

Research from Professor Ah-Ng Tony Kong's Laboratory

Research:

Professor Ah-Ng Tony Kong's current interest focuses on molecular mechanisms of natural, botanical & herbal medicinal products integrating epigenetics/epigenomics; diseases prevention including cancer chemoprevention; Nrf2-mediated anti-oxidative stress and anti-inflammatory signaling; in vivo animal models; drug absorption of various formulations of drug products; drug metabolism (phase I, II drug metabolizing enzymes and phase III transporters); pharmacogenomics (microarray, CHiP-CHIP technology, bioinformatics); pharmacokinetics (PK)/pharmacodynamics (PD); and PK-PD modeling. The current research theme in my laboratory has 3 major foci. (1) Studies of botanicals/dietary/herbal medicinal phytochemicals mediated cellular signaling and diseases prevention such as cancer chemoprevention. Many phytochemicals have been shown to possess health beneficial effects. My laboratory is utilizing the latest molecular, cellular, genomics, epigenetics/epigenomics, and LC-MS-MS to interrogate the biological responses elicited by these health promoting phytochemicals using a combination of various mammalian cell lines coupled with different animal cancer models including TRAMP (prostate), APCmin (intestinal), DSS (colon inflammatory model) AOM-DSS (colon cancer), Nrf2^{-/-} (skin, colon and prostate). (2) Nrf2-mediated redox signaling in anti-oxidative stress and anti-inflammatory. Nrf2 is the key transcription factor regulating the antioxidant response element (ARE)-mediated Phase II drug metabolizing enzymes (DME) /Phase III transporters and anti-oxidative stress genes. The latest molecular, cellular and epigenetics/epigenomics technologies are utilized to study Nrf2-mediated signaling mechanisms in vitro and in vivo. (3) Pharmacokinetics, drug metabolism/transport, pharmacodynamics and personalized medicine. Many phenolic compounds/phytochemicals have poor in vivo bioavailability (systemic absorption) and may render them ineffective and/or required higher doses. We are trying to understand the absorption, metabolism and transport of xenobiotics in vivo resulting in appropriate blood and tissue levels resulting in the pharmacodynamic (PD) responses in tissues to elicit the biological effects. Differences between individuals (due to genetic polymorphism and or epigenetics/epigenomics) in drug metabolizing enzymes, transporters as well as target sites (receptors, enzymes or RNAs) would yield different responses between individuals to the same doses of drugs, botanicals/phytochemicals or xenobiotics in human.

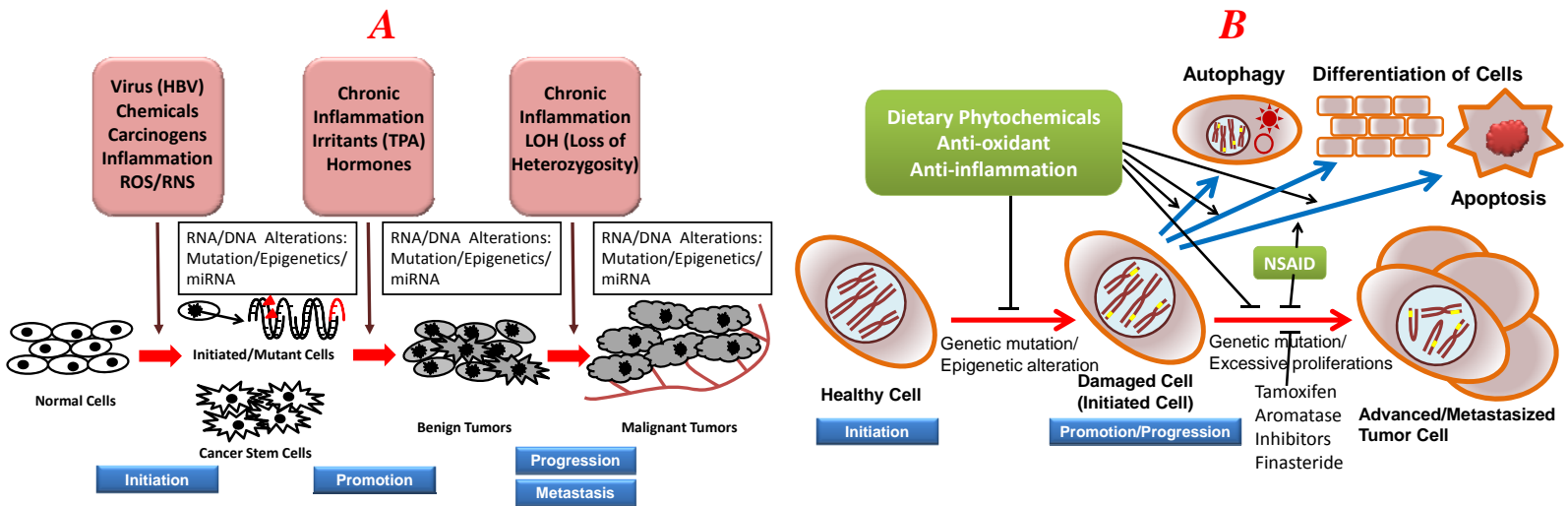
Cancers are Preventable Diseases: Cancer Prevention by Dietary Phytochemicals and NSAIDs

