"You begin saving the world by saving one person at a time; all else is grandiose romanticism or politics"

— Charles Bukowski
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President Obama’s “stimulus package” has challenged the cancer research community to speed discoveries that will have a significant impact on the cancer burden. A substantial influx of funds has been provided for fundamental investigations and translational initiatives. There is enhanced support for the spectrum of critical issues including epidemiology, prevention, early detection, therapy and supportive care. A key component of the package addresses the application of modern biotechnology and informatics to the seminal problems facing our patients.

Few expect to achieve a significant reduction of mortality rates overnight. The barriers are too strong, from observation to application. However, the prospect for meaningful accomplishments is great, and sustained federal contributions to the mission will be essential to maintain the anticipated momentum.

Parallel efforts encouraging measures known to be critical to a healthy lifestyle are equally important, and often neglected. The benefits of proper nutrition, exercise, and eliminating toxic exposures (i.e., tobacco, hormones, sexually transmitted disease, etc.) are well established. Health care delivery will also have a profound effect on the success of the planned investment, balancing the need for universal coverage with the goal of high-quality medicine. The President’s ambitious goals are laudatory, his message of hope inspiring, and his sincere commitment to making an impact should be applauded.
“It was serendipity,” says Seema A. Khan, MD, when asked why she became a breast cancer specialist. But, while chance may have decided her field, her passion for it hasn’t wavered. She continues to be fascinated, rewarded and challenged by the combination of her work as a scientist and clinician.

Khan is Bluhm Family Professor of Cancer Research, Director of the Bluhm Family Program for Breast Cancer Early Detection and Prevention, and Professor of Surgery at the Feinberg School of Medicine. Growing up in Pakistan, she says her mother encouraged her children to do well in school and pursue meaningful careers. This was especially true for her daughters. A self-taught woman, Khan’s mother first became a teacher and then pursued a successful career in radio and television after the birth of her children. “She believed strongly that women should have choices and be independent,” Khan says. “So she pushed us to acquire skills that would allow us to support ourselves.”

Apart from teaching and journalism, Khan says, medicine was one of the few challenging careers available to Pakistani women at the time. With a natural affinity for science, she considered both teaching and medicine, ultimately choosing medicine because “I thought it would be more interesting.”

Co-leader of the Lurie Cancer Center’s Breast Cancer Program, Khan received her medical degree from Dow Medical College in Karachi in
1978 and went on to receive her SM degree in epidemiology from the Harvard School of Public Health. She served on the faculty of the Department of Surgery at the State University of New York (SUNY) until 2000 when she was hired by Northwestern University to develop a research program for breast cancer prevention.

Improving Identification of High-Risk Patients

Dr. Khan has a special interest in identifying women at high risk for breast cancer. “One of the major problems with breast cancer prevention is that we target women based on probability,” says Khan. Currently, doctors assess risk by relying on factors in a woman’s history, such as her age at the onset of menses, how old she was when she had her first child and when menopause occurred. “Our ability to estimate risk this way is poor, just a little better than a coin toss,” Kahn believes. Early and accurate identification of those at high risk will result in better targeting of chemoprevention agents and help physicians avoid treating healthy patients unnecessarily.

Recent research suggests that the breast itself can provide important clues for a woman’s risk for breast cancer. For example, scientists now agree that density is an important indicator. “Women with dense breasts on mammography are at about a four- to five-fold higher risk than women whose breasts are not dense,” Khan says. Atypical hyperplasia is another reliable clue that can help identify those women who are more likely to get breast cancer. Other than a strong family history of the disease, “the best indicators are found in the breast itself,” says Khan.

Dr. Khan notes that her surgical background lends itself to this research approach since surgeons tend to focus on what occurs locally. She is Principal Investigator on a study of “Nipple Fluid Hormone Levels and Breast Cancer Risk,” funded by the National Institutes of Health and the National Cancer Institute, to determine whether there is a relationship between estradiol levels in breast tissue and malignancy risk. By comparing hormone levels in nipple fluid with levels in the blood, and comparing the levels between healthy women and those with cancer, the investigators hope to determine whether local hormone synthesis is an important risk factor for breast malignancy. “We think the breast tissue itself makes hormones and that hormone formation there might be more important for the development of breast cancer than what circulates in the blood,” Khan explains.

Khan is also the PI for an Avon Foundation/Prevention Research Initiative evaluating gene methylation and breast tissue estradiol concentrations. DNA methylation is of interest because it may spur uncontrolled tumor growth. When the promoter sequences of genes (those responsible for gene expression) acquire extra methyl groups, the genes are silenced. When this occurs in tumor suppressor genes, cancer is then allowed to grow unchecked. Khan says that if the investigation confirms the study’s hypothesis, patients who present with hyper-methylation of tumor suppressor genes could be identified as high-risk. Doctors could then administer chemoprevention agents to these high-risk patients, targeting those who may already be in the process of tumor formation.

Khan emphasizes the valuable role breast cancer studies have played in advancing research in other cancers. “Breast cancer has always been in the vanguard of solid tumor research,” she says. Advancements, such as the use of mammography as a screening tool and adjuvant chemotherapy have led to significant progress in other fields as well.

Rewards of Clinical Practice

Dr. Khan finds her work as a clinician complements and enhances her research. “There are so many things that turn upside down in a patient’s life when she is diagnosed with breast cancer, and these women are so brave,” says Khan. “It’s wonderful to help them through this difficult process.” She takes pleasure in watching many of her patients go on to live fulfilling lives. One of the most rewarding aspects of her work as a breast cancer clinician, she says, is “seeing my patients doing well years after treatment and watching their children grow up.”

Dr. Khan and her husband, A. Vania Apkarian, a neuroscientist and Professor of Physiology at Northwestern, enjoy sharing their love of theatre, dance and travel with their own two children.
No quality is more important for a scientist than curiosity, and Thomas O’Halloran, PhD, Associate Director for Basic Sciences for the Lurie Cancer Center, has it in spades. The eldest of nine children, O’Halloran says, “At a very early age, I was fascinated with chemistry and molecules and life, and how things worked.” He and a friend combined their home chemistry sets to create a “Jacob’s ladder”—a high voltage traveling arc that generated 20,000 volts of electricity. Further experiments resulted in laughing gas and disappearing ink, which they sold to their classmates, and a failed attempt to make trinitrotoluene (TNT). “We had too much fun!” he recalls.

As an adult, that same curiosity continues to serve him well. In addition to his position at the Lurie Cancer Center, O’Halloran is the Charles E. and Emma H. Morrison Professor in the Department of Chemistry and in the Department of Biochemistry, Molecular Biology and Cell Biology at Northwestern University. He also serves as Director of the new Chemistry of Life Processes Institute (CLP), a research center on the Evanston campus dedicated to fostering interdisciplinary studies in chemistry, biology, engineering and medicine. His multiple roles on both of Northwestern’s campuses have helped O’Halloran increase the interaction among scientists in different fields and promote interdisciplinary efforts. “There’s a beautiful spectrum of research expertise that cuts across the two campuses,” he says.
Examples of these efforts to foster collaboration include the design of Silverman Hall, the new building that will soon house the CLP. In the new facility, scientists from different disciplines will work in close physical proximity to one another, facilitating interaction and breaking down barriers associated with the more common “silo” arrangement of academic disciplines. “There are still a lot of rich scientific pastures to plow at the interfaces between classical disciplines,” O’Halloran says. And he is hopeful that Silverman Hall will help that happen. He also expects the new facility and the CLP to play “a very important role in implementing the Lurie Cancer Center’s vision of stimulating new types of basic research in the fields of cancer detection, treatment and prevention.”

Discovering Metals: Early Research Experience
O’Halloran came to cancer research by an unusual path. After his freshman year at the University of Missouri at Columbia, he “talked his way into” a summer job at one of the school’s research laboratories, “and they let me start mixing things,” he says. “I didn’t make any TNT, but I did start crystallizing cobalt complexes of deep purples, reds and greens, and iron complexes—and I fell in love with it.” His work that summer stoked O’Halloran’s interest in metals and their functions in the human body. “Metals were clearly needed by living cells,” he learned, “but no one had paid much attention to how they got to where they were going.” After graduating, O’Halloran studied the chemistry of platinum drugs at Columbia University in New York where he earned his PhD. He performed his postdoctoral studies at MIT, and, in 1986, was recruited by Northwestern.

O’Halloran’s research at Northwestern focuses on the regulatory biology and chemistry of transition metal receptors involved in homeostasis and oxidative stress pathways. He is especially interested in the intracellular chemistry of elements essential for growth and proliferation, nanoscale drug delivery systems and the mechanisms of anticancer agents based on arsenic, molybdenum and platinum chemistry.

Targeting Cancer
O’Halloran recently started moving part of his lab forward from examining the basic way molecules work in living systems to creating new drugs that kill cancer cells. As project leader for a Center of Cancer Nanotechnology Excellence (NU-CCNE) study on targeted delivery of multifunctional therapeutic agents, O’Halloran and his team have developed tiny drug delivery vehicles called nanobins that carry drugs directly to malignant cells, where they bind and then unload their drug cargo, killing the cancer cells. (Funding for this study comes from a National Cancer Institute Nanotechnology Excellence Grant.)

To keep drugs from leaking out of these “smart” nanobins, O’Halloran and his team also devised a liposome bilayer that surrounds the vehicles, making them a safe and effective way to get toxic substances to the cancer cells without destroying healthy ones. Along the way, the team discovered a way to reduce the toxicity of arsenic trioxide, a toxic but effective cancer killer that has been used in traditional Chinese medicine for thousands of years, and is one of the drugs transported by the nanobins. The hope is that higher doses will mean more effective treatment and better patient outcomes. (Arsenic trioxide is currently FDA-approved to treat acute promyelocytic leukemia.)

O’Halloran is enthusiastic about the progress they are making and the value of collaboration in their ultimate success. “I simply can’t progress to the next stage with the tools of basic science alone,” he says. “We can’t move these ideas forward without the context of the Lurie Cancer Center, the participation of our clinical colleagues and the phenomenal support of basic cancer research provided by Ann Lurie and groups like the H Foundation, a Chicago-based philanthropic organization.”

In another example of this cooperation, O’Halloran notes that the CLP and the Lurie Cancer Center are working together to recruit a thought leader in the field of proteomics, a branch of molecular biology focused on the study of proteins, and a growing area of cancer research. “Proteomics is an emerging field that is important to both clinical cancer and basic science research, so the CLP and the Lurie Cancer Center are working hard to recruit a senior level faculty member with expertise in this area,” he says. And, once again emphasizing the importance of teamwork, O’Halloran says they are looking for “someone who will work with us arm-in-arm to build something far greater than what we are capable of as individuals toiling away on separate problems.”
O’Halloran credits Lurie Cancer Center Director, Steve Rosen, with fostering this collaborative culture and showing him how to engage colleagues on a personal as well as an intellectual level. “Steve always makes it interesting and fun to join him in a project,” he says. “And I’m trying to employ that skill to bridge really different disciplines in the basic sciences and the medical community.”

Career as Privilege
O’Halloran’s many roles don’t leave him much free time, but he carves out what he can for hiking, camping and fly fishing in the Boundary Waters between Canada and the US. Reflecting on his career, O’Halloran says he is grateful to be able to do the work he does. “It’s such a privilege to be able to explore in these ways, to have our curiosity unleashed on very fundamental questions,” he says. “And, every now and then, when a moment of idle curiosity develops into an obsession and, ultimately, into a discovery that helps cancer patients, you really appreciate how lucky you are.”
“My dad was the smartest man I ever knew,” says William Small, Jr., MD, FACRO, “but he worked 20 hour days driving trucks because he wasn’t able to get an education.” Dr. Small’s father grew up during the Great Depression, joined the U.S. Army at 17 and fought in both World War II and Korea. He then settled in Chicago and went to work to support his family. Small says his father’s experience influenced his career choice. Small loved science and thought medicine would provide both interesting work and job security. “From my earliest memories, I always thought I was going to be a doctor,” he says.

Small graduated magna cum laude from the University of Illinois in 1986 and went on to earn his MD with distinction from Northwestern University in 1990. His academic success is especially remarkable because he achieved it while juggling coursework with a full-time job. Among other jobs, he served as a mental health worker for Northwestern Memorial Hospital’s Institute of Psychiatry, where he monitored patients during the week and took them on outings on weekends. In addition, he got married during medical school and shares credit with his wife for helping him survive those challenging years.

In 1994, Small joined the faculty of the Feinberg School of Medicine, where he now serves as Professor and Vice Chairman of the Department of Radiation Oncology. He is also Associate Medical Director of the Lurie Cancer Center, attending physician and Chairman of
the Cancer Committee for Northwestern Memorial Hospital. He is considered a national leader in the field of radiation oncology. While Small says he spends most of his time taking care of patients, his research is a priority, as well. “Clinical work and research go hand-in-hand,” he asserts. “To be a really good oncologist, part of your work must be in research.”

Improving Patient Outcomes
Small is committed to advancing the understanding and treatment of gynecologic, breast and gastrointestinal malignancies, and to reducing the toxicity of radiation treatments in an effort to improve patient outcomes. Much of his research is focused on unique combinations of radiotherapy with systemic therapies such as biological agents. He recently served as Principal Investigator (PI) for two Radiation Therapy Oncology Group (RTOG) studies investigating the use of cytoprotective agents to reduce radiation toxicity. He also acts as Co-Chair of the RTOG’s gynecologic working group.

Small says radiation is an important part of the oncologist’s arsenal. “It can cure almost any localized cancer—if you can give enough,” he says. “And we treat a lot of cancers with radiation alone.” However, treatments come with serious side effects, such as painful burns that can occur soon after treatment, as well as problems that can appear much later, such as bowel obstructions, mucositis and sexual dysfunction. “Any time you turn a radiation beam on, you will have toxicity,” says Small. Developing ways to administer more radiation with fewer side effects and produce better patient outcomes are among his goals.

Small’s efforts are global in scope. In addition to his work at Northwestern and with the RTOG, he chairs the cervical cancer committee for the Gynecologic Cancer Intergroup (GCIG), an international organization of clinical trials groups tasked with promoting clinical research and furthering international collaboration in the area of gynecologic malignancies. One of the important efforts of the GCIG is to increase enrollment in clinical trials. According to Small, only about five percent of adults currently participate in such trials and many studies have closed because researchers were unable to recruit enough subjects. “Lack of participation in trials makes it hard for us to make progress,” he says. “It takes forever to test new treatments.”

Small is also a member of the National Cancer Institute’s steering committee for gynecologic cancers and is proud of the work he’s done to help guide gynecologic research both here and abroad. He cites the group’s efforts in cervical cancer, a disease that is well controlled in the US but remains a menace in much of the rest of the world. “It’s a huge international problem and we’re working on different ways to move that science forward,” he says.

Small credits his mentor, Bharat Mittal, MD, Professor of Radiation Oncology and Chair of the Department of Radiation Oncology at Northwestern’s Feinberg School of Medicine, with teaching him valuable research skills. While Small was a medical student, he worked on his first oncology paper with Mittal. “Dr. Mittal helped me become aware of all the questions there are in this field and gave me important advice on my first real research project.”

