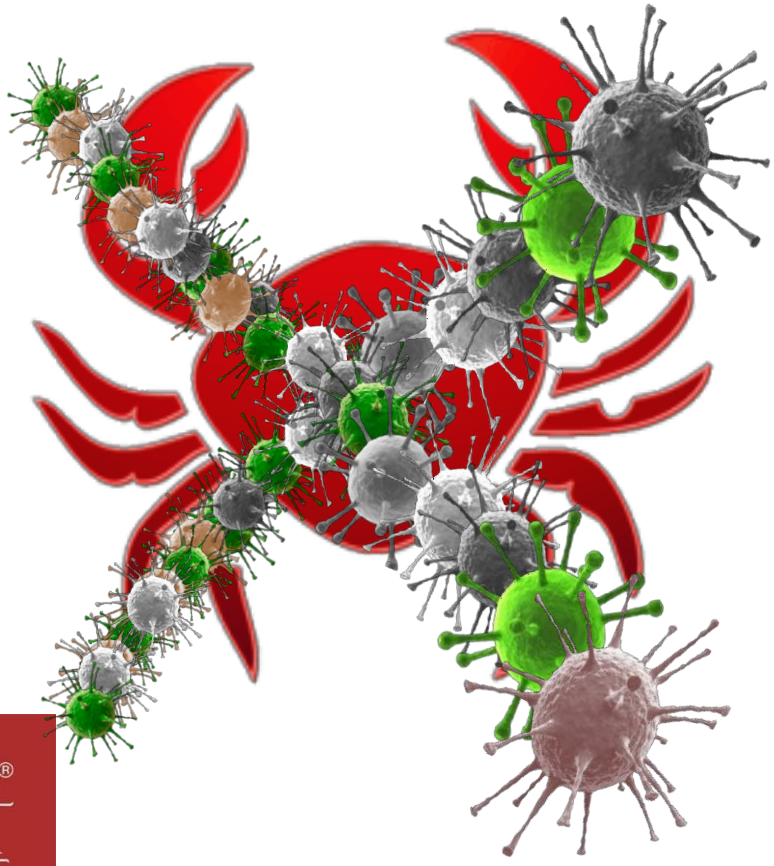
NCI CONFERENCE ON MICROBIAL-BASED CANCER THERANOSTICS



Technologies to Overcome Cancer Challenges

Conference sponsored by the National Cancer Institute

May 25, 2022

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Dear Conference Attendees:

On behalf of the National Cancer Institute and the conference organizing committee, I welcome the attendees and the speakers to the "Microbial-based Cancer Theranostics" conference, which was initiated by the NCI Office of Cancer Complementary and Alternative Medicine (OCCAM).

This meeting follows the "Microbial-based Cancer Therapy" conference which took place at the NIH Bethesda campus in November 2017, a white paper in the journal Cancer Immunology Research, and the publication of two funding opportunity announcements (FOAs) which were recently reissued (PAR-22-085 and PAR-22-086) and have elicited an enthusiastic response from the scientific community.

The new FOAs have added new areas of interest beyond cancer biology including oral cancer, and imaging science which may add a new dimension to microbial-based cancer therapy, advancing towards more targeted precision medicine. Applications with the potential of impacting global health, particularly for low resource settings are encouraged. Also of interest is the utilization of bacteria for cancer diagnosis and early detection.

This conference is a trans NCI effort with program staff from OCCAM, the Cancer Diagnosis Program, the Cancer Imaging Program, the Division of Cancer Biology and the Center for Global Health. The program extends the vision from the 2017 meeting by introducing the concept of microbial-based cancer theranostics, the strategic combination of therapeutics and cancer imaging in one multifunctional microbial agent.

Microbial cancer theranostics may enhance the study of microbial-tumor interactions during microbial cancer therapy while providing direct monitoring of the therapeutic effect. The potential for real-time, *in vivo* imaging of the microbial therapeutic agent raises the possibility of immediate information about the localization of the microbial treatment agent, estimates of the magnitude and duration of the microbial colonization, and an opportunity for treatment modification if necessary.

At this conference, which is the first comprehensive meeting on this topic, speakers will describe the complex nature of the microbe-tumor interaction and discuss recent advances in the field. The goal is to present current research and to stimulate new research to harness the unique unmet potential of viruses and bacteria to invade human cells and induce immune responses to create new safe and effective therapeutic approaches to selectively eliminate cancer cells, while also introducing approaches to imaging these therapeutic interactions.

More than 700 scientists from academia, industry and government, have registered for this multidisciplinary conference which I hope will further stimulate research interest and collaborative activity in the field and unleash new tools based on bacteria and viruses against cancer, augmenting NCI's efforts to find novel approaches to combat cancer.

Sincerely,

Jeffrey D. White, M.D.
Director, Office of Cancer Complementary and Alternative Medicine
Division of Cancer Treatment and Diagnosis
National Cancer Institute, NIH

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National Cancer Institute Conference on Microbial-Based Cancer Theranostics

Purpose

Microbial-based cancer theranostics is a treatment strategy that combines cancer therapeutics with cancer imaging in one multifunctional microbial agent. The purpose of this one-day NCI conference is to discuss the various aspects of the field including the biology of microbial-tumor interaction, microbial-based therapy, microbial-based imaging and diagnosis, microbial-based cancer theranostics and the potential clinical utility of this strategy.

Background

There is a clinical need to develop new cancer treatments (including oral cancer treatment) that are more targeted and effective under conditions where conventional cancer therapies are inadequate, such as metastatic cancer, poorly vascularized hypoxic solid tumors, immunologically "cold" tumors (that do not trigger an immune response), dormant or slowly dividing cells, tumors resistant to treatment, or islands of microinvasive tumor cells buried within normal brain tissues. An attractive characteristic of anaerobic microbial agents for anticancer therapies is their capacity for tumor-specific targeting, and ability to colonize the tumor, deliver a therapeutic payload to the tumor and activate anti-tumor immunity.

