.05$.

A total of 836 participants were recruited into the study, 62.7% were females, 58.9% were aged 15-17 years, 78.4% graduated high school, and 89.1% had no income. Majority were Akha (30.0%), Yao (16.3%), Hmong (15.8%) respectively. 13.2% smoked, 21.5% used alcohol, 13.3% had tattoos, 3.9% experienced drug injection from illegal practitioners, and 35.7% had no history of HBV immunization. The prevalence of HBsAg was 3.0%, anti-HBs was 10.2%, and the combined prevalence of HBsAg and anti-HBs was 13.2%. In the multivariate analysis, four variables were found significantly associated with combined HBsAg and anti-HBs among the hill tribe youths; a) those aged 18-20 years and 21-24 years had a greater chance of hepatitis B infection than those aged 15-17 years at 2.14 times (95%CI=1.35-3.29), and 2.39 times (95%CI=1.05-3.90) respectively, b) Akha, Lahu, and Hmong youths had a greater chance of HBV infection than Lisu youths at 3.21 times (95%CI=1.07-9.21), 3.71 times (95%CI=1.21-11.41), and 3.84 time (95%CI=1.26-11.69) respectively, c) those who had experienced working outside the village had a greater of HBV infection than those who did not at 1.77 times (95%CI=1.18-2.98), and d) those who had ≥ 2 partners had a greater chance of HBV infection than those who did not have at 2.66 time (95%CI=1.96-3.87).

Effective HBV prevention programs should be promoted in Akha, Lahu, and Hmong youths particularly those who have sexual partners, work outside the village, and are aged 18-24 years.
Prevalence and Risk Factors for Significant Liver Fibrosis in HIV-infected Thais

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In the era of combination anti-retroviral therapy (cART), liver-related diseases have been increasingly reported in human immunodeficiency virus (HIV)-infected patients. Our previous study demonstrated high prevalence of hepatitis B and C (HBV and HCV) in Thai HIV-infected patients mostly receiving relatively long-term suppressive ART. Therefore, this study aimed to investigate prevalence and risk factors for liver fibrosis, a pathological sign of chronic liver disease, in the HIV-infected Thais. A cross-sectional study was conducted in 353 individuals attending the ART clinic in Nakorn Nayok hospital from October 2011 to June 2013. Prevalence rates of HIV/HBV, HIV/HCV dual and HIV/HBV/HCV triple infection were 8.2%, 7.2% and 0.3% respectively. Combination ART was received by 77.2% (median duration of cART, 25 (1-50) months and CD4+ cell count, 346 (176-518) cells/μL). The rates of significant fibrosis assessed by non-invasive markers, fibrosis-4 (FIB-4) > 1.45 and aspartate aminotransferase to platelet ratio index (APRI) > 0.5, were 18.1% and 21.2% respectively. Univariate and multivariate logistic regression analyses identified HCV coinfection as a major predictive factor for significant fibrosis by APRI (OR 8.8, 95% CI 3.4-22.4, \( P < 0.001 \)) and by FIB-4 score (OR 7.4, 95% CI 2.7-20.6, \( P < 0.001 \)), together with age difference, being male and CD4+ cell count. The analysis also indicated that treatment with a first-line regimen of lamivudine/zidovudine/nevirapine was a protective factor for liver fibrosis by APRI and FIB-4 score (OR 0.4, 95% CI 0.2-0.8, \( P = 0.020 \) and OR 0.3, 95% CI 0.1-0.7, \( P = 0.008 \) respectively).