Cancer development generally involves multi-stage processes including initiation, promotion and progression (scheme below). In human, depending on the tissues, it takes between 10 to 30 years from the initiation stages to advanced metastasized stages of tumors. Within this period, there are windows of opportunity to block and intervene the progression of cancer using relatively non-toxic dietary phytochemicals and or NSAIDs (such as aspirin) (scheme below). **Reference:** "Dietary phytochemicals and cancer prevention: Nrf2 signaling, epigenetics, and cell death mechanisms in blocking cancer initiation and progression." Lee JH, Khor TO, Shu L, Su ZY, Fuentes F, **Kong AN**. *Pharmacol Ther*. 2013 Feb;137(2):153-71. doi: 10.1016/j.pharmthera.2012.09.008. Epub 2012 Oct 3. <http://www.ncbi.nlm.nih.gov/pubmed/23041058>.

(A) Schematic representation of multi-stage carcinogenesis: Exposure to intrinsic/extrinsic factors, including various toxic chemicals, oncogenes, viruses (e.g., HBV, hepatitis B virus), ROS/RNS, and inflammation, can result in genetic mutations and/or epigenetic alterations that drive the initiation of carcinogenesis in normal cells. The initiated cells and non-neoplastic cancer stem/progenitor cells can first progress to benign tumors, which would be amendable with surgery, radiation and or chemotherapy, if detected early, with subsequent progression to advanced/metastasized/malignant/drug-resistant tumors due to the prolonged effects of chronic inflammation, various irritants and aberrant hormones. Advanced/metastasized/malignant tumors are typically very difficult to treat since they are resistant to radiation and chemotherapy. Hence it is critically important to **PREVENT** cancers from progressing to the latter stages.

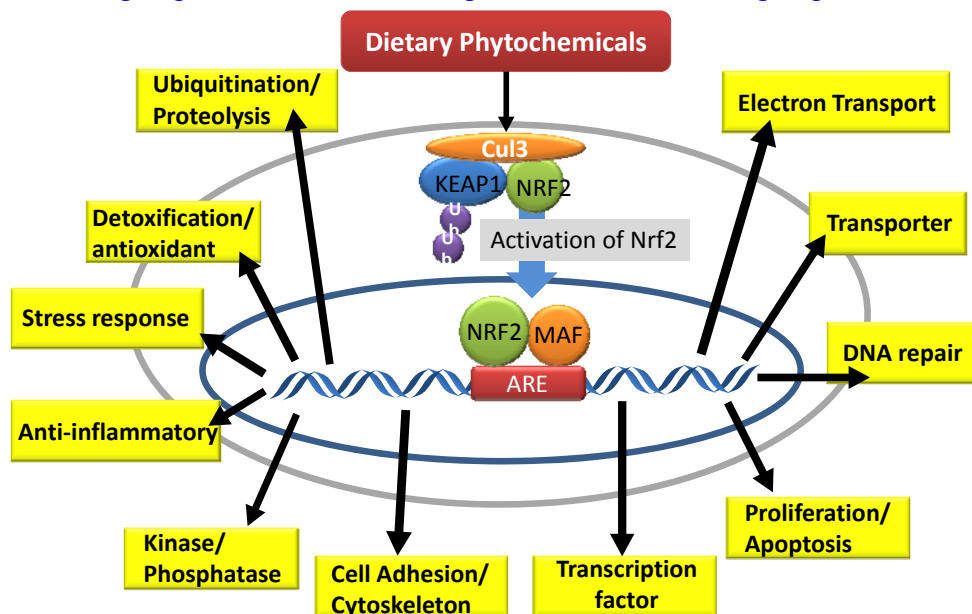
(B) Schematic representation of cancer chemoprevention strategy using dietary phytochemicals and non-toxic therapeutic drugs: Oxidative stress, inflammation and reactive intermediates of carcinogens can cause genetic mutations and epigenetic alterations. Through the promotion/progression stages, initiated cells become advanced/metastatic tumor cells. Applying relatively non-toxic dietary phytochemicals at the early stages of carcinogenesis may block further development of carcinogenesis. Treatment with dietary