Microbial cancer theranostics can be used to study microbial-tumor interactions during microbial cancer therapy and directly monitor the therapeutic effect by engineering them for use in various types of imaging/diagnosis modalities such as, MRI, PET, and ultrasound. Theranostics may allow real-time, *in vivo* imaging of the microbial therapeutic agent in the tumor, potentially providing immediate information about the localization of the microbial treatment agent, estimate of the magnitude of the microbial colonization, its duration, and its impact on the tumor. This information may be used to facilitate timely, interactive adjustment of treatment and to improve microbial based cancer therapy. Finally, the relative ease of microbial genetic manipulation to create microorganisms that have selective tumor cytotoxicity and selective anti-cancer activation of the immune system also offers the prospect of developing relatively simple, low-cost cancer immunotherapy for global health and low resource settings.

Related funding opportunities: Microbial-based Cancer Imaging and Therapy - Bugs as Drugs: PAR-22-086 and PAR-22-085

Jeff Hasty

Professor, Department of Bioengineering, UC San Diego

Academic Biography

Jeff Hasty received his Ph.D. in physics from the Georgia Institute of Technology in 1997, where he learned how to do science from his advisor Kurt Wiesenfeld. He was subsequently a postdoctoral fellow at Boston University, where he learned engineering from Jim Collins ('98-'01). Somewhere during his postdoctoral stay with Jim he mutated from a theoretical physicist into a hybrid computational/molecular biologist. He is currently at the University of California, San Diego, where he is a Professor in the Departments of Bioengineering and Molecular Biology, Director of the Synthetic Biology Institute, and Co-Director of the UCSD qBio Ph.D Specialization Program. Cells, constructs, and microfluidic devices from his lab are in use for research and teaching around the world and he is known for freely sharing genetic constructs and measurement technologies that "just work."

Engineered bacterial population dynamics in solid tumors

An intriguing paradigm for synthetic biology is to engineer small ecologies to maintain stability and release therapies in the complex heterogeneous environment of a solid tumor. We have programmed strains of *E. coli* and *Salmonella* to lyse synchronously at a threshold population density and to release genetically encoded cargo (*Nature*, 536(7614):81, 2016). Following quorum lysis, a small number of surviving bacteria reseed the growing population, thus leading to pulsatile delivery cycles of any therapeutic that the bacteria can produce (*Nat. Rev. Cancer*, 18:727, 2018 and *Nat. Rev. Cancer*, 19:187, 2019). We have demonstrated the therapeutic potential of this platform technology in animal models using fluorescent markers to monitor *in vivo* bacterial population dynamics and tumor progression. We have extended the quorum lysis approach to small ecologies that can generate interesting drug delivery schemes (*Science*, 365(6457):1045, 2019), and to control a small multi-strain consortium using a nutrient-based "inducer" commonly found in many food products (*Nat. Comm.*, 11:1193, 2020). As part of an NCI funded project with Rob Knight at UCSD and Dan Worthley and Susan Woods at the University of Adelaide, we are currently characterizing native bacterial ecologies that colonize human tumors (*Science*, 371:1331, 2021). In a NIBIB supported collaboration with Amir Zarrinpar at UCSD, along with Professors Worthley and Woods, we have begun to explore the population dynamics of small ecologies in solid tumors and the long term stability of *in-vivo* bacterial colonization. This work represents early steps towards probing small engineered ecologies in solid tumors.

9500 Gilman Drive – La Jolla, CA 92093-0349 – USA
+1 (760) 429 4816 • *hasty@ucsd.edu* • *biodynamics.ucsd.edu*

SESSION 1: MICROBIAL-TUMOR INTERACTIONS AND THERAPY

Chair: Neil Forbes, University of Massachusetts
Engineered Salmonella for drug delivery to solid tumors

Dan Littman, New York University
Mechanisms of gut microbiota-directed T cell differentiation programs in homeostasis and inflammation

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Tumor-targeted delivery of childhood vaccine recall antigens by attenuated Listeria as a powerful alternative to neoantigen-mediated cancer immunotherapy

Cammie Lesser, Massachusetts General Hospital, Harvard Medical School
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Q&A Phil Daschner National Cancer Institute, moderator

Engineered Salmonella for drug delivery to solid tumors

Neil Forbes
University of Massachusetts

Biography

Dr. Forbes is a Professor of Chemical Engineering at the University of Massachusetts, Amherst. He is an adjunct member of the Molecular and Cell Biology Program and a member of the Institute for Applied Life Sciences. His laboratory focuses on developing cancer therapeutics and understanding molecular transport in tumors. Dr. Forbes received a BS in Chemical Engineering from Case Western Reserve University and a PhD in Chemical Engineering from the University of California at Berkeley. There he studied cancer metabolism with Harvey Blanch and Douglas Clark. He did postdoctoral training with Rakesh Jain in Radiation Oncology at Harvard Medical School / Massachusetts General Hospital.

Abstract
Engineered bacteria have the potential to overcome the limitations that cause cancer therapies to fail. We have generated bacteria that deliver therapeutic payloads, target hard-to-treat intracellular pathways, and are effective against tumors that are refractory to immunotherapy. Specifically, we have created Salmonella vectors that deliver therapeutic payloads to the (1) extracellular environment in tumors and (2) directly to the cytoplasm of cancer cells. We have developed intracellular delivering (ID) Salmonella that deposit both cytotoxic proteins and immune stimulatory antigen. As one example of extracellular delivery, *Salmonella* that express and secrete α-hemolysin from *Staphylococcus aureus* kill 99% of cancer cells in culture and reduce tumor volume. ID Salmonella contain three genetic circuits that (1) produce the therapeutic protein, (2) activate the regulator *flhDC* to drive cell invasion, and (3) induce lysis to release the protein into cells. Released proteins diffuse from Salmonella containing vacuoles (SCVs) into the cellular cytoplasm where they interact with their therapeutic targets. Control of invasion with *flhDC* increased delivery over 500 times. The autonomous triggering of lysis after invasion makes the platform self-limiting and prevents drug release in healthy organs. Bacterial delivery of constitutively active caspase-3 blocks the growth of hepatocellular carcinoma and lung metastases, and increases survival in mice. We also created ID Salmonella that deliver ovalbumin as a model exogenous antigen to mark cancer cells as foreign. In culture, delivered ovalbumin disperses throughout the cellular cytoplasm and triggers a cytotoxic, ovalbumin-specific CD8 T cell response. After the adoptive transfer of OT-I CD8 T cells, intracellular ovalbumin delivery reduced tumor growth and eliminated tumors. This effect was dependent on the presence of ovalbumin-specific T cells. Following a vaccination regimen in mice, ovalbumin delivery cleared 43% of established pancreatic tumors, increased survival, and prevented tumor re-implantation. This response to re-challenge indicates that intracellular delivery of antigens established new immunity to intrinsic tumor antigens. In the clinic, this bacterial system could deliver an antigen from a childhood vaccine to refocus the pre-existing immunity towards tumors and generate antitumor immunity. As an off-the-shelf immunotherapy, this system would be less dependent on intrinsic tumor characteristics and has the potential to be effective in a broad range of cancer patients. These techniques establish *Salmonella* as a tunable platform for cancer therapy and provides critical evidence that this approach will be applicable to a wide range of protein drugs for the treatment of solid tumors