The Best Profession
With one foot in research and another in patient care, Small’s hours are long, and include as much time as possible with his wife and two daughters. “I always laugh when someone asks me to break up my 40 hour week. Break up my 100 hour week you mean?” But, despite a challenging schedule, Small says he’s glad he chose to become a physician. “There’s no greater profession.” The best part is “when I’ve done something that has made a difference in patients’ lives, such as enrolling an individual in a clinical trial, or the times when taking a chance and not giving up on a patient leads to a good outcome that they may not have had if they hadn’t come to me.” He is gratified when he learns that other physicians’ treatment plans were influenced by his research. “With research, you want to improve therapies, and sometimes I think I’ve done that, which is very fulfilling.”
David Cella Named Head of New Medical Social Sciences Department

David Cella, PhD, a top researcher on how to measure outcomes among cancer patients and the quality of life for patients in medical clinical trials, has been named chair of a newly created Department of Medical Social Sciences at Northwestern’s Feinberg School of Medicine.

Cella, currently professor of Psychiatry and Behavioral Sciences at Feinberg and in the Institute for Healthcare Studies, and Associate Director for Cancer Prevention and Control at the Lurie Cancer Center, also served as executive director of the Center on Outcomes, Research & Education at NorthShore University HealthSystem, formerly known as Evanston Northwestern Healthcare. The new department will focus on health measurement, quality of life measures, outcomes science and statistical tools used to support clinical research. He developed, and is continually refining, the Functional Assessment of Chronic Illness Therapy Measurement System for outcome evaluation in patients with chronic medical conditions.

Chad Mirkin Named to Obama’s Science and Technology Advisory Council

Chad A. Mirkin, the world’s top-cited researcher in nanomedicine and one of the most widely cited chemists, has been named to the President’s Council of Advisors on Science and Technology (PCAST).

President Barack Obama announced the names of the 20 members in a speech at the National Academy of Sciences in April. “This council represents leaders from many scientific disciplines who will bring a diversity of experience and views,” said President Obama. “I will charge PCAST with advising me about national strategies to nurture and sustain a culture of scientific innovation.”

PCAST is an advisory group of the nation’s leading scientists and engineers who advise the president and vice president and formulate policy in many areas where understanding of science, technology and innovation is key to strengthening the U.S. economy and forming policy that works for the American people.

Mirkin Receives the $500,000 Lemelson-MIT Prize for Invention

In addition, Mirkin has been awarded the prestigious 2009 $500,000 Lemelson-MIT Prize, which recognizes outstanding inventors. Mirkin, George B. Rathmann Professor of Chemistry in the Weinberg College of Arts and Sciences, director of the International Institute for Nanotechnology at Northwestern and member of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, is being honored for his revolutionary discoveries and sizeable contributions to science and invention.

A leader in the burgeoning field of nanotechnology, Mirkin is the author of 380 manuscripts and more than 350 patents and applications. He is best known for the invention, development and commercialization of two revolutionary technologies: the nanoparticle-based medical diagnostic assays underlying the FDA-approved Verigene IDTM system, and Dip-Pen Nanolithography, an ultra-high-resolution molecule-based printing technique.
Prostate Cancer SPORE Receives Five-Year Grant from the NCI

Northwestern One of Just 11 SPORE Sites in US

The Lurie Cancer Center’s Specialized Programs of Research Excellence (SPORE) in prostate cancer, one of just 11 in the country, will receive $11.5 million over the next five years from the National Cancer Institute (NCI). The SPORE brings together a multidisciplinary team of basic scientists, epidemiologists, urologists, oncologists, pathologists and statisticians, who are working together to develop innovative approaches to prostate cancer research.

First funded in 2001, the Northwestern-based SPORE represents a consortium of investigators from Northwestern University’s Feinberg School of Medicine, The University of Chicago, and NorthShore University Health System (formerly Evanston Northwestern Healthcare). The principal investigator is Chung Lee, PhD, the John Grayhack Professor of Urology, Feinberg. Co-principal investigators are William Catalona, MD, Professor of Urology, Feinberg, and Walter Stadler, MD, Professor of Medicine, University of Chicago. The scientific administrator is Robin Leikin, PhD, Research Assistant Professor, Feinberg, and Scientific Program Director, Robert H. Lurie Comprehensive Cancer Center.

“Five years of new funding from the NCI is a major investment in our efforts to understand prostate cancer,” says Dr. Lee. “It allows us to continue to tackle projects aimed at all aspects of the disease, including prevention, early detection, and finding innovative ways to treat patients with disease and improve their quality of life.” Dr. Lee says the SPORE funding supports four major research projects and underscores the fact that all are translational in nature. “One of the most significant features of a SPORE such as ours is that it unites basic and clinical researchers. It is a link from the bench to the bedside.”

In addition to the four research projects, the prostate cancer SPORE includes Core facilities that support the research; the Developmental Research Program that funds promising pilot projects; and the Career Development Program that recruits junior investigators.

Rituximab Linked to Often Fatal Virus

Research from the RADAR project (Research on Adverse Drug Events and Reports), led by Charles Bennett, MD, PhD, links the popular cancer drug rituximab (brand name Rituxan) to progressive multifocal leukoencephalitis, or PML. Widely used for lymphoma, it is also approved for treatment of rheumatoid arthritis and is widely used off-label to treat multiple sclerosis, lupus erythematosus and autoimmune anemias. Bennett reports on 57 cases from 1997 to 2008 in which patients developed the fatal brain disease after taking rituximab. They died an average of two months after being diagnosed.

"Rituximab is one of the most prominent drugs in a new class called monoclonal antibodies. It’s now the third monoclonal antibody that is associated with PML," said Bennett, the A.C. Buehler Professor in Economics and Aging at Feinberg, an oncologist at the Jesse Brown VA Medical Center, and Director of the Cancer Control Program at the Lurie Cancer Center. The RADAR project is an international consortium of physicians that collaborate to identify adverse reactions to medications and devices.
Less Toxic Drug Prolongs Survival in Metastatic Breast Cancer

A national study led by William Gradishar, MD, Director of Breast Medical Oncology at the Lurie Cancer Center and Professor of Medicine at the Feinberg School of Medicine, found that the drug Abraxane prolonged progression-free survival by almost seven months compared with Taxotere, which is part of a class of solvent-based drugs called taxanes. “It nearly doubled progression-free survival,” said Gradishar.

The study showed Abraxane also was much less toxic to patients. Gradishar said solvents are responsible for many of the side effects of chemotherapy including a drop in the white blood cell count and numbness or tingling in the fingertips. In the study, the Abraxane was administered on a weekly schedule compared to injections every three weeks of Taxotere.

"This is a win-win finding," Gradishar said. "The weekly schedule of Abraxane has more anti-tumor effects and is better tolerated than Taxotere. There is also evidence that Abraxane is able to deliver the chemotherapy drug more effectively to the tumor. These results suggest that weekly Abraxane may be an appropriate alternative to docetaxel (Taxotere) in the first-line treatment of patients with metastatic breast cancer."

The Phase II, open-label, randomized clinical study involved 300 patients with previously untreated metastatic, stage 4 breast cancer. The results were assessed by an independent radiology company and study investigators. The study was designed to evaluate the safety and efficacy of three doses of Abraxane versus the highest standard dose of Taxotere.

William W. Wirtz Cancer Innovation Fund Allocates $19.5 Million to Cancer Care and Research at Northwestern

Northwestern Memorial Foundation announced the inception of the William W. Wirtz Cancer Innovation Fund, a new initiative in support of continued research and clinical advancements within the Robert H. Lurie Comprehensive Cancer Center of Northwestern University at Northwestern Memorial Hospital. The late William W. Wirtz designated more than $19.5 million to seed the program prior to his passing in 2007.

In recognition of Wirtz’s generosity, Northwestern Memorial formed this pivotal oncology fund in his honor. The William W. Wirtz Cancer Innovation Fund will support efforts to enhance patient access to a wide range of essential services for individuals with cancer; recruit leading physicians and physician-scientists specializing in cancer research and treatment; and allow Northwestern Memorial to remain at the vanguard of emerging technologies.
Stewart Goldman Honored by Children’s Brain Tumor Foundation

Stewart Goldman, MD, was presented with the Children’s Brain Tumor Foundation’s (CBTF) Pioneer Award for outstanding contributions in pediatric neuro-oncology and brain tumor research. Goldman is medical director of neuro-oncology at Children’s Memorial Hospital, director of the Center for Clinical Trials Research for the Children’s Memorial Research Center, associate professor of pediatrics at Northwestern University’s Feinberg School of Medicine, and a member of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University.

Goldman serves as a director of Children’s Memorial Hospital’s Falk Brain Tumor Center, one of the world’s leading centers for pediatric brain tumor diagnosis and treatment. Established in 1986, the center was one of the first of its kind in the nation to create a comprehensive multidisciplinary program, offering specialists in more than 30 pediatric and surgical subspecialties. Last year, the brain tumor team cared for more than 140 new patients and performed more than 100 surgeries.

Goldman is a member of the Children’s Oncology Group CNS disease committee and is the Children’s Memorial principal investigator for the National Cancer Institute-sponsored Pediatric Brain Tumor Consortium where he is a member of the consortium’s steering, scientific, new agents and angiogenesis committees. He is also the site Principal Investigator for the Children’s Oncology Group Phase I Consortium.

Cryoelectron Microscope Finds Home at Northwestern

The NIH has awarded Northwestern University a $1.9 million grant to purchase a 300 kV (kilovolt) cryoelectron microscope. The JEOL 3200FS field-emission electron microscope will be one of less than a dozen of its kind in the United States.

The microscope, which is being built in Japan, is designed to allow high-resolution examination of biological specimens at low temperatures. Researchers from the Weinberg College of Arts and Sciences, the McCormick School of Engineering and Applied Science and the Feinberg School of Medicine will take advantage of the instrument’s features. The microscope’s primary use will be for single-molecule imaging and cryotomography with frozen specimens. One medical application is to predict the onset of cancer.

The microscope includes a wide range of features aimed at performing high-quality tomography, STEM (scanning transmission electron microscopy), dark field microscopy, EDS (energy-dispersive spectrometry) and EELS (electron energy-loss spectrometry), and will be installed in the new Richard and Barbara Silverman Hall for Molecular Therapeutics and Diagnostics on the Evanston campus.
Rebecca Caires Named to NCCN Best Practices Committee
Rebecca Caires, Administrative Director, the Robert H. Lurie Comprehensive Cancer Center of Northwestern University at Northwestern Medical Faculty Foundation, has been named to the Best Practices Committee of the National Comprehensive Cancer Network (NCCN). The purpose of the Committee is to share successes and challenges in areas related to cancer center operations management and the delivery of oncology services. To this end, the Committee develops research studies and shares experiences in areas such as quality and patient safety, oncology workforce productivity, inpatient and ambulatory clinical operations, and clinical research/clinical trials operations.

The National Comprehensive Cancer Network (NCCN), a not-for-profit alliance of 21 of the world’s leading cancer centers, is dedicated to improving the quality and effectiveness of care provided to patients with cancer. The primary goal of all NCCN initiatives is to improve the quality, effectiveness, and efficiency of oncology practice so patients can live better lives.

Jamie Von Roenn Receives 2009 ASCO Statesman Award
Jamie Von Roenn, MD, Professor of Medicine at Northwestern University’s Feinberg School of Medicine, member of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, and Medical Director of Northwestern Memorial Hospital’s Palliative Care and Home Hospice Program, was honored with the 2009 American Society of Clinical Oncology (ASCO) Statesman Award.

The ASCO Statesman Award recognizes ASCO members for their extraordinary volunteer service, dedication and commitment to the Society. Dr. Von Roenn’s longstanding interest in palliative care, end-of-life issues and the supportive care of oncology patients has led to important initiatives in both the inpatient and outpatient setting.

Dean Ho Wins Prestigious NSF Award for Young Faculty
Dean Ho, PhD, assistant professor of biomedical engineering and mechanical engineering in Northwestern’s McCormick School of Engineering and Applied Science, and a Lurie Cancer Center member, has received a Faculty Early Career Development (CAREER) award from the National Science Foundation. The CAREER program recognizes and supports early career development of those teacher-scholars who are most likely to become the academic leaders of the 21st century. CAREER awardees are selected on the basis of creative, career-development plans that effectively integrate research and education within the context of the mission of their respective institutions.

In his research, Ho is pushing the frontier of biomedical and nanotechnology research towards the realization of tailored biology: the ability to configure desired bio-functionality into non-biological materials rapidly and on demand. Ho and his research group are developing nanodiamond- and polymer-based platforms for sustained drug delivery to address a broad array of medical challenges. These strategies have resulted in nanoparticle and microfilm device approaches towards both systemic and localized treatment.
Quality Measures and Translational Efforts in GI Cancer Surgery

David Bentrem, MD

A great deal of research time and resources are spent on development of new treatments for GI cancers with little return on these investments. Less time is spent evaluating the effectiveness of current treatment and improving/ensuring delivery of those treatments and best practices. More lives of cancer patients would be saved if we focused on delivery of current standards of care, ensuring that all patients received treatment in adherence with current recommendations\(^1\). Since the Institute of Medicine report “To Err Is Human” in 1999, measuring and improving quality in healthcare has become a dominant issue. This report estimated that as many as 98,000 people may die each year from preventable harm in hospitals. Its follow-up report, “Crossing the Quality Chasm: A New Health System for the 21\(^{st}\) Century” (2001) introduced aims for improvement: care that is safe, timely, effective, efficient, equitable and patient-centered. Despite the advances made during the past decade, daunting problems still confront clinicians as they work to make care better and safer. The Centers for Medicare and Medicaid Services (CMS) Physician Quality Reporting Initiative (PQRI) is now underway. This program rewards reporting of process and quality information to CMS and requires hospitals to demonstrate adherence with several clinical measures. Additional specific, valid indicators are needed to measure performance and to help hospitals direct quality improvement initiatives. Measures must focus on each type of cancer to get a true sense of...
quality care. Moreover, these initiatives and hospital-specific performance and outcome measures will soon be publicly reported in Illinois.

QUALITY MEASURES IN CANCER SURGERY
Commonly performed elective surgical procedures on the alimentary tract are carried out with low morbidity and low mortality in most hospitals in the United States. There are some procedures on the alimentary tract for less common tumors that are performed with a relatively low frequency and are associated with higher morbidity and mortality. Volume is a proxy for unmeasured hospital structural features and processes of care and is associated with outcome with relative differences being dependent on the complexity of the procedure and the frequency with which it is performed. For high-risk cancer procedures, it is well established that patients undergoing surgery at high-volume hospitals have lower risks of complications and perioperative mortality than those at lower-volume centers. Nevertheless, variations in short and long term outcome among hospitals suggest the safety and application of cancer treatment can be improved. Multimodality cancer care requires a high degree of coordination by a hospital and its providers. Furthermore, more specific indicators of surgical quality beyond case volume are needed. Defining and improving on “quality” of cancer care has been a tremendous challenge.