phytochemicals and/or relatively non-toxic therapeutic drugs on cancer cells may induce positive results, including autophagy, cell cycle arrest, apoptosis, and differentiation, and may block tumor development.



Cancer Prevention by Dietary Phytochemicals via Nrf2-signaling Pathway

Nrf2 is a master regulator of anti-oxidative stress and anti-inflammatory responses. Surprisingly, many health beneficial/anti-cancer dietary phytochemicals found abundantly in our fruits, vegetables, nuts, among others, would activate Nrf2 resulting in induction of cellular defense detoxifying/antioxidant enzymes and inhibition of inflammatory responses in many tissues. Consequently, Nrf2 appears to be a critical factor/biomarker in cancer development and cancer prevention. <http://www.ncbi.nlm.nih.gov/pubmed/23041058>; <http://www.ncbi.nlm.nih.gov/pubmed/22836898>; <http://www.ncbi.nlm.nih.gov/pubmed/20486765>



The impact of dietary phytochemicals/botanicals on the regulation of Nrf2-dependent pharmacogenomics

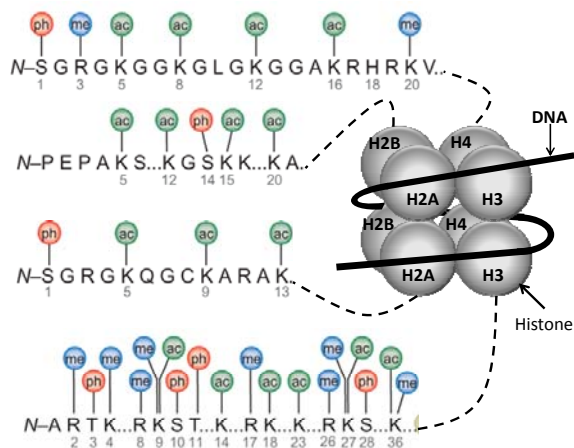
Both Nrf2 KO and WT mice were treated with dietary phytochemicals or with vehicle (control group). DNA was extracted from the organ of interest, such as the liver, prostate, or small intestine, and then hybridized to a DNA microarray. Through a comparison of the compound-treated Nrf2 KO group vs. the treated WT and non-treated Nrf2 KO groups, a large amount of Nrf2-dependant compound-induced genes was found (Barve, et al., 2008; Hu, et al., 2006a, 2006b; Nair, et al., 2006; Shen, et al., 2006; Shen, et al., 2005; Thimmulappa, et al., 2002; Wu, et al., 2011), and some of the representative groups of genes are presented.

Epigenetics and Epigenomics

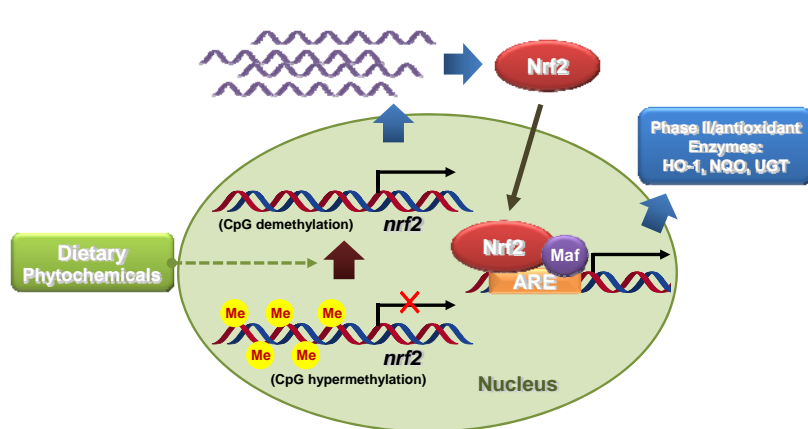
As shown above, cancer appears to be caused by a series of genetic/epigenetic changes in tumor suppressor genes and oncogenes impacted in part by oxidative stress and inflammatory signals. The concept of epigenetics has been defined as “the study of heritable changes in gene expression that occur without a change in DNA sequence” (Wolffe et al., 1999). In cancer, epigenetic hypermethylation of the promoter regions of certain tumor suppressor genes is thought to be the most relevant epigenetic change associated with malignant transformation. These heritable changes occur through the methylation of cytosine bases in the DNA and by post-transcriptional modifications of histones (Baylin et al., 2000).