Mechanisms of gut microbiota-directed T cell differentiation programs in homeostasis and inflammation

Dan R. Littman
Howard Hughes Medical Institute and Skirball Institute

Biography

Dan Littman's laboratory has made multiple contributions towards our understanding of mechanisms that promote immune system development and roles in physiological homeostasis, particularly through interactions with microbiota and with cells of the peripheral and central nervous systems. He received Ph.D. and M.D. degrees from Washington University in St. Louis, and was Professor of Microbiology and Immunology at the University of California, San Francisco, before joining NYU, where he is the Kimmel Professor of Molecular Immunology at the Skirball Institute and an Investigator of the Howard Hughes Medical Institute. Dr. Littman is a member of the U.S. National Academy of Sciences, the National Academy of Medicine, and the American Academy of Arts and Sciences, past president of the American Association of Immunologists, and recipient of several scientific awards, including the Ross Prize in Molecular Medicine and the Vilcek Prize in Biomedical Sciences.

Abstract

Multiple constituents of the intestinal commensal microbiota interact with host immune system cells to promote mutually beneficial functions. Among these, bacterial pathobionts, such as *Helicobacter hepaticus* (Hh), co-exist with the mammalian host under homeostatic conditions, but promote inflammatory bowel disease following diverse perturbations of host immune functions. Hh induces microbe-specific regulatory T cells (iTreg) and follicular helper cells (Tfh) at homeostasis. However, when iTreg cell differentiation is perturbed, e.g. upon blockade of IL-10 signaling or loss of cMaf in $CD4^+$ T cells, Hh instead induces pathogenic Th17 cells and colitis. We sought to determine which antigen-presenting cells (APCs) convey Hh-directed signals for iTreg cell differentiation. We unexpectedly found that antigen presentation by non-myeloid CCR7-dependent migratory ROR□t^+ cells is both required and sufficient to instruct microbiota-specific iTreg cell differentiation. In contrast, pathogenic Hh-specific Th17 cell differentiation required neither CD11c- nor CCR7-expressing APCs, while Tfh cells were dependent on antigen presentation by $CD11c^+$ cells. The precise identity of the RORγt^+ APCs is not yet clear, but they are most likely recently-described $Aire^+$ Janus cells and/or type 3 innate lymphoid cells, both of which express integrins αv and β8, which release TGF-β that is required for iTreg cell differentiation. Our results thus highlight the existence of defined APC subsets that respond to microbiota to direct the differentiation of distinct $CD4^+$ T cell programs. Insights from these studies may allow for future manipulation of APCs to achieve desired compositions of antigen-specific T cells for therapeutic application

Tumor-targeted delivery of childhood vaccine recall antigens by attenuated *Listeria* as a powerful alternative to neoantigen-mediated cancer immunotherapy

Claudia Gravekamp

Albert Einstein College of Medicine

Claudia Gravekamp, PhD, is an Associate Professor in tDepartment of Microbiology and Immunology of Albert Einstein College of Medicine in New York, and member of the Albert Einstein Cancer Center. She received her PhD in 1988 in the field of Tumor Immunology at the Erasmus University in Rotterdam, The Netherlands. From 1987-1993, she served as head of the Laboratory for Leptospirosis at the Royal Tropical Institute in Amsterdam, The Netherlands. From 1993-1998, she was a Research Fellow/Instructor in Medicine at the Channing Laboratory of Harvard Medical School, Boston, MA, where she gained expertise in the development of bacterial vaccines. From 1998-2006, she was an Associate Member of the Cancer Therapy and Research Center, in San Antonio, TX, where she began to develop a program aimed at cancer vaccines. From 2006-2008, she was a Scientist at the California Pacific Medical Center Research Institute in San Francisco, CA, continuing to develop novel immunotherapeutic approaches to cancer utilizing an attenuated bacterium *Listeria monocytogenes* as selective delivery platform for anti-cancer agents. She has been funded by grants from NIH (RO1/R21/RO3), the pancreatic cancer action network (PCAN), and private industry, published 80 scientific articles, is reviewer for various scientific journals, and served as reviewer on several NIH/DOD study sections. She has three patents granted on Listeria-recall antigens and Radioactive Listeria in the US and abroad.