An extensive cross-sectional study was conducted in 105 HIV/HCV coinfected individuals attending Bamrasnaradura Infectious Diseases Institute from November 2016 to December 2017. In this study group, there were 99% patients receiving ART (median CD4+ cell count, 566 (5-1748) cells/μL). The rates of infections with HCV genotypes 1, 3 and 6 were 38.1%, 25.7% and 5.5% consequently, and 43% of the patients had attained HCV therapy. The rates of liver fibrosis evaluated by FIB-4 and APRI in this patient group were 53% and 64% respectively. The logistic regression analysis indicated that the patients naïve to HCV therapy carried 5.4 times higher risk of having significant fibrosis assessed by APRI (OR 5.4, 95% CI 1.7-116.7, \( p = 0.003 \)) than those attained a sustained virologic response, and the other predictive factors, age difference, CD4+ cell count and serum levels of low-density lipoprotein, were also identified. The data generated in this study demonstrated relatively high prevalence and risk factors of liver fibrosis in HIV-infected Thais, particularly HIV/HCV coinfected group, suggesting their potential to develop cirrhosis, end-stage liver disease and ultimately hepatic cellular carcinoma.
Inhibition of T-cell-mediated Immune Response via the PD-L1/PD-1 Axis in Intrahepatic Cholangiocarcinoma Cells

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Cholangiocarcinoma (CCA) is a malignant tumor arising from the biliary tract epithelium. The incidence of CCA, especially intrahepatic CCA, has increased globally over the past few decades and Thailand’s North-East region has the world’s highest incidence rate related to chronic infection with liver fluke. Current available treatment for the disease with chemotherapy is not impressive. Clinical trials using monoclonal antibodies targeting immune checkpoint showed promising results in melanoma and some solid tumors. The present study aimed to assess the expression of programmed cell death protein 1 ligand (PD-L1) on Thai intrahepatic CCA cell lines and its potential to suppress T-cell function. The results showed that most of Thai CCA cell lines including HuCCA-1, RMCCA-1, KKU-100, and KKU-213 but not KKU-M055, expressed PD-L1 higher than that of normal cholangiocyte MMNK-1 cells. PD-L1 expression was highest in HuCCA-1 cells. A 48 h treatment with interferon gamma (IFN-γ) concentration dependently increased the expression of PD-L1 in intrahepatic CCA cell lines both HuCCA-1 and RMCCA-1. Meanwhile, the expression of the cytotoxic T-lymphocyte–associated antigen 4 (CTLA-4) ligands including B7-1/CD80 and B7-2/CD86 did not changed after IFN-γ treatment. The immugenicity of RMCCA-1 cell lysate to induce programmed cell death 1 (PD-1) and CTLA-4 in lymphocyte was higher than HuCCA-1 cell lysate. The results of 48 h co-incubation between activated lymphocytes and IFN-γ-treated CCA cells showed that CD8+ T cell apoptosis significantly increased in all co-culture groups when compared to the control lymphocytes alone group both in HuCCA-1 and RMCCA-1 cells. Pembrolizumab, a commercial anti-PD-1 monoclonal antibody, was successful in blocking escape phenomenon of RMCCA-1 cells. Altogether, the results demonstrate the inhibition of T-cell-mediated immune response via the PD-L1/PD-1 axis in intrahepatic CCA. The immunotherapy with checkpoint inhibitor offers a potential effective therapeutic strategy for intrahepatic CCA patients.
Decreased Argininosuccinate Synthetase Expression in Thai Patients with Cholangiocarcinoma and the Effects of ADI-PEG20 Treatment in CCA Cell Lines