It is apparent that environmental factors, diet and lifestyle have an impact on the development of various cancers in humans. Hence, minimizing exposure to environmental carcinogens, maintaining a healthier lifestyle and consuming a healthy diet are thought to be reasonable approaches for cancer prevention. In addition to genetic mutations, epigenetic alterations play an important role in cancer development. It is believed that epigenetic changes arise before genetic alterations. The potential of dietary phytochemicals as cancer chemopreventive/anti-cancer agents through epigenetic modification has been demonstrated in many studies.

<http://www.ncbi.nlm.nih.gov/pubmed/23041058>; <http://www.ncbi.nlm.nih.gov/pubmed/21938566>;
<http://www.ncbi.nlm.nih.gov/pubmed/21787756>; <http://www.ncbi.nlm.nih.gov/pubmed/22457388>;
<http://www.ncbi.nlm.nih.gov/pubmed/23416117>; <http://www.ncbi.nlm.nih.gov/pubmed/23441843>



(A)



(B)

(A) Histone tails affecting chromatin modification: Chromatin modifications usually appear in the amino acids in the N-terminal tails of histones (H2A, H2B, H3, H4), which provide the site for a wide range of posttranslational modifications. Various enzymes, such as histone acetyltransferases (HATs), histone methyltransferases (HMTs), histone deacetylases (HDACs), and histone demethylases (HDMs), are involved in these modifications, which cause covalent changes at the marked amino acids. ac: acetyl group, me: methyl group, ph: phosphate group. Modified from (Bhaumik, et al., 2007).

(B) Dietary phytochemicals sulforaphane and herbal medicine Z-ligustilide reverse CpG methylation of Nrf2:

References: Sulforaphane enhances Nrf2 expression in prostate cancer TRAMP C1 cells through epigenetic regulation. Zhang C, Su ZY, Khor TO, Shu L, Kong AN. *Biochem Pharmacol*. 2013 Feb 14. doi:pii: S0006-2952(13)00114-7. 10.1016/j.bcp.2013.02.010. [Epub ahead of print]; Epigenetic Reactivation of Nrf2 in Murine Prostate Cancer TRAMP C1 Cells by Natural Phytochemicals Z-Ligustilide and Radix Angelica Sinensis via Promoter CpG Demethylation. Su ZY, Khor TO, Shu L, Lee JH, Saw CL, Wu TY, Huang Y, Suh N, Yang CS, Conney AH, Wu Q, Kong AN. *Chem Res Toxicol*. 2013 Mar 18;26(3):477-85. doi: 10.1021/tx300524p. Epub 2013 Mar 8.