Abstract

Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic disease. Tumors are poorly immunogenic and immune suppressing, preventing T cell activation in the tumor microenvironment. We developed a novel microbial-based immunotherapeutic treatment for selective delivery of an immunogenic tetanus toxoid protein ($TT_{856-1313}$) into tumor cells by attenuated *Listeria monocytogenes*. This treatment reactivates pre-existing TT-specific memory T cells generated during childhood to kill infected tumor cells. Treatment of *Kras*$^{G12D/+}$;*LSL-Trp53*$^{R172H/+}$ (KPC) mice with *Listeria*-TT resulted in TT accumulation inside tumor cells, attraction of TT-specific memory CD4 T cells to the tumor microenvironment and the production of perforin and granzyme B in tumors. Low doses of gemcitabine (GEM) increased the immune effects of *Listeria*-TT, turning immunologically cold into hot tumors in mice. In vivo depletion of T cells from *Listeria*-TT+GEM-treated mice demonstrated a CD4 T cell-mediated reduction in tumor burden. CD4 T cells from TT-vaccinated mice were able to kill TT-expressing Panc-02 tumor cells in vitro. In addition, peritumoral lymph node like structures (LNS) were observed in

KPC mice treated with *Listeria*-TT or *Listeria*-TT+GEM, in close contact with the pancreatic tumors. These LNS displayed CD4 and CD8 T cells producing perforin and granzyme B. Whereas CD4 T cells efficiently infiltrated the KPC tumors, CD8 T cells did not. *Listeria*-TT+GEM treatment of KPC mice with advanced PDAC reduced tumor burden by 80% and metastases by 87% after treatment, and increased survival by 40% compared to non-treated mice. These results support the use of *Listeria*-delivered recall antigens as a powerful alternative to neoantigen-mediated cancer immunotherapy.

STAMPing out cancer

Cammie Lesser

Massachusetts General Hospital, Division of Infectious Diseases
Harvard Medical School, Department of Microbiology

Biography

The Lesser Lab studies aspects related to type III secretion systems, complex nanomachines used by many pathogenic bacteria to directly inject tens of proteins into host cells. Efforts focus on studying how secreted proteins are defined within bacteria and their roles in promoting bacterial spread and survival once inject inside host cells. In addition, the lab is working to translate these studies towards the development of designer probiotics that encode modified type III secretion systems that enable them to deliver therapeutic payloads, rather than virulence proteins, directly into the gut lumen. It is their hope that these bacteria will lead to a new paradigm that increases therapeutic efficacy while decreasing off-target side effects.

Abstract

Multiple bacterial species selectively colonize and proliferate to high titers in tumors where some like *Salmonella* and *Clostridial* species promote tumor regression and even clearance, at least in part by inducing host inflammatory responses. Attenuated versions of these bacteria are observed to effectively eradicate tumors in mouse models, but have shown limited success in human trials, potentially because they are rapidly cleared from the systemic circulation such that the bacteria never reach and establish residence in the tumors. The overall goal of our work is to develop tumor-targeting non-pathogenic *Escherichia coli* capable of site-specific delivery of therapeutic payloads directly into the tumor microenvironment. Towards this goal, we are currently working towards identifying bacterial determinants that promote their homing to and replication within tumors. Here, we will describe our payload delivery system as well as our initial investigators regarding the population dynamics of *E. coli* within the tumor microenvironment.

SESSION 2: MICROBIAL-BASED IMAGING AND DIAGNOSIS

Chair: Guanshu Liu, Johns Hopkins School of Medicine
Molecular imaging of bacteria by their inherent CEST MRI signal

Tal Danino, Columbia University.
Engineering probiotics for colorectal cancer screening and prevention

David Wilson, University of California, San Francisco
Targeting bacteria-specific metabolic pathways for infection imaging

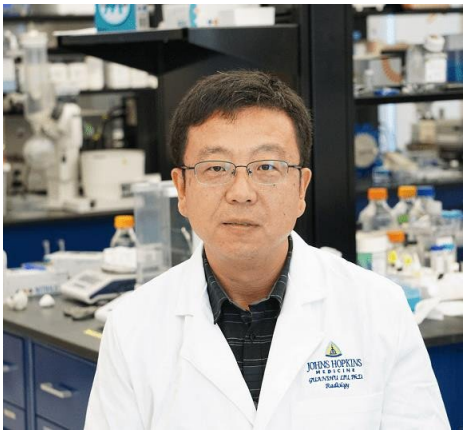
Lacey McNally, University of Oklahoma
Bacterial-based contrast agents for monitoring disease

Q&A Charles Lin. National Cancer Institute, moderator

Molecular imaging of bacteria by their inherent CEST MRI signal

Guanshu Liu
Johns Hopkins School of Medicine.

Biography



Guanshu Liu, Ph.D., currently is an Associate Professor in the F.M. Kirby Center at Kennedy Krieger Institute and Russell H. Morgan Department of Radiology at Johns Hopkins School of Medicine. His primary research interest is to develop new MR molecular imaging technologies for the precision diagnosis and treatment monitoring for cancer and central nervous system diseases. His laboratory currently focuses on the development and biomedical application of Chemical Exchange Saturation Transfer (CEST) MRI that can make bioorganic and biocompatible compounds MRI visible. He and his colleagues have invented a variety of CEST MRI approaches and CEST-guided multifunctional nanoparticle systems that can be tracked with MRI without the need for metallic- or radioactive- labeling for a broad spectrum of biomedical applications and, more importantly, with great potential to be rapidly translated into the clinic. More recently, he also has been working on an efficient labeling strategy that uses superparamagnetic iron oxide nanoparticles for tracking therapeutic extracellular vesicles.

Abstract

Detection of bacterial infection is of important clinical significance. While numerous tracer-based molecular imaging technologies have been developed, with some having entered clinical testing, their clinical utility is still limited for detecting bacterial infection in deeply-seated organs where perfusion is limited. To date, real-time detecting of therapeutic aerobic bacteria in the hypo-perfused tumor core is still an unmet need. In this presentation, I will focus on our recent development of a tracer-free molecular MR imaging method for monitoring the germination of anticancer bacterium C. novyi-NT in a tumor mouse model and S. aureus infection in a rat model of brain abscess. In particular, we utilized the unique Chemical Exchange Saturation Transfer (CEST) contrast generated by the exchangeable protons inherently carried on bacterial cells to quantitively detect bacteria in vivo. This novel approach will provide new imaging guidance that can ultimately improve the success rate of cancer therapies using anaerobic bacteria, including but not limited to C. novyi-NT. This MRI platform technology, once translated to human scanners, will address an unmet need in bacterial treatment and can accelerate the development and clinical testing of bacterial therapies. It will also benefit other areas in medicine (e.g., infection medicine/sepsis), thereby pushing clinical capabilities forward.