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Cholangiocarcinoma (CCA) is a severe cancer with poor prognosis. The aim of the present study was to explore the expression of argininosuccinate synthetase (ASS), as well as the possibility of using pegylated arginine deiminase (ADI-PEG20) for the treatment of CCA. ASS expression was determined in CCA specimens from 40 patients in Thailand. Immunohistochemical detection of ASS and determination of the proliferative index, Ki-67, were carried out in paraffin-embedded sections of these specimens, as well as in two CCA cell lines, HuCCA and RmCCA-1, derived from CCA samples from patients in Thailand. In total, ~45% of the CCA specimens had low ASS expression, and the level of expression was significantly negatively associated with cell differentiation (P<0.05) and Ki-67 expression (P<0.05). The level of ASS expression in tumor cells was significantly lower than that in non-tumor cells (1.3-fold, P<0.05). The HuCCA cell line had significantly lower levels (P<0.05) of ASS expression at the mRNA and protein levels relative to those of normal human immortalized fibroblast cells (BJ-1). By contrast, the RmCCA-1 cell line showed no significant difference. In addition, the effects of ADI-PEG20 on growth inhibition, apoptosis and cell cycle arrest were determined in HuCCA and RmCCA-1 cells. ADI-PEG20 treatment reduced cell viability and cell proliferation in the two CCA cell lines, though it had no effect in immortalized BJ-1 cells. Furthermore, ADI-PEG20 treatment significantly increased G0/G1 cell cycle arrest in HuCCA, though not in RmCCA-1 cells. ASS silencing in the RmCCA-1 cell line significantly enhanced its sensitivity to ADI-PEG20 treatment. Results from the in vitro study demonstrated that ADI-PEG20 has antitumor activity against CCA with low ASS expression.
Thai Noni Juice Products Combined with 5-Fluorouracil Synergistically Induced Apoptosis in Cholangiocarcinoma Cells

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Cholangiocarcinoma (CCA) is a devastating malignancy with a poor prognosis, a high mortality, and a public health concern in the northeastern of Thailand. Clinical application of 5-fluorouracil (5-FU) is often associated with resistance and toxicity posing an urgent demand for combination therapy. Noni (\textit{Morinda citrifolia}) is a medicinal herb has been reported to possess anticancer activity. From our previous study, Thai noni juice product 3 and product 4 enhanced the antiproliferative activities of 5-FU against human CCA cell lines. Therefore, in this study, we investigated the anticancer effects and the possible mechanisms of ethanolic extracts of Thai noni juice product 3 or product 4 in combination with 5-FU against human CCA cell line KKU-100. Phenolic acid composition of Thai noni juice products was determined using reverse phase HPLC. Antiproliferative activity was measured using MTT assay. Cell cycle arrest and apoptosis induction were analyzed by flow cytometry. The results of HPLC analysis revealed that phenolic acids, including gallic, protocatechuic, \(p\)-hydroxybenzoic, vanillic, syringic, \(p\)-coumaric, and ferulic acids were identified in ethanolic extracts of both Thai noni juice product 3 and product 4, while caffeic acid was identified only in product 3. The ethanolic extracts of both Thai noni juice product 3 and product 4 reduced the viability of KKU-100 cells in a dose- and time-dependent manner. Combined effects of ethanolic extracts of Thai noni juice product 3 or product 4 with 5-FU exerted more cytotoxicity than either agent alone at 48 hours after treatment. Synergistic cytotoxic effects of drug combinations of Thai noni juice product 3 or product 4 with 5-FU were represented by the combination index (CI) values below 1.0. Treatment of Thai noni juice products and 5-FU either alone or in combination did not cause the cell cycle arrest but caused an increase of sub-G1 population, especially in combination treatment indicating the increased apoptotic cell death. Apoptosis assay confirmed that Thai noni juice products combined with 5-FU induced more apoptotic cell death than the single drug. Our results showed that ethanolic extracts of Thai noni juice product 3 and product 4 had synergistic effects with 5-FU in the growth inhibition of human CCA cell line KKU-100 through apoptosis induction.
Anticancer Activity of Biphenyls from *Streptomyces* sp. BO-07: An Endophyte in *Boesenbergia rotunda* (L.) Mansf A

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Strain BO-07 was isolated from the root tissue of *Boesenbergia rotunda* (L.) Mansf A. and identified as *Streptomyces* sp. on the basis of morphology, chemotaxonomy and 16S rDNA sequencing. The fractionation of the crude extract from strain BO-07 cultures led to the isolation of two biphenyls: 3′-hydroxy-5-methoxy-3,4-Methylene-dioxybiphenyl (1) and 3′-hydroxy-5,5′-dimethoxy-3,4-methylenedioxybiphenyl (2); these compounds and the crude extract had potent anticancer activity. These compounds showed strong cytotoxicity against all the three cancer cell lines (HeLa, HepG2 and Huh7) at an IC50 value of 3.04–20.30 µg/ml. Both the compounds were less toxic on normal cells (L929) than on the investigated cancer cell lines.
A Thai Herbal Formula Benja Amarit Efficiently Suppresses Lung and Liver Cancer Cells Proliferation through Inducing ROS-mediated Apoptosis