Quantitative System Pharmacology - Pharmacokinetics-Pharmacodynamics Modeling

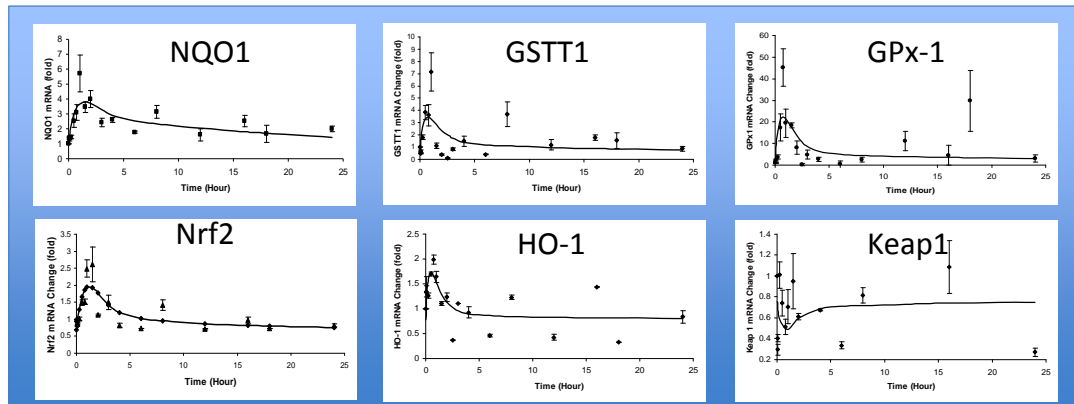
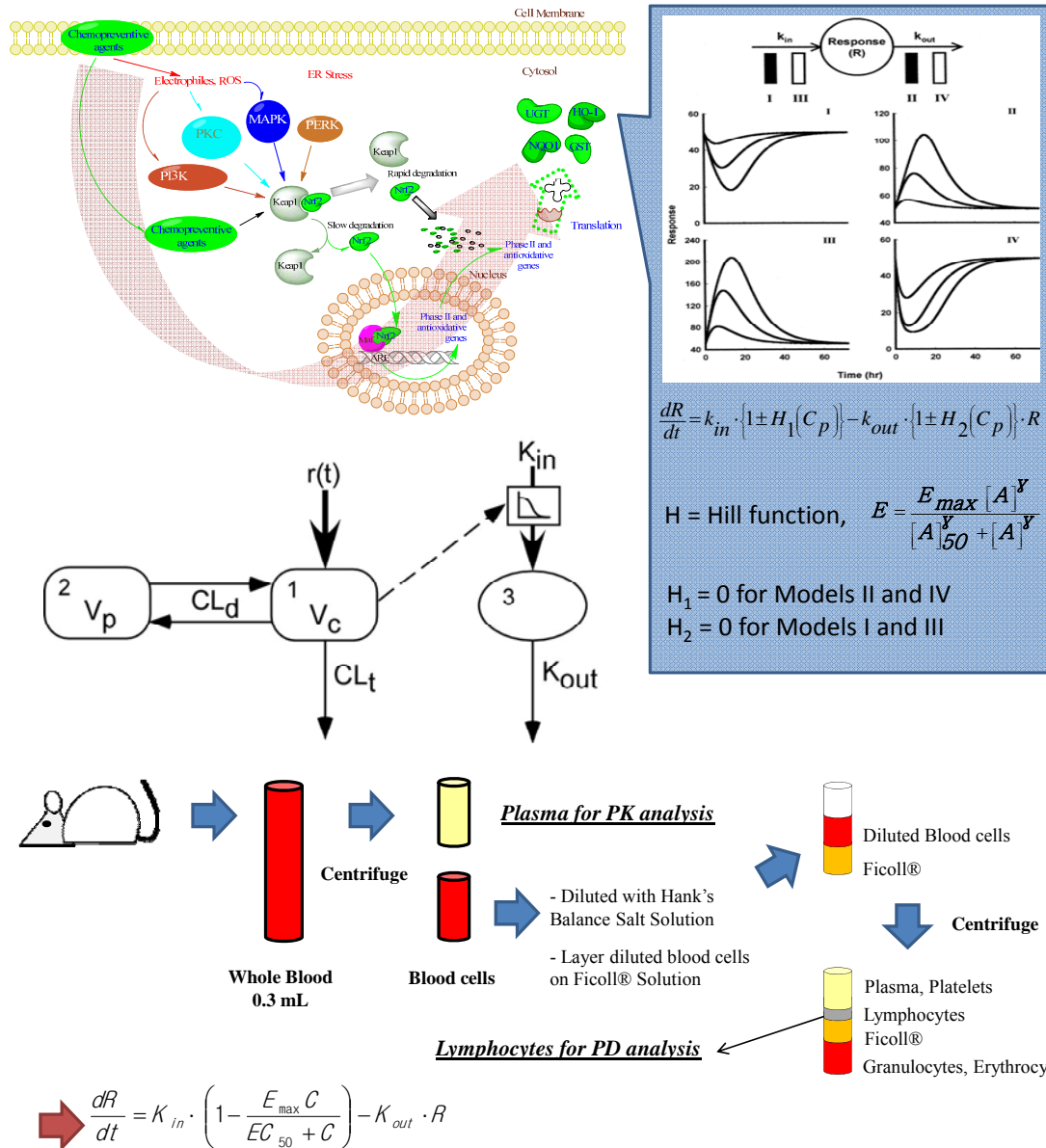
The current challenges in developing new pharmaceutical agents lie in part with the failure of phase II and phase III clinical trials, due to either toxicity or lack of efficacy. With the advent of genomics/pharmacogenomic biomarkers and pharmacokinetic (PK)-pharmacodynamics (PD) modeling effort, one could envision to utilize modeling and simulation (M&S) technology to enhance the success of phase II and III