Engineering probiotics for colorectal cancer screening and prevention

Tal Danino
Columbia University

Biography

Tal Danino is an Associate Professor in the Department of Biomedical Engineering at Columbia University. His lab focuses on engineering bacteria for biomedical applications, with a particular emphasis on developingbacteria as a cancer therapy. Tal received a Ph.D. in Bioengineering from UCSD in Jeff Hasty's lab and was a postdoctoral fellow at the Koch Institute for Integrative Cancer Research with Sangeeta Bhatia. He is the recipient of awards including the NSF CAREER Award, Era of Hope Scholar Award, CRI Lloyd J Old STARS Award, Pershing Prize, and is a TED Fellow. He directs the Synthetic Biological Systems Laboratory and is a member of the Herbert Irving Comprehensive Cancer Center and Data Science Institute.

Abstract:
Synthetic biology is driving a new era of medicine through the genetic programming of living cells. This transformative approach allows for the creation of engineered systems that intelligently sense and respond to diverse environments, ultimately adding specificity and efficacy that extends beyond the capabilities of molecular-based therapeutics. One particular focus has been on engineering bacteria for cancer therapy, where a multitude of studies has demonstrated selective colonization of solid tumors by bacteria, primarily due to reduced immune surveillance in tumor cores. In this talk, I will describe our laboratory's progress in building and developing engineering bacteria as diagnostics and therapeutics for colorectal cancer. I will highlight recent examples of bacteria programmed to sense and respond to tumor environments and release specific diagnostic molecules and therapeutic payloads ranging from cytotoxic to immunomodulatory agents.

Targeting bacteria-specific metabolic pathways for infection imaging

David M. Wilson
University of California, San Francisco

Biography

David M. Wilson, M.D., Ph.D. received his B.S. degree from Harvard University in Biochemistry, and completed his M.D./Ph.D. training at Columbia University in New York City. His Ph.D. mentor was Dr. Ronald Breslow, a pioneer in artificial enzymes, the hydrophobic effect, and bio-organic chemistry. Dr. Wilson's subsequent clinical training was in radiology and neuroradiology at the University of California, San Francisco (UCSF), where he trained further in the laboratory of Professor John Kurhanewicz and subsequently became a faculty member in 2010. He has since established a basic science laboratory investigating the detection and characterization of cancer via analyte sensing and developing probes for positron emission tomography (PET) and hyperpolarized 13C spectroscopy. His laboratory has most recently studied imaging infection using bacteria-specific metabolic pathways, and ways to detect ACE2 suppression in SARS-CoV-2.

Abstract

I lead the UCSF effort to develop bacteria-specific imaging methods, using both positron emission tomography (PET) and nonionizing magnetic resonance methods (13C or 2H MRS). We know that radiotracers like D-[3- 11C]ala can produce dramatic images in infected animals, but know less about the precise mechanisms employed, and the behavior of imaging probes in the complex in vivo environment. We are therefore answering three critical questions: (1) how precisely is an exogenous radiotracer like D-[3-11C]ala incorporated into Pseudomonas Aeruginosa (PA), and can we observe different pathways via in vivo isotopomers (i.e. D-[3-11C]ala versus D-[1-11C]ala?) (2) what is the incorporation of pathogen-targeted sensors into biofilmnassociated bacteria? (3) can we target biofilms themselves via specific metabolic tools? Furthermore, we are focusing on PET methods compatible with rapid clinical translation, especially using the 18F radionuclide (t 1/2 = 110 minutes).

Bacterial-based contrast agents for monitoring disease

Lacey McNally
University of Oklahoma

Biography

Lacey McNally is the Stephenson Chair in Cancer Imaging, Professor of Surgery, and Program Leader of Cancer Therapeutics at the Stephenson Cancer Center at the University of Oklahoma. Dr. McNally's overall goal is to develop imaging agents that facilitate the detection and monitoring of both inflammation and cancer using multispectral optoacoustic tomography and fluorescent imaging. She has been continuously funded by NIH for the last 13 years and is currently awarded 3 R01s from NCI and NIBIB. Dr. McNally is a Standing Member of the Imaging Probes and Contrast Agents study section. While Dr. McNally was the 1st adopter of MSOT technology in the USA for cancer imaging, her work has in contrast agents has recently expanded into inflammation.

Abstract

The ability to monitor diseases such as cancer and inflammation *in vivo* is critical to improving our understanding of the pathophysiology underlying various disease etiologies. Current technologies to image cancer or inflammation frequently suffer from using radiation, lack of specificity, high cost, or invasive procedures. Therefore, there is an urgent need to develop a noninvasive imaging modality that accurately identifies cancer and inflammation that can be used longitudinally to monitor the status of the disease. With the use of a newly emerging imaging technology, multispectral optoacoustic tomography (MSOT), MSOT has the potential for detecting inflammation and has the capability to detect multiple contrast agents simultaneously. While the majority of development of contrast agents detectable for this modality has largely explored nanomaterials, dye-labeled peptides, or dye-labeled antibodies, we have utilized MSOT and NIR fluorescent imaging to track bacteria-based contrast agents in vivo to assess disease specificity and biodistribution. We have utilized both genetically encoded reporters as well as exogenous reporters to facilitate the detection of probiotic bacteria as well as facultative anaerobic bacteria to image areas of inflammation and cancer.