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Cancer is an important cause of illness and mortality around the world. Moreover, cancer is the number one cause of death in Thailand. Intriguingly, lung and liver cancer are the remaining two of the five most common causes of cancer death in Thailand. At the present, there are many ways to treat cancer. However, cancer cells have the ability to resist to the conventional therapies, hence it requires further research for cancer treating development. Benja Amarit (BJA) is a mixture of herbal formulation containing nine ingredients. This formula has been effectively used against diseases for hundreds of years. The properties of this formula include reducing edema and jaundice, increasing the motility of the intestine as well as detoxification. In addition, it is also used for the treatment of liver cancer patients. However, there is no data in an in vitro model on anticancer activity against human lung and liver cancer cell lines of BJA and molecular mechanisms involved. The aim of this study was to investigate the potential of BJA for inhibiting cancer cell proliferation and the molecular mechanisms of cytotoxicity. The antiproliferative effects were determined by using tetrazolium bromide (MTT) assay of 95% ethanolic (EtOH) extract of BJA on the human lung adenocarcinoma (A549) cell lines and human hepatocellular carcinoma (HepG2) cell lines to measure the half-maximal inhibitory concentration (IC50) at 24, 48 and 72 hours after treatment. Interestingly, 95% EtOH extract of BJA inhibited proliferation in predominantly dose-dependent manner on A549 and HepG2 cell lines and presented the nearly equal and quite low of IC50 at 8.57 and 8.59 µg/ml, respectively. The molecular mechanisms of cytotoxicity through apoptosis were investigated and quantitated by annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) staining employing flow cytometry. The result showed that 95% EtOH extract of BJA caused both cell lines to undergo apoptosis. Mechanistically, 95% EtOH extract of BJA treatment significantly induced reactive oxygen species (ROS) generation in both cell lines. In conclusion, the current study demonstrated antiproliferative effects of 95% EtOH extract of BJA on human lung and liver cancer cells by inducing apoptosis via ROS generation.
A Novel Mechanism of Metastasis-associated Paclitaxel Resistance in Floating Lung Cancer Cells

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Drug resistance is frequently found in lung cancer patients with metastatic disease, even though they have never received chemotherapy before. In order to spread out, the metastasizing lung cancer cells have to detach from their extracellular matrix (ECM) niche microenvironment to become floating cells in blood or lymphatic circulation. This study aims to explore whether drug resistance development could be a result of cell adaptation to the loss of ECM attachment. An in vitro model of floating cancer cells was achieved by using polyHEMA-coated culture plates. H460 human lung cancer cells were cultured under adherent and non-adherent conditions, to obtain attached and floating cells, respectively. Drug sensitivity of attached and floating cells was determined against a panel of anticancer drugs including paclitaxel, vinblastine, doxorubicin, and 5-fluorouracil, by using MTT assay. The floating cancer cells exhibited paclitaxel-specific resistance, by 15.6-fold increases in IC50 value compared with attached cancer cells, while there was no resistance against the other drugs. The level of paclitaxel target protein, β–tubulin, was increased in floating cells compared to attached cells. Gene expression analysis of β–tubulin isotypes revealed the 3.0-fold up-regulation of a paclitaxel-resistant β–tubulin isotype, βIVa-tubulin, in the floating cells. ERK phosphorylation was increased in the floating cells compared with attached cells, and blocking ERK activation diminished both βIVa-tubulin up-regulation and paclitaxel-resistance. In conclusion, a novel mechanism of metastasis-associated drug resistance in lung cancer cells was uncovered. Intrinsic paclitaxel resistance of lung cancer cells could result from cell adaptation during metastasis. Thus, this knowledge might be helpful for management of lung cancer chemotherapy.
Effects of Particulate Matter from Biomass Burning on Cytokine Production and Cytotoxicity of A549 Alveolar Epithelial Cells