clinical trials. As a proof of concept, a preclinical PK-PD modeling effort for the anti-cancer chemopreventive agent sulforaphane in rat which could be translated to human clinical studies is illustrated below. **Reference:** Pharmacokinetics and pharmacodynamics of phase II drug metabolizing/antioxidant enzymes gene response by anticancer agent sulforaphane in rat lymphocytes. Wang H, Khor TO, Yang Q, Huang Y, Wu TY, Saw CL, Lin W, Androulakis IP, **Kong AN**. *Mol Pharm*. 2012 Oct 1;9(10):2819-27. Epub 2012 Sep 11.

<http://www.ncbi.nlm.nih.gov/pubmed/22931102>; <http://www.ncbi.nlm.nih.gov/pubmed/23069314>

Pharmacokinetic and pharmacodynamic modeling

Mol Pharm. 2012 Oct 1;9(10):2819-27. Epub 2012 Sep 11.



Seminar Series at Rutgers University on Quantitative Pharmacology and Pharmaceutical Sciences:

Pharmaceutical Science Symposium Celebrating Rutgers 250: A Model of Translational Therapeutics.
September 12-13, 2016

Ioannis P. Androulakis, PhD, Professor & Undergraduate Director, BME, Biomedical and Chemical & Biochemical Eng., Rutgers University. **“Systems engineering meets Quantitative Systems Pharmacology: From low-level targets to engaging the host defenses”**. DATE: Wed, Feb. 04, 2015, TIME: Noon-1PM

Sebastian Polak, PhD, SIMCYP. **“Virtual drug R&D with modeling and simulation - from discovery to safety”**. DATE: Wed, Oct. 29, 2014, TIME: Noon-1 PM

Mini-Symposium on Quantitative Systems Biology-Pharmacology (QSBP)

May 7, 2014 • 10 AM - 4:00 PM

Pharmacy • LCR 202 • Piscataway, NJ

9:55 a.m. – 10:00 a.m. WELCOMING REMARKS

10:00 a.m. – 10:30 a.m. **Richard J Bertz, PhD**, Bristol-Myers Squibb R&D, Vice President and Head Clinical Pharmacology & Pharmacometrics. **“Metabolic/Transporter Drug-Drug Interactions with Some Examples in Virology”**

10:35 a.m. – 11:05 a.m. **A.-N. Tony Kong, PhD**, Distinguished Professor, Ernest Mario School of Pharmacy, Rutgers University. **“Systems Biology of Epi/Genomics, Pharmacokinetics (PK), Pharmacodynamics (PD) and PK-PD modeling & Simulation of Anti-Cancer Phytochemicals”**.

11:10 p.m. – 11:20 a.m. Break

11:20 a.m. – 11:50 a.m. **Terik Leil, PhD**, Bristol-Myers Squibb R&D. **“Quantitative Systems Pharmacology in Pharmaceutical R&D: Application in Exploratory Clinical Development”**.

11:50 am – 12:15 p.m. Discussion #1

12:15 p.m. – 1:30 p.m. Lunch

1:30 p.m. – 2:00 p.m. **Leonid Kagan, PhD**, Assistant Professor, Ernest Mario School of Pharmacy, Rutgers University. **“Model-based interspecies scaling in pharmacokinetics”**.

2:10 p.m. – 2:40 p.m. **Ioannis Androulakis, PhD**, Professor, Biomedical and Chemical & Biochemical Engineering, Rutgers University. **“Systems biology of inflammation: The interplay between circadian rhythms and immune response”**.

2:50 p.m. – 3:00 p.m. Break

3:00 p.m. – 3:30 p.m. **Anirvan Sengupta, PhD**, Professor, Department of Physics, Rutgers University. **“Modeling Epigenetic Silencing”**.