There are ongoing national efforts to identify processes of care to help improve the quality of cancer care. The Leapfrog Group, a consortium of 170 private and public organizations that insure nearly 35 million individuals, incorporated volume standards for 5 operations including complex cancer operations (esophagectomy and pancreatic resection) and advocates selective referral to high volume centers. While this may improve care for select lower-risk, insured individuals, this strategy will likely increase disparities in access to care, and may not improve overall outcomes for all. Thus, alternative mechanisms are needed to improve care. Deficits in the quality of cancer care have encouraged the National Cancer Institute and the National Cancer Policy Board of the Institutes of Medicine to investigate why treatments of known effectiveness are not always used.

The goal of our health services research group at RHLCC has been to promote the study of quality cancer care.

Figure 1. Utilization of surgery by center type & center volume for early stage pancreatic cancer (stage I)

Figure 2. Utilization of completion lymph node dissection (CLND) for Melanoma (stage I-III, 2004-2005)

Treatment Utilization- We have examined utilization of surgery for early stage pancreatic cancer and found low volume or nonspecialized centers are less likely to offer surgery (figure 1). We also found similar associations when examining use of completion nodal dissection for breast cancer or melanoma (figure 2).
Expert Panels - Together with RHLCCC researchers Drs. Jeffrey Wayne and Karl Bilimoria, we have formed two national expert multidisciplinary panels through the American College of Surgeons and developed quality measures for pancreas cancer and melanoma\textsuperscript{7,8}. We continue to work with the American College of Surgeons to incorporate these pancreas and melanoma measures into national standards.

Hospital Benchmarking - We have worked with the American College of Surgeons to examine several measures such as lymph node counts after surgery for pancreas, gastric or colon cancer patients using the National Cancer Data Base (figure 3)\textsuperscript{9-11}. Specifically, we have been able to compare hospitals across a range of quality metrics.

EXAMINING THE TUMOR MICROENVIRONMENT

Additional translational efforts in pancreas and colon cancer involve extensive collaboration. Together with RHLCCC researcher Hidayatullah Munshi, we are investigating the fibrosis-protease cross-talk regulating pancreatic cancer invasion.

We have reported on the upregulation of 5-lipoxygenase in colon cancer\textsuperscript{12} (figure 4). Together with RHLCCC researchers Khash Khazaie and Paul Grippo, we are evaluating the role mast cells and 5-lipoxygenase in polyp development in a transgenic mouse model with mutant APC and 5-lipoxygenase knockout as well as the interaction between mast cells and

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**Figure 4. 5-LO and Mast Cells in Colon Cancer.** (a) 4\textmu m paraffin section of human colon cancer stained for 5-LO by immunohistochemistry. (mag \texttimes 200) (b) 2\textmu m plastic section of APC\textsuperscript{+/-} intestinal polyp stained for mast cells(CAE). (mag \texttimes 200)
pancreatic cancer growth and progression (figure 5). We are finding mast cells contribute to tumor cell proliferation and invasion in an MMP-dependent manner. Further evaluation of specific mast cell functions that contribute to the etiology of pancreatic and colon cancer may open new possibilities for therapeutic interventions and cancer prevention.

References


William J. Catalona, MD, Professor of Urology, is the Director of the Clinical Prostate Cancer Program for the Robert H. Lurie Comprehensive Cancer Center, Northwestern Memorial Hospital and the Northwestern University Feinberg School of Medicine. A graduate of Yale Medical School, Dr. Catalona trained in surgery at the Yale New-Haven Hospital, the University of California San Francisco, and the National Cancer Institute and in urology at the Johns Hopkins Hospital.

He was the first to show that the prostate-specific antigen (PSA) blood test could be used as a first-line screening test for prostate cancer. He is an expert in performing the “nerve-sparing” radical prostatectomy, having performed more than 5,000 of these operations.

He has received the James Ewing Society Award for Cancer Research, 1972; the American Urological Associations Gold Cystoscope Award, 1986, Hugh Hampton Young Award, 1994, and Eugene Fuller Triennial Prostate Medal, 1998; The Johns Hopkins Society of Scholars, 1994; the American Association of Genitourinary Surgeons, James Stockwell Barringer Medal, 1999 and Edward L. Keyes Medal, 2003; Prostate Cancer Foundation, Donald S. Coffey Physician-Scientist Award, 2005; Florida Prostate Cancer Network’s, General Schwarzkopf Pioneer in Prostate Cancer Award, 2005; the Society of Urologic Oncology,

He has served on advisory boards of the American Cancer Society and the editorial boards of several medical journals. He has been principal investigator on grants from the NIH, Department of Defense, American Cancer Society, and CaP CURE. He is author of more than 400 articles and books.

Introduction

At Northwestern, Dr. Catalona directs a highly translational clinical research program designed to move discovery from the laboratory to the patient bedside as rapidly as possible. He brings to the program an in-depth knowledge of prostate cancer genetics that is uncommon among urologists. He also brings the experience in conceiving and executing large-scale clinical trials with a rigor that qualifies for publication in leading scientific journals and satisfies the stringent requirements of regulatory agencies such as the United States Food and Drug Administration (FDA). He has also published extensively in prostate cancer genetics in several of the foremost journals, such as Nature Genetics and has landmark publications on PSA in the New England Journal of Medicine and the Journal of the American Medical Association.

In his current research, Dr. Catalona is a principal investigator in multi-institutional collaborative research programs focused on the genetics of prostate cancer. His collaborators and he have recently discovered several regions in the human genome linked to prostate cancer susceptibility and some linked to its aggressiveness as well. Identification of these risk variants has led to the launch of the first commercial genetic test for prostate cancer susceptibility and holds promise to provide insights into the cause of prostate and possibly other cancers, such as breast, colorectal, bladder, lung cancer and malignant melanoma.

The prostate cancer genetic risk variants discovered by Dr. Catalona’s collaborators have been replicated by leading research groups and have been heralded in Science magazine as the most significant discovery in prostate cancer genetics to date.

It is possible that through genetic testing, physicians will be able to discriminate between tumors that behave aggressively, and, therefore, require early detection and treatment, and indolent tumors that may not require early detection or treatment.

Unraveling the genetic underpinnings of cancer will also provide fundamental insights into the cause of cancer that could lead to new methods for diagnosis and treatment and even means of preventing it. This research has broad implications for bringing personalized health care for all people.

As co-chair of the National Cancer Institute’s (NCI) Prostate Cancer SPORE Genetics Working group, Dr. Catalona has assembled an inventory of thousands of DNA samples and related databases of prostate cancer patients currently available at the other 10 prostate cancer SPORE centers. Dr. Catalona is also Co-Principal Investigator of Northwestern Prostate Cancer SPORE.

As a member of the NCI-funded International Consortium of Prostate Cancer Genetics, he has potential access to DNA samples and data on nearly 3000 hereditary prostate cancer families collected from around the world.

He is also a collaborator in the NCI’s Prostate SPECs project to determine the genetic “signatures” of aggressive prostate cancers.

He has collaborations with industry, including Beckman Coulter, Inc. in developing a new commercial assay for pro-PSA and is currently conducting a pivotal clinical trial for FDA approval of this new blood test.

In addition, Dr. Catalona is collaborating with researchers at Northwestern who have discovered a new chemical compound (patents have been filed) that could be developed into a drug to prevent prostate cancer from metastasizing.

He also collaborates with researchers at Northwestern in evaluating the new nanotechnology PSA assay for identifying early tumor recurrence following prostate cancer surgery.

At Northwestern, he has acquired biologic samples for use in research from more than 1600 of his prostate cancer patients and 2000 age-matched men without prostate cancer. In addition, he has collected DNA samples from
numerous members of hereditary prostate cancer families along with detailed data relating to them in his research database.

Dr. Catalona’s prostate cancer study plan is to identify a large number of prostate cancer patients representing the entire spectrum of disease who have detailed clinical and pathology data available and long-term follow-up. He will obtain genotypes from their DNA samples for the approximately 30 prostate cancer risk variants from the genetic loci recently discovered. He will also type additional genetic marker flanking each of these variants to define so-called risk haplotypes. He will then seek to determine which variants and haplotypes are linked to cancer aggressiveness. By correlating the combinations of genotypes with the patients’ disease status and treatment outcomes, he hopes to be able to construct the genetic profiles of individuals with indolent cancers, intermediate-risk cancers, and aggressive cancers and gain fundamental insights into the development and progression of cancer.

Dr. Catalona’s individual research projects are discussed in more detail below.

The SPORE Grant
Dr. Catalona is Co-Principal Investigator of the SPORE (Specialized Programs of Research Excellence) in Prostate Cancer at the Lurie Cancer Center, funded by a grant from the National Cancer Institute of the National Institute of Health. This program brings together basic scientists, epidemiologists, urologists, oncologists, pathologists and statisticians working to develop new approaches in the prevention, early detection, diagnosis and treatment of prostate cancer. Other key administrative personnel of the SPORE are Chung Lee, PhD, Walter Stadler, MD, and Robin Leikin, PhD.

The themes of the SPORE include the identification of the cellular and molecular alterations in prostate cancer; prevention and risk factors in prostate cancer development; prostate cancer innovative therapeutics and rehabilitation; and quality of life and outcomes research in prostate cancer.

The SPORE Program consists of: core facilities that support the research projects; a Developmental Research Program that funds pilot projects; and a Career Development Program that recruits junior investigators. The Prostate Cancer SPORE received renewal funding from NCI for another 5 years early this year.

Preventing Prostate Cancer Metastases with Soy Protein
In addition to his participation in the administrative and specimen acquisition cores of the Prostate Cancer SPORE, Dr. Catalona is Co-Investigator on a project, entitled, “Modulation of Prostate Cancer Cell Motility by the Chemopreventive Agent, Genistein,” conceived and directed by Raymond Bergan, MD. This project involves a clinical trial testing the ability of a component of soy protein that might prevent prostate cancer cells from invading into adjacent tissues and producing metastases.

Screening and Patient Follow-up Studies
A crucial component of Dr. Catalona’s research program involves collecting biologic samples (blood, urine, tumor tissue, prostate fluid, etc.) from patients enrolled in his prostate cancer screening studies as well as from his patients and their family members. At Northwestern, he has enrolled more than 2000 participants in his screening studies and more than 1600 from his surgery patients.

Screening Van Events 2009
A free prostate cancer screening event staffed by Dr. Catalona’s research group and professional colleagues was held before a Chicago Bulls game at the United Center on April 15, 2009. A total of 203 men participated in the event and 125 of them also signed for the “Genetics of Prostate Cancer” research project. A similar event is scheduled for June 10, 2009, before a Chicago White Sox game at the U.S. Cellular Field. Dr. Catalona’s research group also has organized similar free screening van events in 2007 and 2008.

Pro-PSA Blood Test
For the past decade, Dr. Catalona has been evaluating a new form of PSA, called “pro-PSA,” as potentially a better blood test for detecting prostate cancer and its aggressive forms. For this project, his research group is collaborating with Beckman Coulter Incorporated currently enrolling patients in a pivotal trial for FDA approval in a multi-institutional study, including Northwestern University, Johns Hopkins, UCLA and the University of Michigan. Dr. Catalona’s group will contribute 150 patients to this study.
Ultra-Sensitive Nanotechnology PSA Test

Doctors Chad Mirkin, Shad Thaxton, and others at Nanosphere, Inc. have developed an ultra-sensitive (between 0 and 100 pg/ml) nanotechnology PSA test as an postoperative test for undetectable PSA levels. For this project, Dr. Catalona’s research group is collecting serum samples from patients who have undergone prostate cancer surgery and have had long-term follow-up. A total of 668 serum samples from 262 Northwestern patients were identified and shipped to Nanosphere for ultra-sensitive PSA measurements between March and April of 2009. Additional serum samples from the Washington University PSA Follow-up Study led by Dr. Catalona may be added later for further independent evaluation of the results of this study.

deCODE genetics Collaboration

For this project, Dr. Catalona’s group is collaborating with deCODE genetics, Inc. in Iceland. Dr. Catalona’s research group has sent about 4500 blood samples and clinical data to deCODE genetics for DNA extraction and genotyping. As a result, several genetic variants have been discovered that predispose to prostate cancer (on chromosomes 8q24, 17q12, 17q24, 2p15, Xp11, 5p15), and to other cancers as well, including colorectal, bladder, breast, and malignant melanoma. The results have been published in Nature Genetics (see references). Based upon these and other discoveries, deCODE has launched a commercial clinical genetic cheek-scraping test for prostate cancer susceptibility that types 8 of these genetic markers.

Combining Prostate Cancer Genetic Risk Test and PSA to Improve Screening Accuracy

The deCODE Prostate Cancer test measures 8 genetic markers called “SNPs” that are associated with the risk for prostate cancer. The purpose of this proposed research study is to examine the ability of the genetic risk test and/or family history to increase the specificity of PSA for predicting positive biopsy for prostate. About 1500 patients with positive biopsies and 1000-2000 patients with negative biopsies will be recruited for this study. Currently the documents for Institutional Review Board (IRB) approval are being prepared and will be submitted to Northwestern’s IRB in the near future.

ICPCG Project on Hereditary Prostate Cancer

Dr. Catalona is the principal investigator of Northwestern University’s branch of the International Consortium for Prostate Cancer Genetics (ICPCG) grant funded by the National Cancer Institute, which is one of multiple sites world wide, including U.S., U.K., France, Germany, Norway, Sweden, Finland, Australia, and Canada. The ICPCG recently completed a genetic linkage scan at the Center for Inherited Disease Research (CIDR), based at Johns Hopkins, including about 6000 genome-wide genetic markers (SNPs) for 760 prostate cancer families. Linkage signals on chromosomes 8p and 17q were emphasized as showing the greatest similarity between previous linkage scan using different genetic markers called “microsatellites.” A manuscript has been prepared for the submission.

The ICPCG has also performed a family-based genetic association study for 28 SNPs associated with prostate cancer genotyped in the ICPCG families. The dataset is nearly complete, and preliminary results show some confirmed risk SNPs were overly transmitted in members of prostate cancer families, while others were not. The basis and significance of this differential result is unclear at present. Additional analyses will be performed on this dataset. Follow-up analyses of the most promising linkage signals will be performed. The idea of sequencing all genetic regions called “exons” within a given linkage interval in key patients from linked families using a new technique called “Next Gen” sequencing was discussed at ICPCG’s May, 2009 meeting and was deemed to be a project that might be funded through an administrative supplement to the ICPCG grant.

“SPECs” Grant

Dr. Catalona’s research group is a member of this multi-institutional grant that evaluates tumor tissues removed from prostate cancer patients for genetic markers for prostate cancer aggressiveness. This is a multi-institutional study, based at the University of California, Irvine, and is the only prostate cancer “SPECs” (Strategic Partnering to Evaluate Cancer Signatures) program funded by the National Cancer Institute.