SESSION 3: MICROBIAL-BASED CANCER THERANOSTICS

Chair: Robert Hoffman, AntiCancer and University of California, San Diego
Real-Time Fluorescence Image-Guided Oncolytic Virotherapy for Precise Cancer Treatment

Dong-Hyun Kim, Northwestern University
Catheter directed local delivery of nano-functionalized C. novyi NT bacteriolytic cancer therapy

Avinoam Bar-Zion, California Institute of Technology
Acoustically triggered mechanotherapy using genetically encoded gas vesicles

Assaf A. Gilad, Michigan State University
A Remote magnetic activation of theragnostic genes for cancer therapy

Q&A Miguel Ossandon, National Cancer Institute, moderator

Real-Time Fluorescence Image-Guided Oncolytic Virotherapy for Precise Cancer Treatment

Robert M. Hoffman
AntiCancer Inc. Department of Surgery, UCSD

Robert M. Hoffman completed his Ph.D. in Biology at Harvard University in 1971. His post-doctoral training was at Massachusetts General Hospital, Boston, and the Institutes of Bioorganic Chemistry and Molecular Biology, Moscow, Russia. He has been a member of the University of California San Diego School of Medicine faculty since 1979 and is currently a Professor of Surgery. He began his cancer research career in 1965 and in 1984 he founded AntiCancer, Inc. Dr. Hoffman has been a pioneer in tumor-targeting bacteria; fluorescent protein-expressing patient-derived orthotopic xenograft (PDOX) mouse models of cancer; recombinant protein-based cancer drugs and diagnostics; as well as pluripotent hair follicle stem cells. Dr. Hoffman has published approximately 900 scientific papers which have been cited approximately 40,000 times with an h-index of approximately 100. In 2016, Dr. Hoffman was awarded the Sun Lee Prize from the International Society for Experimental Microsurgery.

Abstract
The telomerase - dependent adenovirus is cancer specific and can target cancer cells in vivo in order to selectively kill them and also selectively label them with GFP. A powerful application is to use the telomerase-dependent adenovirus for fluorescence- guided surgery that not only brightly labels tumors with GFP, but since it is a genetic reporter, it can label tumor recurrence. The future clinical applications of telomerase- dependent adenoviruses will also be discussed.

Catheter directed local delivery of nano-functionalized C. novyi NT bacteriolytic cancer therapy

Dong-Hyun Kim
Northwestern University, Departments of Radiology, Biomedical Engineering

Biography

Dr. Dong-Hyun Kim is an Associate Professor of Radiology and Biomedical Engineering at Northwestern University and the director of Biomaterials for Image-Guided Medicine (BIGMed) lab at Northwestern University. He received his B.S. degree in Materials Science & Engineering and Ph.D. degree in Medical Science, Yonsei University. He got postdoc trainings in Chemical & Biological Engineering at University of Alabama and Materials Science Division at Argonne National Laboratory. He and his group research are focusing on the therapeutic carriers, image guided medicine and new cancer therapy research, which can overcome the limitation of the conventional cancer therapies.

Abstract

Bacteriolytic therapy using the anaerobic bacterium Clostridium novyi-NT (C. novyi-NT) is considered a promising approach for the treatment of various solid tumors. The spores of C. novyi-NT germinate in hypoxic regions of tumor tissue and destroy tumor cells by secreting lipases, proteases, and degradative enzymes. Image-guided local delivery of C. novyi-NT spores in the lesions may improve the therapeutic efficacy while potentially reducing non-specific toxicity. Our efforts on various nano-functionalization of spores for the image guided catheter directed local delivered bacteriolytic therapy will be presented in the session.

Acoustically triggered mechanotherapy using genetically encoded gas vesicles

Avinoam Bar-Zion

California Institute of Technology

Biography

Avinoam Bar-Zion was born in Haifa, Israel. He received a B.Sc. degree (summa cum laude) in biomedical engineering from the Technion–Israel Institute of Technology, Haifa, Israel, in 2010, and the Ph.D. degree in 2016. During his Ph.D., he completed a year of research at Sunnybrook Hospital, Toronto, ON, Canada, as a part of a collaboration between the Technion–Israel Institute of Technology and the Medical Biophysics Department, University of Toronto, Toronto., He is currently a Marie Skłodowska-Curie Post-Doctoral Fellow with the Shapiro Lab, Department of Chemical Engineering, California Institute of Technology, Pasadena, CA, USA. His research interests include contrast-enhanced ultrasound imaging, medical signal and image processing, synthetic biology, and computer-aided diagnosis

Abstract

Recent advances in molecular engineering and synthetic biology provide biomolecular and cell-based therapies with a high degree of molecular specificity, but limited spatiotemporal control. Here we show that biomolecules and cells can be engineered to deliver potent mechanical effects at specific locations inside the body through ultrasound-induced inertial cavitation. This capability is enabled by gas vesicles, a unique class of genetically encodable air-filled protein nanostructures. We show that low-frequency ultrasound can convert these biomolecules into micrometre-scale cavitating bubbles, unleashing strong local mechanical effects. This enables engineered gas vesicles to serve as remotely actuated cell-killing and tissue-disrupting agents, and allows genetically engineered cells to lyse, release molecular payloads and produce local mechanical damage on command. We demonstrate the capabilities of biomolecular inertial cavitation in vitro, in cellulo and in vivo, including in a mouse model of tumour-homing probiotic therapy

Remote magnetic activation of theragnostic genes for cancer therapy

Assaf A. Gilad
Michigan State University and Departments of Chemical Engineering & Materials Science, Radiology, and Neuroscience

Biography

Assaf A. Gilad is a Professor of Chemical Engineering & Material Sciences and Radiology at Michigan State University. The overarching theme of his research program is to utilize synthetic biology to develop new biomedical imaging technologies. Specifically, he works to develop novel genetically encoded and nanoparticles biosensors for both brain imaging, neuromodulation, and cancer research. He received a B.A. from the Technion, Israel Institute of Technology in Haifa, Israel, and an M.Sc. and Ph.D. in Biology from the Weizmann Institute of Science. He spent three years conducting postdoctoral research in the Department of Radiology at Johns Hopkins University. In 2007 he joined the Radiology department at Johns Hopkins University as a faculty member. In 2017 he moved to Michigan State.

Abstract

Recent advancements in synthetic biology can unleash the true potential of microbial-based cancer therapy. Such microorganisms can home to tumors and be used to deliver sophisticated drugs. It is now possible to engineer bacteria to express completely external pathways that result in therapeutic genes, enzymes, or simply the production of toxins that can eradicate tumors.
However, a major challenge in the field is the ability to effectively control those enzymes remotely in a non-invasive manner. Moreover, it would be advantageous if those therapeutic bacteria can be image simultaneously while destroying the tumor. In our lab, we developed a novel technology to remotely activate enzymes. We created an adaptation of the split protein method – where a protein is split into two parts that upon stimulation come together to create a functional enzyme - and utilize magnetic fields as the stimulus for activation. We use a magnetic perceptive protein, derived from the glass catfish (*Kryptopterus vitreous*), as a "biomagnetic switch" controlled remotely by magnetic fields. We have deployed this approach, termed magnetogenetics, to activated enzymes in E. coli and mammalian cells. We have created split NanoLuc, a split Peroxidase, and a split Herpes Simplex Virus type 1-Thymidine Kinase (HSV1-TK). Whether combined with theranostic genes or implemented in therapeutic pathways, this new approach can push the envelope of microbial-based cancer therapy and allow remote control of microorganisms *en route* to more impactful cancer therapy.