3:30 – 4:00 pm Discussion #2

Dr. Partha Nandy, PhD, Senior Scientific Director, Model-Based Drug Development, Janssen Research And Development, JNJ. **“Future of Pharmacometrics”** DATE: Wed, April. 16, 2014, TIME: Noon-1 PM

Dr. Sekhar Surapaneni, PhD, Senior Director, DMPK, Celgene, Summit, NJ. **“Role of DMPK in Drug Development: Emerging Challenges”**. DATE: Wed, Mar. 12, 2014, TIME: Noon-1 PM

Dr. George Shen, PhD, Fellow, Clinical Pharmacokinetics, Oncology Clinical Pharmacology, Novartis Oncology, Hanover, NJ. **“PK-PD Modeling at Big Pharma: Exposure-response analysis with logistic regression model”**. DATE: Wed, Feb. 19, 2014, TIME: Noon-1 PM

Dr. Punit Marathe, PhD, Executive director, Metabolism and Pharmacokinetics, Bristol-Myers Squibb, Princeton, NJ.: **“Low clearance compounds: in vitro, in vivo and IVIVE challenges”**. DATE: Fri, Dec. 13, 2013, TIME: 10-11:00 AM

Dr. Francis L. Tse, PhD, FCP, Vice President, Drug Metabolism & Bioanalytics, Novartis Institutes for Biomedical Research, East Hanover, NJ.: “**Role of DMPK in the characterization phase of drug discovery**”. DATE: Wed, March. 27, 2013, TIME: Noon-1:00 PM

Dr. Chris H. Takimoto, MD, PhD, FACP, Vice President, Translational Medicine Early Development, Janssen Research & Development, JNJ, Raritan, NJ.: “**Translational Science in Oncology Drug Development: An Industry Perspective**”. DATE: Wed, Feb. 13, 2013, TIME: Noon-1:00 PM

Dr. Saeho Chong, PhD, Director/DMPK. Millennium Pharmaceuticals, Boston, MA.: “**Role of DMPK in Drug Discovery and Development: Industry Perspectives**”. DATE: Wed, Feb.. 6, 2013. TIME: Noon-1:00 PM

Dr. Sandy Allerheiligen, PhD, Vice President, Modeling & Simulation-ESD , Merck, West Point, PA.: “**Modeling and Simulation in Drug Discovery and Development: It is all about the Question** ”. DATE: Mon, Sept. 17, 2012, TIME: Noon-1:00 PM

Dr. Wen Lin, PhD, Principal Scientist, Novartis Institutes for BioMedical Research, DMPK – Translational Sciences, East Hanover, NJ.: “**Proven predictive preclinical models to predict clinical outcome and maximize success in the clinic**”. DATE: Wed, Oct. 3, 2012, TIME: Noon-1:00 PM

Professor William J. Jusko, PhD, Chair, Department of Pharmaceutical Sciences, SUNY Distinguished Professor, School of Pharmacy and Pharmaceutical Sciences, SUNY Buffalo, Buffalo, NY. : “**PK/PD/Disease Modeling in Cancer Therapeutics**”. DATE: Wed, April 25, 2012, TIME: Noon-1:00PM

Dr. David Rodrigues, PhD, Executive Director, Metabolism & Pharmacokinetics, Bristol-Myers Squibb, Princeton, NJ. : “**Trends in PK-PD-ADME science: increasing complexity and integration**”. DATE: Wed, April 18, 2012, TIME: Noon-1:00PM.

Dr. Dan Howard, PhD, Vice President & Head of Global Pharmacokinetics & Pharmacodynamics for DMPK Novartis, East Hanover, NJ. : “**Vocational Etiquette**”. DATE: Wed, Feb. 1st, 2012, TIME: Noon-1:00PM

Dr. Amin A. Nomeir, PhD, Amin Nomeir Pharmaceutical Consulting, LLC, NJ.: “**The Importance of Understanding Metabolic Pathways in Drug Delivery**”. DATE: Wed, March 7th, 2012, TIME: Noon-1:00PM

Dr. Eric Rubin, MD, Vice President of Clinical Oncology Research, Merck, West Point, PA. : “**Challenges in Oncology Drug Development**”. DATE: Wed, Feb. 8, 2012, TIME: Noon-1:00PM

Dr. Norman D. Huebert, Ph.D., Research Fellow, Team Leader ADME, JNJ PRD, Spring House, PA. : “**The ADME Toolbox in Drug Discovery – Using the tools to make rational decisions**”. DATE: Wed, Sept. 14, 2011, TIME: Noon-1:00PM