SPORE Genetics Working Group Study

Dr. Catalona is a co-chair of this SPORE working group. The first study a case-case genetic association study that would genotype
the approximately 29 SNPs known to be associated with prostate cancer susceptibility of 1000-25000 prostate cancer patients from Prostate SPORE sites and correlate with clinical and pathological features for aggressiveness. Dr. Catalona’s research team has visited 8 (Harvard, Johns Hopkins, Memorial Sloan-Kettering, Michigan, UCLA, UCSF, Fred Hutchinson, and Mayo Clinic) of the other 10 SPORE sites and have established working relationships with these groups. Visits to the remaining two SPORE sites (Baylor and MD Anderson) are in the process of being scheduled. The protocol for the study is being prepared and will soon be distributed to all 11 SPOREs for review and IRB approval. After obtaining IRB approval, DNA samples will be collected and genotyped (or the previously determined genotypes will be collected), and data analyses will be performed according to the research protocol.

Vitamin D Levels and Related Genetic Polymorphisms, Sun Exposure, Skin Color and Risk of Aggressive Prostate Cancer

After obtaining IRB approval, Dr. Catalona’s research group has been helping recruit patients for this project directed by Dr. Rick Kittles at the University of Chicago and Dr. Adam Murphy at Northwestern and have accrued 45 patients from Northwestern Memorial Hospital. Our preliminary data is on the most recent 30 patients revealed that all patients but one were considered vitamin D deficient. This is consistent with the fact that Chicago is a low UV exposure climate and the samples were collected in the winter months.

Conclusion

These studies hold promise to help devise new clinical tests to guide difficult patient management decisions, such as whether or not to screen or biopsy men at risk or what the most appropriate management would be for a man with any given risk profile. Dr. Catalona believes that taking prostate cancer genetics into the clinical realm would potentially change the scope of diagnostics, risk stratification, treatment, follow-up strategies, and prevention of this tumor that is the most common non-skin cancer in men and second-leading cause from cancer death.

Dr. Catalona’s Publications from 2006 to 2009


1. Is Statin Use Associated with Prostate Cancer Aggressiveness? Stacy Loeb*, Baltimore, MD; Donghui Kan, Brian T. Helfand, Sara N. Gashiri, Robert B. Nadler, William J. Catalona, Chicago, IL.

2. Prostate-Specific Antigen Velocity Risk Count for Prediction of Radical Prostatectomy Outcomes Stacy Loeb*, Baltimore, MD; Kimberly A. Roehl, St. Louis, MO; Robert B. Nadler, William J. Catalona, Chicago, IL.

3. Are Diabetes Mellitus and Chromosome 17q12 Genetic Variants Associated with Prostate Cancer Aggressiveness? Stacy Loeb*, Baltimore, MD; Brian T. Helfand, Donghui Kan, St. Louis, MO; William B. Isaacs, Chicago, MD; William J. Catalona, Chicago, IL.

4. Is PSA Velocity Useful for Prostate Cancer Detection or Prognostication in Men with a PSA > 10 ng/ml? Stacy Loeb*, Baltimore, MD; Kimberly A. Roehl, St. Louis, MO; William J. Catalona, Chicago, IL.

5. Does a Lower PSA Velocity Threshold Lead to the Detection of More Insignificant Cancers? Stacy Loeb*, Baltimore, MD; Brian T. Helfand, Chicago, IL; Kimberly A. Roehl, St. Louis, MO; Donghui Kan, Robert B. Nadler, William J. Catalona, Chicago, IL.

6. Genetic Prostate Cancer Risk Assessment: Common Variants in 9 Genomic Regions Are Associated with Cumulative Prostate Cancer Risk and Aggressive Disease Brian T. Helfand*, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, Angela J. Fought, William J. Catalona, Chicago, IL.

7. Genetic Variants May Explain Increased Risk of Other Cancers in Prostate Cancer Patients Brian T. Helfand*, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, Angela J. Fought, William J. Catalona, Chicago, IL.

8. Prostate Cancer Genetic Variants Can Help Identify Patients with Possibly “Insignificant” Prostate Cancer Brian T. Helfand*, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, Angela J. Fought, William J. Catalona, Chicago, IL.

9. Age- and Race-Specific Results of a Large Community Based Prostate Cancer Screening Study Kimberly A Roehl*, Saint Louis, MO; Angel Desai, William J Catalona, Chicago, IL.

10. Correlation of PSA to Cancer Volume in Prostate Glands of Different Sizes Saima Daudi*, Kimberly A Roehl, Saint Louis, MO; Stacy Loeb, Baltimore, MD; William J. Catalona, Chicago, IL.

11. Causes of Death in Patients with Screen-Detected Prostate Cancer Angel Desai*, Kimberly A Roehl, Brian K Suarez, Saint Louis, MO; William J Catalona, Chicago, IL.

12. Preoperative Leuprolide May Affect Potency Following Nerve-Sparing Radical Prostatectomy Brian T. Helfand, Dae-Yun Kim*, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, Chicago, IL; Kimberly A Roehl, Saint Louis, MO; William J Catalona, Chicago, IL.

13. Association of Prostate Cancer Genetic Risk Alleles with Prostate Biopsy Results. Donghui Kan*, Brian T Helfand, Chicago, IL; Stacy Loeb, Baltimore, MD; William J Catalona, Chicago, IL.

14. ProPSA is More Accurate:Prospective Prostate Cancer Screening Study Comparing [-2]-ProPSA with Free and Total PSA Brian V Le*, Christopher R Griffin, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, William J Catalona, Chicago, IL.

15. Reducing Blood Loss with Radical Retropubic Prostatectomy with Prophylactic Peri-Prostatic Sutures Christopher R Griffin*, Donghui Kan, Saima Daudi, Angel Desai, Chicago, IL; Stacy Loeb, Baltimore, MD; William J Catalona, Chicago, IL.

16. Trial of Empiric Antibiotics Prior to Recommending Biopsy for PSA Elevation Helps Stratify Prostate Cancer Risk Chris Griffin, Hannah H Alphs*, Angel Desai, Chicago, IL; Stacy Loeb, Baltimore, MD; Jessica T Casey, Saima Daudi, Donghui Kan, William J Catalona, Chicago, IL.

17. First-Degree Family History of Prostate Cancer and Carrier Status of Prostate Cancer Risk Alleles Joshua J Meeks*, Brian T Helfand, Chicago, IL; Stacy Loeb, Baltimore, MD; Donghui Kan, Angela J Fought, William J Catalona, Chicago, IL.

18. In Men with Rising PSA after Radical Prostatectomy, 8q24 Prostate Cancer Risk Alleles Help Predict Threatening Cancer Progression and Need for Salvage Therapy William J. Catalona*, Chicago, IL; Carol H Jin, Kimberly A Roehl, Saint Louis, MO; Stacy Loeb, Baltimore, MD; Brian T. Helfand, Jessica T Casey, Chicago, IL; Brian K Suarez, Saint Louis, MO.
Primary Myelofibrosis (PMF), Essential Thrombocythemia (ET), and Acute Megakaryocytic Leukemia (AMKL) are hematologic diseases that involve abnormal proliferation and/or differentiation of megakaryocytes (MKs), the cells that give rise to platelets. These diseases are related to one another in several respects. First, megakaryocytes are abnormal in all three disorders. For example, in AMKL, megakaryocyte progenitors show both a block in terminal differentiation and aberrant proliferation. The bone marrows of these patients harbor immature, dysplastic megakaryocytes and are frequently fibrotic. Similarly, PMF is associated with marked marrow fibrosis and the presence of large, dysplastic megakaryocytes. These MKs show an altered nuclear:cytoplasmic ratio and other morphological abnormalities. MKs in ET are also morphologically abnormal, with a more segmented nucleus that resembles those of neutrophils. Second, these three disorders share common alterations in JAK/STAT signaling. Mutations in \textit{JAK2} are found in nearly half of ET patients while \textit{JAK2} and \textit{c-MPL} mutations account for 30-50% and 10% of PMF cases respectively. AMKL blasts also have been found to harbor \textit{JAK2} and/or \textit{JAK3} mutations.

Finally, these diseases are difficult to manage and have poor prognoses. Although patients with ET can be treated with hydroxy and aspirin to decrease their platelet counts and reduce the risk of thrombosis, many patients become refractory to these therapies or develop PMF or
Similarly, our ability to cure PMF and AMKL is limited. In this report, I will describe some of our recent efforts to define the regulation of megakaryocyte growth and maturation and then discuss how these new insights will lead to the development of new therapies for megakaryocytic neoplasms.

Megakaryocyte Biology
Megakaryocytes arise from the Megakaryocyte-Erythroid Progenitor (MEP) and progress through discrete maturation stages (Fig 1). Committed megakaryocyte progenitors, including the colony-forming unit megakaryocyte (CFU-MK), proliferate to a limited extent, giving rise to megakaryoblasts. Individual cells then undergo terminal differentiation and eventually shed platelets. In concert with cytoplasmic maturation that leads to platelet production, megakaryocyte nuclei undergo a maturation process that involves repeated rounds of DNA synthesis without cell division, a variant cell cycle termed polyploidization, or endomitosis 1. This phenomenon allows megakaryocytes to accumulate DNA content up to 64N and greatly increases their size and protein production. These increases in cell size, DNA content, and protein levels are associated with the development of long cytoplasmic extensions, termed proplatelet forms, that eventually shed platelets. Although it has been well established that megakaryocytes normally become highly polyploid, the specific point at which endomitotic cell cycle diverges from the proliferative cell cycle is largely undefined. Moreover, the signaling pathways that govern this crucial switch remain unknown.

Insights into megakaryocyte maturation
My lab seeks to define the transcriptional network that controls megakaryocyte polyploidization, the changes in cell cycle machinery that accompany endomitosis, and the signaling cascades that regulate the commitment of precursors to polyploidization and differentiation.

One of the genes we study is GATA-1, a key regulator of megakaryocyte development. GATA1 is mutated in AMKL patients with Down syndrome and its expression is significantly downregulated in PMF 2,3. Mice with reduced GATA-1 expression in MKs (Gata1-low) develop a disease that resembles human PMF with myelofibrosis, extramedullary hematopoiesis, circulating CD34+ cells, poikilocytosis and anemia 4. In order to study how GATA-1 regulates the proliferation and differentiation of megakaryocytes, we performed a genome wide expression array comparison between wild-type and Gata1-low murine megakaryocytes 5. As part of our strategy to identify GATA-1 target genes in this lineage, we utilized Ingenuity Pathways software to define regulatory networks that were significantly affected by the loss of GATA-1. One of the prominent networks that we found to be affected included cyclin D1, GATA-2 and Ets1. In teasing apart this network we have made the following discoveries: 1) GATA-1 directly regulates the expression of cyclin D1 during MK maturation: We showed that cyclinD1 is a direct GATA-1 target gene that serves to increase the extent of polyploidization of MKs 6. Reduced expression of cyclinD1, as seen in Gata1-low cells, was associated with reduced DNA content and size. Overexpression of cyclinD1, in concert with its partner CDK4/6, rescued both cell growth and polyploidization of the Gata1-low cells. Chromatin immunoprecipitation (ChIP) assays
confirmed that the cyclinD1 promoter binds GATA-1 in megakaryocytes, but not erythroid cells, which do not undergo polyploidization. Taken together, our studies show that one way in which GATA-1 regulates megakaryocyte maturation is by promoting cyclin D1 expression, which in turn drives the endomitotic cell cycle. 2) Overexpression of ETS proteins greatly enhances megakaryopoiesis and also immortalizes hematopoietic progenitors: ETS2 and ERG, which are located on human chromosome 21, are promising oncogenes in AMKL. We found that overexpression of either gene immortalizes Gata1-low fetal liver hematopoietic progenitors and leads to activated JAK/STAT signaling7. 3) GATA-2 contributes to the excessive proliferation of GATA-1 mutant cells: By knocking down GATA-2 in primary megakaryocyte progenitors, we discovered that its expression is required for the expansion of Gata1-mutant fetal liver derived megakaryocytes (Z. Huang and JDC, submitted). These findings are consistent with the hypothesis that overexpression of GATA-2 directly contributes to AMKL.

In addition to the network described above, we noticed that Gata1-low megakaryocytes expressed reduced levels of the signaling molecule STAT1. This was interesting to us because, although many studies have addressed the requirements for STAT3 and STAT5 in megakaryopoiesis, few have examined a possible role for STAT1 in this lineage. After demonstrating that STAT1 is indeed expressed in MKs, we studied the effects of loss and gain of function on MK development. We found that ectopic expression of STAT1, or its downstream effector IRF1, rescued features of megakaryocyte maturation in primary Gata1-low cells and G1ME cells, a GATA-1 deficient megakaryocytic cell line that is dependent upon TPO8. These effects included induction of polyploidization and expression of a subset of MK-specific genes9. Furthermore, IFN-γ, through its activation of STAT1, induced polyploidization of both wild-type and Gata1-low megakaryocytes as well as G1ME cells. Importantly, we noted that the MKs in STAT1-deficient mice achieve a lower degree of polyploidization as compared to their wild-type littermates. Together, our data show that STAT1 contributes to MK polyploidization and maturation and identify a new regulatory hierarchy through which GATA-1 promotes megakaryopoiesis in part via activation of IFN-γ/STAT1 signaling.

Megakaryocytic Diseases

Disorders that are characterized by aberrant megakaryopoiesis include acute megakaryocytic leukemia (AMKL), essential thrombocytopenia (ET), and primary myelofibrosis (PMF).

Although rare, megakaryocytic leukemia is an aggressive and deadly form of cancer that, in general, does not respond to current treatment regimens10-12. AMKL affects three groups of patients: children with Down syndrome (DS), infants without Down syndrome, and adults. AMKL comprises approximately 5-7% of AML in children without DS and approximately 1% of adult AML (for review, see13. In the context of DS, however, megakaryocytic disorders are relatively common. Nearly 10% of newborns with DS show evidence of Transient Myeloproliferative Disorder (TMD), a disease that is characterized by a temporary expansion of abnormal megakaryocyte precursors14 (Fig. 2). Children with TMD are predisposed to developing AMKL by the age of 4.

Each of these AMKL subtypes has unique clinical and genetic features. i) TMD and DS-AMKL blasts harbor mutations in GATA1 that block expression of the full-length protein, but allow for expression of a shorter isoform named GATA-1s. We hypothesize that the combination of trisomy 21 and a GATA1 mutation contribute to initiation of the disease, but that additional genetic mutations are needed for the evolution to acute leukemia2. Although the current treatment regimen has resulted in
favorable outcomes for this group of patients, with an overall survival of 77% at 5 years\textsuperscript{15}, 10-20% of children with DS-AMKL die from this leukemia and/or from the toxicity of the treatment. ii) Many infant cases of non-DS AMKL are associated with the (1;22) translocation, which was initially discovered in 1991 and recently found to result in fusion of the RBM15 and MKL1 genes\textsuperscript{13}. In other cases of childhood non-DS AMKL, different cytogenetic abnormalities are observed, including t(10;11), t(9;11), +8 or +21. Of note, all groups of children with non-DS AMKL show significantly inferior overall survival and event free survival compared to children diagnosed with other myeloid leukemias (FAB M0-M5) or with DS-AMKL. iii) Much less is known about the etiology of adult AMKL, as no specific chromosomal rearrangements or genetic mutations have been described, apart from rare detection of mutations in JAK2 or JAK3\textsuperscript{13,16}. Although some patients achieve complete remission, the long-term outcome is significantly worse for AMKL than other forms of adult AML, with a median survival of 40 weeks or less\textsuperscript{11}. In summary, new therapeutics are desperately needed for this leukemia.