PANEL DISCUSSION: CHALLENGES AND OPPORTUNITIES FOR MICROBIAL- BASED CANCER THERANOSTICS

Moderators

Jeff HastyLuis	University of California San Diego
Partha Basu	WHO, International Agency for Research on Cancer
Alejandro Salicrup	National Cancer Institute, Center of Global Health
Miguel Ossandon	National Cancer Institute, Division of Cancer Therapy and Diagnosis, Cancer Imaging Program

International Agency Research on Cancer
World Health Organization

Panel discussion: challenges and opportunities for microbial- based cancer theranostics

Partha Basu MD, PhD

Deputy Head, Early Detection, Prevention & Infections Branch

Dr. Partha Basu, MD, PhD, is presently the Acting Head of Cancer Early Detection, Prevention & Infections Branch at International Agency for Research on Cancer, the specialized cancer agency of the World Health Organization.
Dr. Basu's pioneering research in cervical cancer prevention includes evaluation of efficacy and safety of different HPV vaccines, evaluation of efficacy of a single dose of the vaccine, assessment of performance of cervical cancer screening and triaging tests in the limited resourced settings and development of a novel technology (thermal ablation) to treat cervical precancers. Evidence generated from these studies have informed several guidelines developed by the World Health Organization.

FUNDING OPPORTUNITIES AND AREAS OF INTEREST

Phil Daschner	National Cancer Institute, Division of Cancer Biology, Cancer Immunology, Hematology, and Etiology Branch
Luis Alejandro Salicrup	National Cancer Institute, Center of Global Health
Miguel Ossandon	National Cancer Institute, Division of Cancer Therapy and Diagnosis, Cancer Imaging Program
Yisong Wang	National Cancer Institute, Division of Cancer Therapy and Diagnosis, Cancer Imaging Program
Avi Rasooly	National Cancer Institute Division of Cancer Therapy and Diagnosis, Office of Cancer Complementary and Alternative Medicine
Zhong Chen	National Institute of Dental and Craniofacial Research, Oral & Salivary Cancer Biology Program

REISSUE OF "MICROBIAL-BASED CANCER IMAGING AND THERAPY - BUGS AS DRUGS" (PAR-22-085 AND PAR-22-086)

The initial funding opportunities PAR-19-193 and PAR-19-194 focused on:

- **microbial-tumor interactions**, studying the biological and mechanistic basis of microbial-based cancer therapy
- **microbial-based cancer therapy**, advancing research on microorganisms for cancer therapy

The program attracted nearly 300 applications, significantly increasing the interest and NCI investment in microbial-based cancer therapy.

The reissued funding opportunities added several new topics:

- **microbial-based cancer imaging** to provide better analytical capabilities for microbial-based cancer therapy and to develop new cancer imaging modalities
- **microbial-based cancer diagnosis** to provide new diagnostics capabilities including non-invasive early detection of asymptomatic cancers
- **microbial-based cancer therapy for global health**, promoting the development of microbial-based cancer therapies as potential low-cost cancer therapy for global health and low resource settings

The new broadened funding opportunities address scientific, clinical, and technology areas related to cancer therapy. In addition, they encourage research aimed at addressing urgent issues of access to cancer therapy, especially cancer immunotherapy, in low resource settings and the development of low-cost microbial anti-tumor agents as immunotherapy approaches to activate an anti-tumor-immune response.

The right study section for your application

Appropriate review with expertise **and experience** with the topic of your application is important. The NIH Center for Scientific Review has provided tools that can be used to recommend potential study sections for your application.

How to identify appropriate study sections

1. Assisted Referral Tool (ART): ART was developed by CSR to recommend and target potentially appropriate study sections.

- Input: You can enter the title and the abstract/aims of your project.
- Output: A ranked list of review groups appropriate for your project.

2. NIH RePORTER: RePORTER is an NIH-wide tool designed to provide information on all NIH funded projects. RePORTER can be used to identify study sections that reviewed applications in your area of research.

- **Input:** In RePORTER's Advanced Search field, enter the FOA numbers. Since RePORTER only provides information on funded projects, is advisable to use the previously issued FOA number. For example, for the Bugs and Drugs FOAs, the previous issued FOAs are PAR-19-193 and PAR-19-194.
- **Output:** A list of funded applications: Application information, including title, abstract, and a list of study sections that reviewed successful applications, can be downloaded to an excel file and analyzed in ART.

What to do with the information and the list of recommended study sections?

1. Learn about the study sections at https://public.csr.nih.gov/StudySections/StandingStudySections. Take a look at the previous review panel rosters and determine if the study section matches your area of research and if it had the relevant expertise to review your application.

2. Better understand the factors contributing to the success of applications (e.g. topics, study sections, scientific approaches, etc.)

3. Request your selected study section for your project: Use the Assignment Request Form in your application package to recommend the appropriate study section, or to suggest scientific expertise needed in the review group for your project (using keywords relevant to your project)

4. If CSR assigned your application to a study section that you believe lacks the scientific expertise to review your project, you can request reassignment, but the final decision rests with CSR.

While it is important to recommend an appropriate review home, it is the quality of the application that will ultimately determine the outcome.

Microbial-based Cancer Imaging and Therapy Bugs as Drugs

(PAR-22-086 for R21 and PAR-22-085 for R01)

The funding opportunity aims to stimulate research focused on the underlying mechanisms of the complex interactions between microorganisms, tumors, and the immune system. The major focus of the program is the utilization of microorganisms for cancer therapy, diagnosis, and imaging including potential applications for oral cancer and global health.