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Particulate matter (PM) has been recognized as one of the key risk factors of lung cancer. Chiang Mai has been encountered with critical air pollution especially during burning season. The level of PM is higher than the safety limit at almost every air quality testing station. Most of PM sources in Chiang Mai come from biomass burning. Previous studies have shown PM from each type of biomass burning contain different amount of polycyclic aromatic hydrocarbons (PAHs). However, the effects of PM from biomass burning on alveolar epithelial cells is still lacking. This study aimed to investigate the effects of PM from different types of biomass burning on A549 alveolar epithelial cell viability and cytokine release. Rice straw, maize residue and leaf litter were burn in a chamber and PM2.5 from biomass burning was collected using MiniVol air sampler. PM samples were then extracted by dichloromethane/ isopropanol. A549 cells were treated with different concentration of extracts. Cell viability was detected by MTT assay. Interleukin-6 (IL-6) and interleukin-8 (IL-8) cytokine release were detected by ELISA. The results showed that all extracts did not toxic to A549 cells at concentration of 0.5 µg/ml. However, biomass burning extracts significantly stimulated IL-6 and IL-8 release in dose dependent manner. The release of these cytokines was correlated to the level of PAHs found in the biomass burning extracts. These results indicated that the release of pro-inflammatory cytokines might be one mechanism of PM-induced lung inflammation and cancer.
Combating Multidrug Resistance of Lung Cancer by Photodynamic Therapy with PLGA-lipid Hybrid Nanoparticles

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Multidrug resistance (MDR) continues to be a critical hurdle to cancer therapy. Photodynamic therapy (PDT) is a treatment using light to activate a photosensitizing agent to produce reactive oxygen species that will kill cancer cells. However, an obstacle to using PDT in cancer treatment is the delivery of hydrophobic photosensitizers. In this study, we demonstrate a strategy for overcoming MDR in human lung cancer cells by using PLGA-lipid hybrid nanoparticles to deliver a hydrophobic photosensitizer 5,10,15,20-Tetrakis(4-hydroxy-phenyl)-21H,23H-porphine (pTHPP). Thus, the photocytotoxic effect of the pTHPP-loaded nanoparticles was studied in the A549 human lung adenocarcinoma cell line and an MDR subline A549RT-eto, which showed 17.4- and 1.8-fold increased resistance to etoposide and paclitaxel, respectively. Treatment with pTHPP-loaded nanoparticles showed no significant differences between the MDR and parental cell lines in terms of pTHPP cellular uptake and light-induced superoxide anion generation. However, in contrast to the results with anticancer drugs, both cell lines were equally sensitive to PDT-induced cytotoxicity when treated with pTHPP-loaded nanoparticles. Analysis of the mode of cell death by flow cytometry revealed that PDT/nanoparticle treatment induced apoptosis in both MDR and parental cells. These results indicate the potential of PDT using pTHPP-loaded nanoparticles to overcome MDR in lung cancer cells.
Astaxanthin Attenuates Migration and Invasion of Human Glioblastoma Cells

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Glioblastoma is the most aggressive central nervous system tumor. Chemotherapeutic resistance is the recent problem for glioblastoma treatment. Astaxanthin (ATX) is a carotenoid derived from various plants and algae with potential antioxidant activity. ATX also possesses anti-proliferative, anti-migration as well as cell death promoting activities in various cancers. This study was tested cellular effect of ATX for inhibition of migration and invasion on A172 human glioblastoma cells by scratch and transwell invasion assays, respectively. We found that ATX has cytotoxic effect at 24 h post-treatment by decreasing cell viability lowered to 50% at concentration between 50 – 150 $\mu$M using MTT assays. But their viability was not further decreased at higher ATX concentration. ATX at 50 and 100 $\mu$M significantly decreased migration and invasion of A172 cells for 24 and 48 h post-treatment. This finding indicated that ATX might be applicable to protective against glioblastoma.
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LIST OF INVITED SPEAKERS

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