PMF and ET are myeloproliferative diseases that are characterized by an abnormal expansion of megakaryocytes. Due to high platelet counts, patients with ET are at significantly increased risk of thrombotic events. PMF patients suffer from cytopenias that result from bone marrow fibrosis and also show a risk of evolving to AML. Recent molecular studies have shown that JAK2 mutations are found in nearly half of ET patients, while JAK2 and c-MPL mutations account for 30-50% and 10% of PMF cases, respectively\textsuperscript{17}. This discovery has fueled the development of a new generation of JAK2 inhibitors, which will likely serve as targeted therapeutics for MPDs.

Development of targeted therapies for MK neoplasms

The paradigm for successful, targeted differentiation therapy is the use of all trans retinoic acid (ATRA) for treatment of Acute Promyelocytic Leukemia (APL). Prior to the development of ATRA therapy, the prognosis for patients with APL, which represents 5-10% of adult AML, was very poor. In contrast, the vast majority of APL patients treated with ATRA achieve hematologic remission\textsuperscript{18-20}. Given that megakaryocytes are programmed to undergo polyploidization as a normal event in their terminal maturation, we believe forced induction of polyploidization would serve as a “differentiation therapy” for AMKL. In collaboration with the Broad Institute and the Samuel Waxman Cancer Research Foundation, we have recently identified a collection of small molecules that induce differentiation of malignant megakaryocytes. We are working closely with colleagues in the Robert H. Lurie Comprehensive Cancer Center to test these new agents in animal models and patient specimens. We anticipate that our research will lead to new effective therapeutics for megakaryocytic neoplasms in the near future.

Acknowledgements:

I thank the many talented members of my laboratory for their hard work and dedication. I am very grateful for the support of the many outstanding clinical and basic science colleagues in the Robert H. Lurie Comprehensive Cancer Center. Special thanks to Drs. Martin Tallman, Jessica Altman, Ana Adriana Zakarija, Brandon McMahon, and Olga Frankfurt for assistance with obtaining clinical specimens and to our patients for their contributions to this research. Finally, I would also like to thank my many collaborators at Northwestern, including Drs. Jon Licht and William Miller.

References:


The incidence of melanoma is rapidly rising. Accurate diagnosis is based on histopathologic exam and while in the majority of cases this is straightforward, a significant number of biopsy specimens obtained for histopathologic examination to exclude melanoma may show ambiguous histopathologic features making it difficult to render a definitive diagnosis of benign or malignant. Over-diagnosis of melanoma can lead to inappropriate therapy and psychological burdens, while under-diagnosis can lead to inadequate treatment of a deadly cancer.

Previous studies with comparative genomic hybridization (CGH) show that the great majority of melanomas have recurrent clonal chromosomal copy number changes not seen in benign nevi. Using CGH data, our group has been involved in a collaborative study which has successfully developed a targeted fluorescence in situ hybridization (FISH) probe assay suitable for the analysis of paraffin-embedded tissue samples of melanocytic neoplasms. Using FISH data from a training set of 301 tumors, we established a discriminatory algorithm and validated it on an independent set of 169 unequivocal nevi and melanomas as well as 27 cases with ambiguous pathology, for which we had long-term follow-up data. An algorithm using signal counts from a combination of four probes targeting chromosome 6p25, 6 centromere, 6q23 and 11q13 provided the highest diagnostic discrimination. This algorithm correctly classified melanoma with 86.7% sensitivity and 95.4% specificity in the
validation cohort. The test also correctly identified as melanoma all six of six cases with ambiguous pathology that later metastasized. There was a significant difference in the metastasis free survival between test-positive and negative cases with ambiguous pathology (p=0.003). Further we have applied this assay to a number of problematic areas in melanocytic pathology such as the accurate diagnosis of nevoid melanomas, blue nevus like metastases, and lentiginous junctional melanoma of the elderly as well as microstaging in melanoma and have shown it to be of high utility in these problematic areas.

INTRODUCTION
The incidence of melanoma is rising at a rate of 3-7% per year for fair-skinned Caucasian populations, a faster rise than known for any other major cancer. The American Cancer Society (ACS) projects 62,480 new diagnoses of invasive melanoma and 54,020 in situ melanomas in 2008 the number of biopsies to rule out melanoma is estimated to range between one to two million per year or above in the United States alone. In most cases histopathologic evaluation alone can readily distinguish between malignant melanoma and benign nevi, however, there are a considerable number of cases which may show ambiguous histopathologic features making it difficult to render a definitive diagnosis. There are many types of melanocytic nevi that histopathologically simulate melanoma, and variants of melanoma that resemble nevi. Some specific problematic areas include distinguishing melanoma from spitz nevi or dysplastic nevi, diagnosis of nevoid melanomas, diagnosis of blue nevus like metastases, diagnosis of lentiginous junctional melanoma of the elderly and microstaging of melanoma. The current literature is rich with examples of the challenges put forth with such cases. In light of the medicolegal risks and potential loss of life with the mis-diagnosis of melanoma, there is an incentive for classifying ambiguous or borderline lesions as melanoma. However, over calling can also result in significant morbidity for the patient in the form of excessive surgery or adjuvant therapy as well as unnecessary psychologic stress. Recently our lab has been involved in the development of a fluorescence in situ hybridization assay and has studied this technique extensively for its ability to distinguish malignant melanoma from benign nevi and to assist in the diagnosis of these diagnostically challenging cases.

Development of the targeted 6p25, 6q23, Cep 6 and 11q13 FISH assay
The development of this assay originated from a combinatorial analysis of the existing CGH data set on melanoma which was developed from Dr Boris Bastian. This analysis yielded thirteen regions on eight different chromosomes, 1, 6, 7, 9, 10, 11, 17, and 20, which in combination yielded the best discriminatory ability between melanomas and nevi. FISH probes targeting these 13 regions were hybridized in panels of three to four probes, each probe within a panel labeled with a different spectrally distinct fluorophore to an extensive set of melanomas and nevi. The probe combinations with the highest discriminatory capability between melanoma and nevi included three probes targeting chromosome 6 at the short arm (6p25), centromere (CEP6), and long arm (6q23) in combination with a probe for chromosome 11q13. The validation of this probe set in our lab resulted in a sensitivity of 86.7% and specificity of 95.4% in distinguishing melanoma from nevi in a set of 82 melanomas and 86 nevi.

Clinical Utility
Ambiguous or Spitzoid Neoplasms
Additionally, we analyzed a series of cases that could not be reliably classified as nevus or melanoma by histopathology to determine the ability of the test to distinguish cases that later did or did not reveal their malignant potential by metastasis or death of disease. This cohort consisted of 27 histopathologically ambiguous tumors (Figure 1 and 2) in which most cases the differential diagnosis was spitz nevus versus spitzoid melanoma. In general this group consisted of patients with thicker tumors with

![Figure 1. Low power (40X) hematoxylin and eosin stained section of a melanocytic neoplasm with conflicting histopathologic features.](image-url)
12 of 27 patients having tumors with a Breslow depth > 2 mm, 12 of 27 with Breslow depth between 1 and 2 mm and just 3 patients with tumors less than 1 mm in Breslow depth. Six patients developed metastasis while the remaining 21 remained event free with a follow up period of at least five years in each case. The metastases were both regional or distant. Cases with only regional metastasis had to show effacement of the lymph node parenchyma. All six primary tumors (100%) that metastasized were positive by FISH (Figure 3). In addition, six of the 21 tumors (29%) that did not metastasize during the follow-up period were positive, suggesting that they were also melanomas that had either been cured by the removal of the primary tumor or were not followed long enough to detect metastasis (Figure 4). The disease-free survival times between FISH-positive and -negative was significantly different (p=0.003, Fisher’s Exact Test).8

These lesions lack a prominent epidermal component, significant pagetoid changes and destructive features of the epidermal-dermal contour commonly identified in more conventional melanomas. Additionally the dermal component is often well circumscribed, shows some degree of maturation, and lacks striking pleomorphism or high mitotic activity. Despite these banal features the outcome of nevoid melanomas has not been shown to be any better than that seen with other subtypes of melanomas with similar Breslow’s depth. Previous reviews of the literature have reported a mortality of 24% among 24 reported cases with at least 3 years follow up and average Breslow’s depth of 2.1 mm. This is also supported by the findings in our series in which 4/9 (44%) developed metastatic disease. Hence, as these patients not infrequently suffer fatal outcomes it is critical to accurately identify these cases. In our own studies we have shown that this FISH assay targeting 6p25, 6q23, Cep 6 and 11q13 was able to distinguish between 9 nevoid melanomas and 10 mitotically active nevi with a sensitivity and specificity of 100% showing tremendous potential utility in this diagnostic scenario.

Blue nevus-like metastasis
Blue-nevus like cutaneous melanoma metastasis is a well recognized variant of melanoma metastasis. These lesions may clinically and histologically simulate benign nevi. The histologic changes may be indistinguishable from epithelioid blue nevi (BN), a benign dermal based melanocytic neoplasm with epithelioid morphology and heavily pigmented...
cytoplasm. Distinguishing BN-like cutaneous melanoma metastasis and epithelioid BN is important for staging and treatment since a blue nevus-like metastasis is equivocal to stage III disease while and epithelioid blue nevus is a completely benign neoplasm. In a blinded analysis of 10 blue nevus-like metastases and 10 epithelioid blue nevi using the same targeted FISH assay, we were able to detect 9 of 10 blue nevus-like metastases while all 10 epithelioid blue nevi were negative.

Lentiginous Junctional Melanoma of the Elderly
Several authors have recently proposed the term lentiginous melanoma to describe a pattern of melanoma histologically characterized by prominent lentiginous single cell growth with focal and poorly cohesive nesting, suprabasilar migration of melanocytes and preservation of the rete ridges. These cases typically occur in elderly patients, commonly lack solar elastosis and have a prolonged in situ or radial growth phase but can eventually result in invasive and progressive melanoma. As features commonly associated with lentigo maligna are absent in these lesions, they are frequently mis-diagnosed as benign junctional nevi. FISH targeting the same set of chromosomal loci discussed above was able to identify 16 of 19 of these lesions in our own studies with no false positive results in the control set of lentiginous junctional nevi.

Microstaging
Approximately 30 to 50% of melanomas arise in association with a nevus. Accurately defining the nevus from the melanoma can significantly affect microstaging. We performed FISH on 36 cases of melanoma occurring in association with a nevus. In the melanomas with associated benign nevi, FISH enumeration was performed separately on the histologically malignant and benign components. Among melanomas with associated nevi, 28 of 36 cases (78%) tested positively in the histologically malignant areas. The benign nevus components were uniformly negative for all criteria. FISH analysis with probes targeting 6p25, 6q23, 11q13 and CEP6 can effectively discriminate the malignant and benign components of melanomas with associated nevi and be used as an adjunctive tool for microstaging. The assay has high sensitivity for the malignant areas of nevus associated melanomas and outstanding specificity for the benign areas.

In summary, our study demonstrates that FISH using a combination of probes for regions commonly affected by DNA copy number alterations in melanoma can distinguish between melanomas and nevi and assist in the diagnostic classification of melanocytic tumors that cannot be classified reliably by current methods. This test should be performed in conjunction with standard clinical and histopathologic evaluation.

References
1. ACS. melanoma. ACS . 7-27-2007. 3-23-2008. Ref Type: Internet Communication
Acute leukemia is the most common cancer in children less than 18 years of age.\textsuperscript{1} Advances in effective therapy have improved outcomes in recent years such that five-year survival rates now approach 87 percent for ALL and 54 percent for AML.\textsuperscript{1} However, the prognosis remains poor for children who experience relapses or who are refractory to frontline therapy.\textsuperscript{2,3} The selection of effective salvage treatments for relapsed leukemia is difficult because this patient population often has multidrug resistance and lower tolerance of treatment that results from prior chemotherapy. Induction of remission is the first critical step for successful salvage treatment. Increased intensity of modern frontline therapy may make retrieval therapy more challenging, as leukemic blast cells may become more resistant to chemotherapy than those treated less intensively. A new agent that has a novel mechanism of cytotoxicity and preferably less cross-resistance is urgently needed in this population to improve outcome and there are numerous agents under investigation.\textsuperscript{4}

**Clofarabine in childhood leukemia**

Clofarabine (2-chloro-9-[2-deoxy-2-fluoro-\beta-D-arabinofuranosyl]adenine) is a second-generation deoxyadenosine analogue synthesized to overcome the limitations but retain the favorable properties of fludarabine and cladribine.\textsuperscript{5} (Figure 1) It is one of the most promising new agents in the treatment of childhood leukemia. In both phase I and phase II studies, single agent clofarabine has been shown to be effective in inducing remission at
well-tolerated doses in pediatric patients with multiple relapsed or refractory leukemia.\textsuperscript{6,7} In a phase II study of clofarabine in pediatric patients with multiple relapsed or refractory ALL, 20\% of patients achieved a remission (complete remission [CR] + CR in the absence of total platelet recovery [CRp]) and remissions were observed in patients who were refractory to standard salvage treatments.\textsuperscript{7}

Given the encouraging single agent activity and acceptable safety profile of clofarabine, a mechanism-based combination with cyclophosphamide and etoposide was evaluated. In vitro, biochemical synergy has been demonstrated between the triphosphate form of clofarabine which inhibits DNA polymerases and ribonucleotide reductase, and the alkylating agent cyclophosphamide.\textsuperscript{8} Cells pretreated with clofarabine impeded repair of cyclophosphamide-induced DNA damage, which in turn resulted in a more than additive apoptotic cell death.\textsuperscript{9} Furthermore, in clinical studies, etoposide, another DNA-damaging agent, has been shown to have anti-leukemic activity when used in combination with cyclophosphamide and has been incorporated in many protocols for high risk or relapsed ALL.\textsuperscript{10}

In CLO21800205 study (A Multi-Center Phase I/II Study of Clofarabine in Combination with Etoposide and Cyclophosphamide in Pediatric Patients with Refractory or Relapsed Acute Leukemia),\textsuperscript{11} patients between 2 and 21 years of age with refractory or relapsed acute lymphoblastic leukemia (ALL) or acute myelogenous leukemia (AML) (Phase I portion only) were enrolled. (Table 1) Five dosing cohorts evaluated escalating doses of clofarabine 20-40 mg/m\textsuperscript{2} /day, etoposide 75-100 mg/m\textsuperscript{2} /day and cyclophosphamide 340-440 mg/m\textsuperscript{2} /day. All 3 study drugs were administered via IV infusion daily for 5 consecutive days in induction and 4 consecutive days in consolidation. Patients received up to 2 induction cycles, followed by consolidation (up to a maximum of 8 cycles in total). Dose-limiting toxicities in the phase I portion have been previously reported.\textsuperscript{6,7} The recommended phase II doses were clofarabine 40 mg/m\textsuperscript{2} /day, cyclophosphamide 440 mg/m\textsuperscript{2} /day, and etoposide 100 mg/m\textsuperscript{2} /day.