Program major areas of interest:

Biology of microbial-tumor interaction, Phillip J. Daschner
The importance of host-microbe interactions to carcinogenesis is now established, and there are several examples of microorganisms that have been modified to enhance their natural anti-tumor activities for therapeutic purposes, either as a single agent or in combination with established therapies to improve their efficacy, (including improvements to immunotherapy, radiotherapy, chemotherapy, and mechanotherapy).

However, there are many questions about the mechanism of action for many of these anti-tumor microbial agents. An improved understanding of the basic biology of tumor-microbe interactions is needed for the rational development of microbial-based cancer therapy either as single agents or adjuvants.

The Division of Cancer Biology (DCB) supports basic research in all areas of cancer biology including the tumor microbiome, tumor immunology, synthetic biology, physical oncology, and tumor metastasis. Potential relevant topics for this initiative include, but are not limited to:

- The basic biology of tumor/microbe Interactions and signaling
- Features of the TME that allow homing and colonization of microbial agents
- Effects of microbial agents on tumor immunity
- Innate or engineered features of microbial agents (e.g. quorum sensing, auxotrophy) can be exploited to improve their colonization, persistence, and efficacy in tumor control
- Alterations of the tumor-associated microbiome effect on metastatic disease
- Effects of the tumor-associated microbiome on physical oncology (e.g., tumor stiffness and fibrosis)

Microbial based cancer therapy, Avi Rasooly
The microbial-based cancer therapy program aims to stimulate research on mechanisms of microbial-based cancer therapy and support the development of microorganisms for cancer therapy to target solid tumors, metastasis, microinvasion, and conditions not treatable with conventional cancer treatment. Potential topics of interest include, but are not limited to:

- Exploring novel microbial species that might have therapeutic potential
- Enhancing microbial tumor-selective targeting and activation of the antitumor immune system
- Use of microbes to reduce carcinogenic inflammation
- Microbial-mediated inhibition of tumor cell invasion and migration

Microbial-based imaging, Yisong Wang
The Cancer Imaging Program promotes and supports Cancer-related basic, translational and clinical research in imaging sciences and technology, and the integration and application of these imaging discoveries and developments to the understanding of cancer biology and to the clinical management of cancer and cancer risk. For more information see https://imaging.cancer.gov/. Potential topics of interest include, but are not limited to:

- Enhancing bacterial tumor colonization and penetration for imaging applications
- Microbial-specific contrast agents and molecular imaging probes for the various in vivo imaging modalities
- Microbial labeling for microbial-based cancer detection
- Developing precise spatiotemporal resolution (including computation and image manipulation) of microbial-based cancer imaging
- Microbial-based image-guided drug, gene, or radiation therapy *in vivo*

Microbial-based cancer detection and diagnosis, Miguel Ossandon
The interest of the program is to stimulate research on the various approaches to microbial-based cancer diagnosis to support the development of microorganisms for cancer diagnosis to provide information about the tumor organ site and characteristics and facilitate early detection or analysis of asymptomatic cancers. Potential relevant topics include, but are not limited to:

- Exploring novel microbial species that might have diagnostic potential
- Engineering of microorganisms with detectible markers of interactions with tumor cells
- Exploring microorganisms as biomarkers for cancer
- Monitoring shifts in microbial populations associated with cancer.

Microbial based cancer therapy and imaging for global health, Luis Alejandro Salicrup
Cancer is rapidly becoming a major health care problem in low- and middle countries (LMICs), where 60% of the world's total new cases are diagnosed. High cost, limited access to cancer care, manpower and training limitations, and a lack of awareness are some of the challenges for cancer therapy in LMICs. Microbial-based cancer therapy may offer an opportunity to address the issue of global cancer therapy disparity and introduce a more suitable cancer immunotherapy approach to LMICs. Potential relevant topics of interest for the Center for Global Health include, but are not limited to:

- Low-cost microbial-based cancer therapies and diagnosis for low resource settings
- Low-cost imaging technologies such as ultrasound for microbial-based cancer therapy to enable monitoring of microbial-based cancer therapy
- Novel cancer-targeting approaches suitable for LMICs
- Microbial cancer therapy delivery approaches suitable to LMICs.

Oral cancer, Zhong Chen
The National Institute of Dental and Craniofacial Research (NIDCR) supports research on dental, oral, and craniofacial health and disease, including the treatment of cancers of the oral cavity, oropharynx, and salivary glands. Potential relevant topics of interest for NIDCR include, but are not limited to:

- Microbial-based treatment of oral, salivary gland, or oropharyngeal cancers
- Microbial delivery of anti-cancer therapeutics to oral, salivary gland, or oropharyngeal tissues
- Microbial manipulation of the oral microbiome to reduce the risk of developing cancers of the oral cavity, oropharynx, and salivary gland
- Microbial or microbial metabolite-based inhibition of HPV acquisition, infection, and persistence in the oral cavity to prevent oral cancer

Organizing committee

Phil Daschner	NCI Division of Cancer Biology, daschnep@mail.nih.gov
Luis Alejandro Salicrup	NCI Center of Global Health, luis.salicrup@nih.gov
Miguel Ossandon	NCI Division of Cancer Treatment and Diagnosis, Cancer Diagnosis Program, ossandom@mail.nih.gov
Chiayeng Wang	NCI Division of Cancer Treatment and Diagnosis, Cancer Imaging Program, chiayeng.wang@nih.gov
Charles (Pengnian) Lin	NCI Division of Cancer Treatment and Diagnosis, Cancer Imaging Program, p.lin@nih.gov
Yisong Wang	NCI Division of Cancer Treatment and Diagnosis, Cancer Imaging Program, yisong.wang@nih.gov
Farah Zia	NCI Division of Cancer Treatment and Diagnosis, Office of Complementary and Alternative Medicine, ziaf@mail.nih.gov
Avi Rasooly	NCI Division of Cancer Treatment and Diagnosis, Office of Complementary and Alternative Medicine, rasoolya@mail.nih.gov
Zhong Chen	NDCR Oral/Salivary Gland Biology zhong.chen@nih.gov