Twenty-five patients (ALL: 20 patients; AML: 5 patients) were enrolled in the 5 phase I dose cohorts. The median number of prior induction regimens was 2, 7 patients (5 ALL, 2 AML) were refractory to their immediately preceding regimen, and 4 patients (1 ALL, 3 AML) had a prior hematopoietic stem cell transplant (HSCT). Data showed complete remission (CR) in 10 patients (9 ALL, 1 AML) and complete remission without platelet recovery (CRp) in 6 patients (2 ALL, 4 AML) for an overall response rate of 64\% (ALL: 55\%; AML: 100\%). Of the 16 responders (CR + CRp), 9 patients proceeded to HSCT after treatment. The median duration of remission (censored at the last known date of follow-up regardless of alternative therapy) for the 16 responders was 18.2 weeks (range 6.6 to 105.1+ weeks) (ALL: median 22.6 weeks, range 6.6 to 105.1+ weeks; AML: median 15.6 weeks, range 7.3 to 61.0+ weeks), including 27.0 weeks for the 10 patients with CR and 15.3 weeks for the 6 patients with CRp. At the last known date of follow-up, 5 of the 16 responders were alive. Of these 5 patients, 1 patient remained in CR and 1 remained in CRp (both patients had undergone post-therapy HSCT). One patient

---

**Figure 1**

![Fludarabine](image1.png) ![Cladribine](image2.png) ![Clofarabine](image3.png)
with ALL completed 8 cycles of therapy, with a duration of remission of 61.4 weeks.

In phase II, 3 of the first 8 ALL patients enrolled achieved a response (1 CR, 2 CRp). However, 4 patients developed severe hepatotoxicity. The study was amended to exclude patients with a history of prior HSCT, viral hepatitis and/or cirrhosis, or elevated conjugated bilirubin levels. Eight additional patients have since been enrolled in the amended phase II portion of the study and no cases of severe hepatotoxicity have been observed to date.

This highly encouraging result in ALL and AML prompted us to study this regimen in larger cohorts.

Other clofarabine studies
The combination of clofarabine and cytarabine has been tested in adult studies.\textsuperscript{12} Clofarabine is a potent inhibitor of ribonucleotide reductase. Inhibition of ribonucleotide reductase causes decrease of deoxynucleotides, which leads to a subsequent feedback inhibition of deoxycytidine kinase. Therefore, clofarabine modulates accumulation of intracellular ara-CTP, which is the biologically active form of cytarabine.\textsuperscript{13} This combination is currently under study in the pediatric population to determine the optimal dose and efficacy.

Clofarabine has enhanced oral bioavailability compared to other adenosine nucleoside analogues. This is due to stability in gastric acid due to substitution of a fluorine at the C-2’ position of the arabinofuranosyl moiety of

<table>
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<th>Total (N=25)</th>
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Table 1

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<tr>
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</tr>
<tr>
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<td>-</td>
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Table 2: CR = Complete Remission; CRp = Complete Remission in the absence of total platelet recovery; PR = Partial Remission; SD = Stable Disease; the patient fails to qualify for either CR, CRp, PR, or PD; PD = Progressive Disease; an increase of at least 25% of the absolute number of bone marrow or circulating leukemic blasts, development of extramedullary disease, or other laboratory or clinical evidence of progression; NE = Not Evaluable. *Patient died before bone marrow recovery.
clofarabine.\textsuperscript{14} Oral clofarabine is being tested in adult patients with MDS. \textsuperscript{15}

Summary
Treatmen tof relapsed leukemia in children is challenging. The combination of clofarabine, cyclophosphamide and etoposide induced durable remission in children with relapsed or refractory acute leukemia. This promising combination is now in phase II portion and will be also studied in a randomized fashion.

References

The Journal of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Vol. XIII
My laboratory focuses on the study of the cell division cycle in normal and cancer cells. The overall goal of our work is to define how cancer cells exploit mechanisms that regulate normal cell division in order to survive and proliferate. Normal cell cycle phase transitions are carefully regulated by multiple, partially redundant mechanisms; however, loss of function of several key tumor suppressor pathways that regulate cell cycle progression occurs in virtually all cancer cells. In our research, we focus in particular on the genes and proteins that control the cell’s decision to replicate its genome during the G1-S phase transition (Figure 1). Cancer cells nearly uniformly have lost the regulatory controls that normally govern this transition, which are components of the Retinoblastoma (Rb) and p53 tumor suppressor pathways. In our studies, we are dissecting some of the molecular mechanisms within the Rb and p53 pathways that we believe are critically important for the emergence of cancer, and our major interests currently center on the functions and regulation of cyclin E.

The Fbw7 ubiquitin ligase – a multi-tasking tumor suppressor
Cyclin E, which positively regulates S-phase entry, is frequently over-expressed in cancer cells. Cyclin E overexpression in tumors can occur due to increased gene expression or impairment of its normal degradation, which is controlled by the SCF\textsuperscript{Fbw7} ubiquitin ligase. Fbw7 (also known as hCdc4) is a tumor
suppressor protein that is frequently mutated in solid and hematopoietic tumors, and these mutations impair its ability to bind substrates, including cyclin E, and thus its ability to catalyze their ubiquitination\textsuperscript{2-6}. Within the multi-protein SCF complex, Fbw7 conveys substrate specificity by interacting directly with phosphorylated substrates, bringing them in contact with the rest of the ubiquitination machinery\textsuperscript{2, 6-12}. The most frequently occurring cancer-associated mutations in Fbw7 occur at the substrate-contacting arginine residues within the WD40 domain, and other commonly occurring mutations produce deletions that truncate Fbw7 before or within this region\textsuperscript{13-15}. Fbw7 targets other proteins for ubiquitination and degradation that are important in cancers, in addition to cyclin E, including c-Myc, c-Jun, and Notch\textsuperscript{10, 11, 22, 23}. Moreover, c-Myc, Notch1, and c-Jun are directly targeted at their CPDs by cancer-associated mutations, consistent with the notion that impaired Fbw7-mediated degradation of these substrates is positively selected for during tumorigenesis\textsuperscript{10, 24-26}. In contrast, cyclin E, which is the only currently known Fbw7 substrate containing dual CPDs, is not directly targeted for mutations in cancers, perhaps because two independent events would be required to disable both degrons while preserving its intervening functional domains.

Currently, it is unknown to what extent each Fbw7 substrate contributes to the development of a particular tumor type in which Fbw7-loss occurs. In ongoing work, we are attempting to “deconvolute” the combinations of Fbw7 substrates that are critically dysregulated downstream of Fbw7-loss in animal models of tumorigenesis. In addition to inactivating Fbw7 mutations in cancers, hyper-activation of signal transduction pathways that negatively regulate GSK-3, among these, the Ras/mitogen-activated protein kinase (MAPK), Wnt, and PI-3 kinase/AKT pathways, can potentially decrease Fbw7-mediated ubiquitination of multiple oncoprotein substrates. For example, loss-of-function mutations in the PTEN tumor suppressor, frequently occurring in cancers, including in T-ALL, can decrease GSK-3 activity by up-regulating AKT-mediated inactivation of GSK-3\textsuperscript{15, 27}. The extent to which impaired Fbw7 function is important to
tumorigenesis associated with hyper-activation of these oncogenic signaling pathways has yet to be experimentally determined.

**Cyclin E in cancers – driving cell proliferation and genomic instability**

Cyclin E appears to play a critical role during cell transformation, as cyclin E-null mouse fibroblasts are resistant to Ras-mediated transformation. Chromosome instability is a hallmark feature of inappropriately high cyclin E expression in cells and is thought to be a major way in which cyclin E exerts oncogenic activity in vivo (Figure 2 and refs. 29-33). Cyclin E-associated genome damage manifests as chromosome instability, and this may occur both from the production of unstable replication fork structures and from defective chromosome segregation resulting from aberrant pro-metaphase. Cyclin E-induced genome instability is dependent on its kinase activity and is greatly exacerbated by loss of p53. In fact, p53 and its downstream target, p21, represent a major physiologic barrier against deregulated cyclin E activity in primary cells. Aside from its link to genome instability, how cyclin E overexpression promotes cell transformation and cancer remains unclear. Does cyclin E overexpression in cancer cells signify particular biological properties or does it more often only indicate loss of Rb pathway control? Is p53-loss absolutely required for deregulated cyclin E to promote tumors? What are other collaborating events in cyclin E-associated tumorigenesis? These are key questions we are addressing in my laboratory.

In addition to its role in promoting genome instability, we have found that cyclin E, when deregulated, is able to promote cellular hyper-proliferation in vivo. Specific cell lineages seem to be especially vulnerable to the consequences of deregulated cyclin E activity. Using a novel mouse knock-in model to study the physiologic consequences of impaired Fbw7-mediated cyclin E degradation, we found that deregulated cyclin E produces multiple abnormalities in erythroid progenitors, including greatly increased proliferation, impaired maturation, increased apoptosis, and dysplastic morphologies (Figure 3 and ref. 36). We see similar evidence of increased proliferation, counter-balanced by increased apoptosis, in mammary epithelial cells as well. Currently, we are elucidating the mechanisms by which impaired Fbw7-mediated cyclin E degradation promotes defective erythroid maturation in vivo. A long-term goal of our research is to determine whether these mechanisms are involved in the pathogenesis of human hematopoietic diseases, such as myelodysplasia and leukemia.

**Defective cell cycle control as a potential Achilles’ heel in cancer cells?**

In our studies of the responses to impaired Fbw7-mediated cyclin E degradation in vivo, we have learned that cells of different lineages utilize distinct homeostatic mechanisms to counteract the deleterious consequences of unchecked cyclin E activity. In epithelial cells of...
cyclin ET74A T393A tissues we found increased apoptosis, which may prevent hyperplastic lesions comprised of genomically unstable cells from developing36. Deregulated cyclin E also has been shown to induce cellular senescence in vivo, and in tumors the mechanisms that establish and maintain oncogene-induced senescence appear to be circumvented34, 37, 38. A long-term goal of our laboratory is to develop a better understanding of how cancer cells adapt to these and other mechanisms that normally halt unscheduled DNA replication and cell division in response to oncogenic signaling.

We hypothesize that high cyclin E expression may provide a point of vulnerability that can be exploited in some cancer cells. In tumors in which cyclin E is highly expressed, it would be especially advantageous to understand the mechanisms that permit cell survival and proliferation, since disabling these mechanisms may restore sensitivity to homeostatic responses that promote senescence or apoptosis in response to high cyclin E. One linchpin for survival in cancer cells expressing high amounts of cyclin E may be p21Cip1. A major target of p53 in its induction of cell cycle arrest in response to oncogenic or genotoxic stress, p21

Figure 3: Dysplastic erythroid cell morphologies in cyclin ET74A T393A mice. Erythroid precursors from cyclin ET74A T393A mice express inappropriately high cyclin E protein and enzymatic activity levels, are hyper-proliferative, and show increased apoptosis. A number of morphologic abnormalities are also present, including abnormally large nuclear size in cells with hemoglobinized cytoplasm (nuclear/cytoplasmic dysynchrony, as shown above in 100x micrographs of Wright-Giemsa stained cyclin ET74A T393A cells, compared to wild-type cells), frequent nuclear buds with extracellular and intracellular nuclear lobes, and many mitotic figures. Pointers indicate erythroid cells.

Figure 4: Apoptosis in cyclin E over-expressing cells with reduced p21Cip1 levels. Wi-38 (normal, human diploid fibroblasts) cells were transduced with a retroviral construct expressing a small hairpin targeting p21 expression. Cells were then transduced with control or cyclin E(T380A)-expressing retroviruses, then fixed and DAPI stained. Nuclei appear normal in the sh-p21 cells expressing the control vector. In contrast, those expressing cyclin E-T380A showed frequent nuclear blebbing (top of field) and chromatin fragmentation (bottom of field, indicated by pointers). Additionally, cell proliferation was rapidly halted in the sh-p21, cyclin E-expressing cells and apoptosis in these was confirmed by identification of PARP-cleavage35. A major target of p53 in its induction of cell cycle arrest in response to oncogenic or genotoxic stress, p21

Primary human fibroblasts, p21 reduced by small hairpin RNA
is also induced by a number of p53-independent mechanisms. Further, p21 appears to have CDK-independent functions, and among these, is the buffering of pro-apoptotic signaling. We previously found that p21 is a critical safeguard against cyclin E-induced apoptosis in primary fibroblasts. Of note, in control primary cells, p21 reduction has no appreciable negative impact on survival and proliferation, but in the presence of ectopic cyclin E, p21 reduction lead to profound, rapid cell death. In these cells, cyclin E-induced apoptosis almost certainly is due to p53 activation, and it is known that p53 activation can result in dichotomous cellular responses (cell cycle arrest vs. apoptosis), with the net outcome dependent on the level of p53-dependent p21 expression. However, p21 may promote cell survival even in the absence of p53, by mitigating p53-independent apoptotic signaling. In ongoing work, we are learning the specific cellular contexts in which p21 promotes cell survival in the setting of deregulated cell cycle controls and the precise mechanisms through which p21 exerts anti-apoptotic activity in different cell types.

We expect that determining how tumor cells circumvent the various checkpoints against unrestricted cell proliferation will be a daunting task because divergent adaptive mechanisms to oncogenic dysregulation of cell cycle controls are likely utilized in different tumor cell types. Thus, in our work we wish to develop not only a detailed understanding of the responses to specific cell cycle perturbations (e.g. impaired Fbw7-mediated ubiquitination) but also practical approaches to probing and exploiting underlying vulnerabilities in cancer cells when much more complex aberrations (combinations of oncogene gain-of-function and tumor suppressor loss-of-function mutations) are present. The latter approach will involve synthetic lethality and chemical genomics methods to identify new (likely unexpected and unpredictable) targets, which will hopefully point to effective therapeutic strategies with improved specificity for cancer cells, compared to most existing anti-cancer treatments.

References


There is strong epidemiologic evidence that obesity increases breast cancer risk\(^1\)\(^-\)\(^3\). In addition, several studies have shown that women undergoing treatment for breast cancer develop significant weight gain\(^3\)\(^-\)\(^6\). This weight gain is associated with relatively poor prognosis and decreased response to chemotherapy\(^7\)\(^-\)\(^9\).

Several factors can affect weight gain in women being diagnosed with breast cancer such as chemotherapy, use of steroids and tamoxifen. However, to date there is very little evidence on the exact role of chemotherapy in weight gain. Furthermore, given the recent discovery through genome-wide association (GWA) studies, of obesity-related genes, there is an opportunity to study whether weight gain in newly diagnosed breast cancer patients is related to their genetic background.

Adiponectin, a protein secreted by the adipose tissue, has been found to be an endogenous insulin sensitizer, the circulating levels of which are lower in obese and diabetic subjects than in normal weight subjects. Recently, circulating levels of adiponectin, have been found to be associated with breast cancer risk\(^10\)\(^-\)\(^12\). Since its levels are inversely correlated with BMI it has been suggested that decreased levels of adiponectin may explain the increased risk of breast cancer in obesity\(^13\)\(^,\)\(^14\). In fact, several groups have shown that, after adjustment for BMI, women with higher adiponectin levels had a 65% lower risk for breast cancer compared to women with lower levels\(^10\)\(^,\)\(^11\)\(^,\)\(^15\). Furthermore, exposure of T47D breast cancer
The proliferation of cells to adiponectin significantly inhibited their proliferation\(^{10,15}\). ADIPOQ, the gene encoding for adiponectin, contains several functional SNPs. These functional SNPs have been associated with several conditions including insulin resistance, type II diabetes and coronary artery disease\(^{13,16-20}\). For example rs2241766*TT (45*TT) and rs1501299*GG (276*GG) are associated with increased risk for insulin resistance and type 2 diabetes\(^ {16,18}\), as well as early onset coronary artery disease\(^ {18}\).

Through a case control study we evaluated the associations of ten haplotype tagging SNPs of ADIPOQ and ADIPOR1 with breast cancer risk\(^ {21}\). A total of 733 cases and 839 controls were included in the analysis. As shown in table 1 below, three SNPs were significantly associated with breast cancer risk. Interestingly rs2241766 and rs1501299 are functional SNPs associated with obesity, risk of insulin resistance, hypertension and cardiovascular disease\(^ {16,18}\). Rs2232853, a haplotype tagging SNP of ADIPOR1 has also been associated with risk for diabetes and cardiovascular disease. The T allele, which in our study was associated with lower breast cancer risk, has been associated with high mRNA levels. In the final analysis we divided individuals based on their genotyping combination of rs2241766 and rs1501299 into high, intermediate and low signalers and found that compared to low signalers, intermediate (OR: 0.64) and high signalers (OR: 0.15) had a significantly lower risk for breast cancer (p for

<table>
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Table 1: Crude and adjusted ORs (95% CI) of breast cancer by ADIPOQ and ADIPOR1 SNP genotypes. *p<0.05, **p<0.01, ***p<0.001 without Bonferroni adjustment and *p<0.10, **p<0.05, ***p<0.005 with Bonferroni adjustment. †age adjustment was done by using categorical age groups (age >50 and age <50). ‡adjustment for SNPs was only done with SNPs from the same gene.
These results suggest a significant role of polymorphisms of the adiponectin pathway for predicting breast cancer risk. These data suggest that the link between obesity, diabetes and breast cancer is adiponectin. Furthermore, several functional adiponectin SNPs may significantly alter a woman’s risk for breast cancer.

Having found a potential link between obesity and breast cancer we are currently moving toward identifying risk factors, both genetic and treatment-related which may contribute to weight gain in newly diagnosed breast cancer patients. The fat mass and obesity associated gene, FTO, was recently identified from several GWAS studies to be implicated in both juvenile and adult-onset obesity. Although the function of FTO is largely unknown it is expressed in the hypothalamus and influences appetite. Several SNPs of FTO have been associated with obesity. More specifically rs9939609*AA has been shown to be associated with increased BMI. Other SNPs in the FTO gene, such as the rs1477196*C allele have also been associated with changes in BMI.

Our ongoing clinical study is enrolling women with a recent diagnosis of breast cancer and following them for at least two years. Women will have frequent weight assessments and will complete detailed questionnaires on lifestyle factors. All participants will have genetic testing for adiponectin and FTO. The aim of this study is to identify a group of women who are most likely to gain weight after breast cancer diagnosis. Given the association of adiponectin and FTO with obesity and breast cancer we consider it likely that we will find an association between the functional polymorphisms of the adiponectin pathway and FTO and weight gain in breast cancer patients. This will allow us to identify the group of women most likely to gain weight and in the future offer them dietary modifications and physical activity programs to prevent the weight gain and hopefully improve their overall prognosis and quality of life.

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Herpesviruses are ubiquitous infectious agents, having over 200 natural hosts encompassing all species. Pathological signs of herpesvirus infection in humans include cold sores, chicken pox and shingles. Epstein-Barr Virus (EBV) is one of two known oncogenic human herpesviruses.

The Oncogenic Herpesvirus EBV Infects Epithelial and B cells

EBV is transmitted in saliva and first encounters the epithelium of the oral pharynx where it can infect tonsillar epithelial cells (25, 34). Malignancies of epithelial origin such as nasopharyngeal carcinoma are associated with EBV infection. EBV in saliva or produced in epithelial cells can encounter B cells in the oral cavity. Transfer occurs between the two cell types and may be essential for virus propagation and transmission in vivo (28). EBV infection of adolescents often results in symptoms of infectious mononucleosis. By adulthood, upwards of 90% of the population is EBV-positive. The B cell population serves as a reservoir for the virus in infected individuals, where virus persists in a dormant, non-replicative state termed latency. Viral latency proteins promote aberrant proliferation of infected B cells (33). EBV infection of B cells is implicated in development of endemic Burkitt’s lymphoma and is associated with other B cell malignancies including Hodgkin’s lymphoma and post-transplant lymphoproliferative disorders (PTLD) (reviewed in (40)).

This review highlights new findings about the
function of EBV entry glycoproteins during infection.

EBV Utilizes Cell Surface Receptors To Infect Target Cells

Herpesviruses infect a wide variety of cells, including neurons, epithelial and lymphoid cells. Proteins on the exterior membrane of the virus, the envelope glycoproteins, interact with cell surface molecules leading to infection. Thus, tropism of the virus is determined by cell surface components. After interaction with the cell surface, infection occurs through fusion of the viral envelope with the cell membrane, leading to release of the viral genome into the cytoplasm. The process of virus attachment, receptor engagement and fusion is termed entry.

For herpesviruses, including EBV, infection of target cells is especially complex and requires the coordinated effort of many viral glycoproteins. Components used for attachment differ from those that engage entry receptors. EBV utilizes distinct glycoprotein complexes to enter the main target cell types of infection, epithelial and B cells (37). EBV entry complexes are illustrated in Figure 1.

During the initial step of entry, attachment, the viral glycoprotein gp350 binds to CD21 on epithelial or B cell surfaces (35) (Figure 1A and 1B). This interaction increases the efficiency of infection but is not required. EBV also infects epithelial cells that do not express CD21; the viral protein BMRF2 can substitute for gp350 and facilitate attachment via interaction with β1 integrins on CD21-negative cells (12, 39) (Figure 1A). The minimal viral requirements to elicit epithelial fusion are glycoprotein B (gB) and the heterodimeric complex of glycoprotein H (gH)/glycoprotein L (gL) (21). After attachment, interactions occur between EBV and co-receptors on the target cell surface. gH/gL can interact with unidentified secondary receptors found on the epithelial cell surface (22) (Figure 1A). Even though no cell surface receptors for EBV gB have been identified, the possibility of receptor engagement by gB during infection cannot be ruled out (Figure 1A).

For B cells, the minimal viral requirements for fusion are gB, gH/gL and glycoprotein 42 (gp42) (7, 21) (Figure 1B). The gp42 interaction with human leukocyte antigen (HLA) class II on the B cell surface is essential for infection (6, 19, 36) (Figure 1B). A stick figure illustration of gp42 is shown in Figure 2. gp42 does not possess characteristics of a fusion protein, but extensive study of functional domains indicates how this glycoprotein facilitates the fusion process. gp42 must be cleaved at an internal site to function in fusion.
Soluble gp42 encompassing residues 33-223 was crystallized and used to identify residues that interact with HLA class II (23). The C-terminal C-type lectin domain (CTLD) contains the discontinuous HLA class II binding domain, which was confirmed by mutagenesis (Figure 2) (23, 29). A hydrophobic pocket was identified upon crystallization with Class II that may bind an unidentified ligand. Linker insertion mutations in the pocket region abolished fusion activity with B cells but did not impact binding to HLA class II (29). Mutagenesis studies in concert with crystallographic analysis provide additional insight into how gB, gH/gL and gp42 cooperate during viral entry.

EBV Glycoprotein Interactions Are Required for Fusion

EBV gH/gL is a heterodimeric complex expressed on the cell surface that is comprised of the membrane-bound gH and its chaperone gL. In the absence of gL, gH remains cytoplasmic; in the absence of gH it is unclear if gL is secreted or retained at the plasma membrane (18, 20). The gH/gL complex is required for fusion with B cells and epithelial cells.

gp42 binds to gH and the gp42/gH/gL complex exhibits 1:1 stoichiometry (15, 24). This interaction is also essential for fusion to occur, gp42 that is unable to bind gH/gL (and vice versa) is non-functional (14, 24, 29). Regions involved in gp42 binding to HLA class II and gH/gL are mutually exclusive (Figure 2). Two gH/gL binding regions in gp42 have been identified as residues 47-61 and 67-81 (Figure 2) (14). Structural analysis suggests that the gp42 hydrophobic pocket “opens” after binding to HLA class II; this opening is caused by the shift of a loop that borders the pocket (Figure 2) (16, 23). gH/gL is proposed as a possible ligand for this region, and movement of the loop may allow for alterations in the interaction between gH/gL and gp42 during fusion (16). Mutagenesis of loop residue 158 (which rotates upon receptor engagement to yield the more open conformation) demonstrated that this particular amino acid is not critical for fusion or receptor binding (16). However, mutation of other residues in the loop prevented Class II binding and fusion (16). The functional significance of this region is being further explored.

Recent work performed in the Longnecker laboratory suggests that gL not only transports gH to the cell surface, it plays an active role in the fusion process with B cells. Chimeric proteins were generated between EBV gL and the related, highly homologous rhesus lymphocryptovirus (Rh-LCV) gL. This approach was taken based on the observation that fusion did not occur with B cells when Rh-LCV gL was substituted for EBV gL with EBV gH, gp42 and gB in a cell-based fusion assay (24, 26). The fusion defect was not a result of abrogated glycoprotein interactions; Rh-LCV gL bound gH and the heterodimeric complex associated with gp42 (24, 26). Site-specific mutagenesis showed that residues 54 and 94 of
EBV gL restored Rh-LCV gL function in fusion with B cells (26). Of note, Rh-LCV gL and Rh-LCV gB function in fusion with EBV gH and gp42 at levels well above background (26). Taken together, this suggests that gL influences recruitment of gB to the B cell fusion machinery and may trigger gB structural rearrangement during fusion.

Structural Rearrangement of EBV gB Contributes to Fusion

Membrane fusion is thermodynamically unfavorable and bending of the two opposing membranes requires considerable energy; viral fusion proteins undergo structural conformation changes to accomplish this. Structural rearrangement can be triggered by proteolytic cleavage (ex. influenza hemagglutinin), receptor engagement (ex. HIV gp120/gp41) and decreases in endosomal pH (ex. Vesicular Stomatitis virus G (VSV G)) (9, 38). In all situations, movement of the fusion protein brings the viral and cellular membrane into close proximity, which allows for pore formation and uptake of the viral genome.

The structures of Herpes Simplex virus gB and EBV gB were recently resolved; gB shares properties with Class III viral fusion proteins which implies that it may function as the herpesvirus fusogen (reviewed in both (2, 10)) (5, 11) (Figure 3).

gB is modified by addition of N-linked glycans (17, 27). The maturation of these side chains from high mannose to complex linkages is an indicator of proper protein folding, this in turn is a critical determinant of gB function (27). Further processing of EBV gB occurs in the form of proteolytic cleavage. gB is detected in full-length and cleaved forms in the virion and in transfected cells (4, 13). An initial hypothesis proposed that cleavage exposes hydrophobic fusion peptides that could insert into the target membrane to initiate fusion, much like that seen for influenza hemagglutinin. However, while enzymatic digestion of gB occurs at an internal furin cleavage site, cleavage is not essential for fusion to occur (4, 31). pH changes are also not required for EBV fusion, and as yet no receptor for gB has been identified on either epithelial or B cell surfaces. Significant support for gB as the herpesvirus fusogen comes from mutagenesis studies of the proposed fusion peptides. These residues, which form bipartite loop structures, are highly hydrophobic in EBV gB (3). Swapping of these residues with the less hydrophobic fusion loop residues of HSV-1 gB completely abolished fusion with both target cell types (3). In HSV-1 gB, these loops and surrounding residues can interact with lipid membranes, this remains to

Figure 3: Structural Rearrangement Undergone by the Proposed EBV Fusogen. The predicted pre-fusion structure of EBV gB is shown to the left, with the crystallized post-fusion structure shown to the right (pre-fusion structure courtesy of Marija Backovic, post-fusion structure PDB ID: 3fvc). Triggering of conformational change may occur after interaction with another viral protein or cell surface receptor engagement. A dramatic rearrangement is undergone which positions the fusion loops close to the C-terminal portion of the ectodomain and the transmembrane region. This structural transition serves to bring the opposing cellular and viral membranes into close proximity, leading to membrane fusion.
be seen with EBV gB (8).

Based on the known pre- and post-fusion structures of the Class III viral fusion protein VSV G, a model of EBV gB in the pre-fusion form was generated (5) (Figure 3). The resolved crystal structure is thought to be in a post-fusion conformation (5) (Figure 3). Since none of the other classical triggering mechanisms used by other fusion proteins seem essential for gB function, the Longnecker laboratory and others have hypothesized that interaction with another glycoprotein prompts structural rearrangement (reviewed for Herpesviruses in (10)).

Future Directions
EBV glycoprotein interactions and structural rearrangement are vital for infection. Current models of EBV entry are predicated on what is known for HSV-1, suggesting sequential involvement of entry glycoproteins with gB as the fusogen (32). In addition, HSV-1 gB is known to interact with gH/gL after receptor engagement (1). Further understanding of EBV entry awaits confirmation of interaction between gB and gH/gL, as well as a structure of gH/gL. Visualization of the alterations undergone by this critical intermediary will provide insight into mechanisms of triggering gB, as well as how gp42 may translate signals leading to likely gH/gL alteration. In addition, gp42 small molecule inhibitor studies may yield valuable information about how interaction between glycoproteins influences infection.

Acknowledgements
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Brain tumors are presently the leading cause of cancer death in children under the age of 20, only recently surpassing acute lymphoblastic leukemia (ALL). In addition, they are the third leading cause of cancer death in young adults ages 20-39 (1). They are phenotypically and genotypically diverse, with over 120 different types of brain tumors currently classified. Glioblastomas represent 23% of all primary brain tumors, rapidly approaching the incidence of meningiomas, which are the most common primary brain tumor and represent 26% of all primary brain tumors (2). Glioblastoma multiforme is nearly uniformly fatal, with median survival between 9 and 12 months from initial diagnosis.

Glycolbiology and Brain Tumors
There are many cell-type specific, developmentally regulated and tumor-type specific cell-surface proteins and lipids containing covalently-linked carbohydrates (glycoconjugates). These glycoconjugates play a key role in regulating cell-cell recognition, adhesion, and migration, and include molecules such as integrins, selectins, cell-adhesion molecules (CAMs) and cadherins. They can modulate critical processes such as growth factor receptor function, intracellular protein trafficking, protease activity and secretion.

It is now clear that virtually all types of human cancers have altered patterns of glycosylation and express aberrant glycosyl moieties on their cell-surface glycoconjugates. Moreover, a significant number of studies have shown that
these carbohydrates play key functional roles in oncogenic transformation, tumor progression and metastasis [reviewed in (3)]. Early descriptive work by Traylor and Hogan (4), examining a series of human glioblastomas, showed that total ganglioside concentration was reduced in tumors and that there was an increase in simpler ganglioside structures and a reduction in complex, polysialogangliosides. For example Yates and co-workers (5) have shown that GD1b ganglioside correlated with tumor grade in astrocytomas. And a series of reports by Ladisch and co-workers (6) and Nakamura et.al (7) has shown that human neuroblastomas, medulloblastomas and astrocytomas shed gangliosides that can be detected in patient serum and cerebrospinal fluid. Li et al. (8) showed that shed ganglioside GD2 was markedly immunosuppressive and as such may facilitate tumor formation and progression. Cell-surface glycoproteins, too, have been identified that are associated with the invasive potential of malignant gliomas, with studies by Paulus et al. (9) showing that β1 integrins play a key role in modulating glioma invasivity.

It is well established that the biosynthetic machinery-the glycosyltransferases, glycosylhydrolases, and the genes that regulate their expression-has been significantly altered in all forms of oncogenic transformation [for recent review see (10)]. Surprisingly, however, there have been very few such studies with brain tumors. Moskal and co-workers (11) reported on the expression of α2,6 sialyltransferase in a variety of human brain
tumors, the altered expression of $\alpha_{2,3}$ sialyltransferase mRNA in malignant gliomas (12), the ability of $\alpha_{2,6}$ sialyltransferase gene transfection to inhibit glioma invasivity in vitro and in vivo (13, 14), the ability of N-acetylglucosaminyltransferase III and V to play a role in regulating glioma invasivity in a stable transfectant of a human glioma cell line (15), as well as the ability of a variety of glycosyltransferase gene transfection studies on the ability to enhance cell death induced by staurosporine, C2-ceramide or etoposide (16).

Xu et al. (17) have reported that $\beta_{1,4}$ galactosyltransferase-I, -II, and -V are overexpressed in human astrocytomas and Hoan et al. (18) have shown that ganglioside GM2/GD2 synthetase mRNA can be used as a marker for detecting metastatic neuroblastoma cells in bone marrow.

There have been many reports over the years in which some aspect of glycoconjugate biochemistry (e.g., inhibitors of biosynthesis, addition of cell-surface glycosphingolipids, addition of oligosaccharides or glycopeptides, etc.) has been manipulated leading to an inhibition of tumor growth, metastasis, etc. (19). Of all of these studies, however, only two therapeutic candidates have progressed into clinical trials; swainsonine, an inhibitor of the Golgi-associated $\alpha$-mannosidase II leading to the inhibition of or alterations in normal N-glycan biosynthesis on many glycoproteins (20) and a GD2-based immunotherapy specifically targeting gliomas (21) that, while showing no toxic side effects, was unable to stimulate antibody formation or effect any tumor regression. A recent review by Rebbaa et al. (22) discuss the modulation of growth factor receptors in brain tumors by complex carbohydrates, yet another interesting pathway for the development of therapeutics.

**Glyco-gene expression in brain tumors**

Our program, aimed specifically at developing glyco-biology-based therapeutics for malignant brain tumors is based primarily on the idea that glioma invasivity can be disrupted by altering the aberrant cell-surface glycosylation patterns found in these cells. We hypothesized that the most direct way to do this would be by directly manipulating glycosyltransferase and/or glycosylhydrolase gene expression in these cells. Our initial strategy was to attempt to identify a glycosyltransferase gene, for example, that was markedly altered in its expression in primary specimens of human brain tumors compared to normal human brain. We hypothesized that the most direct way to do this would be by directly manipulating glycosyltransferase and/or glycosylhydrolase gene expression in these cells. Our initial strategy was to attempt to identify a glycosyltransferase gene, for example, that was markedly altered in its expression in primary specimens of human brain tumors compared to normal human brain. By starting with the measurement of gene expression changes, the identification of multiple therapeutic targets would be possible: from the transcriptional regulation of gene expression to post-translational modification of the gene products themselves. Clearly for each altered gene there are an abundance of approaches to modulating its expression and the function of its product(s).

To date there are approximately twenty sialyltransferases that have been cloned.

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### Table 1: histochemical examinations and Northern analyses of human brain tumors (SNA, Sambucus nigra agglutinin)

<table>
<thead>
<tr>
<th>Case</th>
<th>2,6ST mRNA</th>
<th>SNA</th>
<th>2,6ST mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumors of neuroepithelial tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytic tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Astrocytoma (fibrillary)</td>
<td>0/4</td>
<td>0/4</td>
<td>0/1</td>
</tr>
<tr>
<td>Anaplastic astrocytoma</td>
<td>0/4</td>
<td>0/4</td>
<td>0/1</td>
</tr>
<tr>
<td>Glioblastoma</td>
<td>0/8</td>
<td>0/8</td>
<td>2/4 (0/4)</td>
</tr>
<tr>
<td>Pilocytic astrocytoma</td>
<td>0/1</td>
<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Oligodendrogial tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oligodendroglioma</td>
<td>0/4</td>
<td>0/4</td>
<td></td>
</tr>
<tr>
<td>Anaplastic oligodendroglioma</td>
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<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Ependymal tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ependymoma</td>
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<td>0/5</td>
<td></td>
</tr>
<tr>
<td>Myxopapillary ependymoma</td>
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<td>0/1</td>
<td></td>
</tr>
<tr>
<td>Choroidplexus tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choroidplexus papilloma</td>
<td>2/2</td>
<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Choroidplexus carcinoma</td>
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<td>1/1</td>
<td></td>
</tr>
<tr>
<td>Embryonal tumors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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</tr>
<tr>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>0/3</td>
<td>0/1</td>
</tr>
<tr>
<td>Tumors of the meninges</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Meningiomas</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Meningothelial type</td>
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<td>6/6 (5/6)</td>
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<td>3/4 (1/4)</td>
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<td>Tumors of uncertain origin</td>
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<td>Hemangioblastoma</td>
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<td>Haemopoetic neoplasms</td>
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<td></td>
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<td>2/2</td>
<td></td>
</tr>
<tr>
<td>Tumors of the anterior pituitary</td>
<td></td>
<td></td>
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</tr>
<tr>
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</tr>
<tr>
<td>adamantinomatous type</td>
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<td>0/2</td>
<td></td>
</tr>
<tr>
<td>Squamous papilloma</td>
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<td></td>
</tr>
<tr>
<td>Chordomas</td>
<td>5/5</td>
<td>5/5</td>
<td></td>
</tr>
<tr>
<td>Metastatic tumors</td>
<td></td>
<td></td>
<td></td>
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<td>Adenocarcinomas</td>
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<td>0/2</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
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<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>1/1</td>
<td>0/1</td>
<td>0/1</td>
</tr>
<tr>
<td>Mixed mesodermal tumor</td>
<td>0/1</td>
<td>0/1</td>
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</tr>
<tr>
<td>Adenosquamous carcinoma</td>
<td>1/1</td>
<td>1/1</td>
<td></td>
</tr>
</tbody>
</table>

Parenthesis shows the number of cases which expressed a high level of $\alpha_{2,6}$ST mRNA
including O-linked and N-linked as well as the entire series a and b ganglioside sialyltransferases (23). They comprise a structurally related family of molecules that display substrate specificity, tissue specificity, and are all developmentally regulated (24). Studies by Recchi et al. (25) and Marcos et al. (26) showed that sialyltransferase expression in breast cancer cells is markedly altered and that the modulation of their expression can impact tumor cell behavior in vivo as well. Alterations in the expression of terminal sialic acid residues on glycoconjugates are typically found in cells undergoing or that have undergone oncogenic transformation (27, 28). Increased cell-surface sialylation has been associated with invasivity, metastatic potential, adhesion to endothelial cells and extracellular matrices and resistance to T-cell-mediated cell death (29).

In our first series of studies, glycosyltransferase and glycosylhydrolase gene expression was evaluated in freshly dissected brain tumor specimens using Northern analyses. Figure 1 shows a synopsis of some of these data. The x-axis of this figure represents ten arbitrarily chosen malignant, grade IV gliomas. The y-axis is the quantity of gene expressed and the z-axis gives the name of the gene measured. The purpose of depicting our data in this way was 3-fold. First, it gave us some sense of the patterning of each gene in a sample of tumors and addressed the question: “What is each gene’s expression profile?” Second, it gave us a sense of the patterning of the ensemble of genes, addressing the question: “Is there a pattern that emerges by examining the gene expression patterns of more than one gene at a time?”. And third, “What do the relative quantitative values of the gene expression patterns look like compared to each other?”.

Glioblastomas are complex tumors comprised of heterogeneous cell types. Glyco-genes are abundant—approximately 400—and are differentially expressed and developmentally regulated. Thus, it seemed likely that a complex pattern of gene expression might be expected in this family of tumors as well as a different pattern for each tumor. The data depicted in Figure 1 showed that indeed both the qualitative and quantitative expression of the glyco-genes that we measured were indeed complex and quite variable from tumor to tumor. However, it was also clear that the α2,6 sialyltransferase (α2,6ST) transcript that we measured was virtually absent from all gliomas measured.

This finding led us to our second set of studies in which we directly evaluated the expression of α2,6ST mRNA and the cell-surface expression of α2,6-linked sialic acids in a variety of brain tumors. These results are shown in Table 1. Epithelial-like tumors such as meningiomas, chordomas and craniopharyngiomas often expressed α2,6ST and α2,6-linked sialic acids. However, glioblastomas, oligodendrogliomas, ependymomas, medulloblastomas, and brain metastases were essentially devoid of: (1) detectable α2,6ST mRNA (2) α2,6ST immunohistochemical staining; and (3) cell surface α2,6-linked sialic acids (11).

The lack of expression of the α2,6 ST in malignant gliomas and the fact that the α2,6ST together with the α2,3 sialyltransferase (CMP-NeuAc:Galβ1,3(4)GlcNAc α2,3 sialyltransferase (30); α2,3ST) are the two enzymes responsible for effectively all terminal sialylation of N-linked glycoprotein oligosaccharides led us to the next series of experiments. We examined α2,3ST mRNA expression in panels of primary human
brain tumors, cell lines and fetal astrocytes along with the expression of α2,3-linked cell surface sialic acids (12). Figure 2 shows Northern blots from these experiments. It was concluded from these studies and others, using lectins to examine the expression of α2,3-linked sialic acids (12), that gliomas markedly over-express terminal sialic acids compared to normal human brain controls and that it is the α2,3ST as opposed to the α2,6ST that is the principal enzyme involved in this increase in terminal glycoprotein sialylation.

The next step toward the development of our glycobiology-based brain tumor program was to create a model system that reflected the results described above and was based on two working hypotheses: (1) alterations in glioma cell-surface glycosylation should affect tumor cell invasivity and (2) altering the expression of glycosyltransferase gene expression in glioma cells should modify cell-surface tumor cell glycosylation patterns. We thus began by creating a stable, α2,6ST-expressing, human, glioma cell line and evaluating it for a number of properties. We chose the cell line, U373MG because it is derived from a human glioma, is tumorigenic, and does not express α2,6-linked sialic acids. Moreover, adhesion of this cell line to fibronectin or collagen matrices is mediated by the α3β1 integrin receptor since no other □-integrins could be detected. This was important because α3β1 integrin expression is increased in glioblastomas compared to normal brain and likely plays an important role in glioma invasivity.

The results from these studies can be summarized as follows: stable transfectants were created that now expressed: (1) α2,6ST mRNA; (2) measurable amounts of α2,6ST enzyme activity; and (3) cell-surface, α2,6-linked-sialic acids. These transfectants showed a significant reduction in adhesivity to the extracellular matrix molecules fibronectin and collagen compared to mock transfected controls and the parental glioma cell line. The α3β1 integrin was found to contain α2,6-linked sialic acids and the tyrosine phosphorylation of p125fak was blocked in the transfectants despite increased expression of p125fak mRNA (13). Integrins interact with the extracellular milieu and act as signal transducers that can mediate glioma cell migration by regulating the function of focal

Figure 3: Sialylation-dependent α3β1 integrin signaling.

- In parental, tumorigenic, U373MG cells, p125fak is present in a phosphorylated form and leads to activation of intracellular signaling cascades mediated by the IP3, PI3K, JNK, and MAPK pathways.
- Activation of these pathways impacts the increased adhesivity and invasivity in these cells.
- Expression of α2,6ST via stable transfection;
  - increases cell surface α2,6-linked sialic acids specifically on the β1 subunit of the α3β1 integrin receptor,
  - decreases adherence to fibronectin and collagen matrices,
  - decreases adhesion-mediated phosphorylation of p125fak and alters the pattern of focal adhesions,
  - decreases intracellular signaling through the IP3, PI3K, JNK, and MAPK pathways, and
  - decreases invasivity in vitro and tumorigenesis in vivo.
adhesion proteins such as p125fak through control of their phosphorylation state (13). Thus, our results suggested that by simply stably expressing the α2,6ST gene, we could indeed alter the adhesivity/invasivity of these tumor cells via a well characterized molecular mechanism (Figure 3 and (13)).

In the next set of studies (Figure 4), the α2,6ST U373MG stable transfectants were evaluated for their invasive potential using Biocoat Matrigel Invasion Chambers, as described in the legend. Clearly, various clones of the α2,6ST expressing stable transfectants showed marked inhibition of invasivity compared to parental or mock transfectants. These results were robust enough to merit in vivo studies which are shown in Figure 5.

Using the severe combined immunodeficient, SCID mouse model (the details of the methods are described in the Figure 5 legend) we found that U373MG clones expressing the α2,6ST gene showed virtually no brain tumor formation whereas parental cell or mock transfectant-injected SCID mice typically had quite large tumors by comparison. The right-hand panel shows tumor cross sectional area as a percent of control versus tumor cell type injected. And the left-hand panel shows a typical SCID mouse brain six weeks after intracranial injection of either parental cells or transfectants, as indicated. Again, it can be seen that SCID mice injected with the α2,6ST expressing stable transfectants have no detectable tumor formation (14).

From these results it was felt that our approach had clear therapeutic potential. The α2,6ST transfectants completely suppressed human glioma invasivity in vitro and tumor formation itself in vivo. Mechanistically the data strongly suggested that we had modified the glycosylation of the key integrin in gliomas, which, in turn, had altered its signal transducing capabilities and modified cell-extracellular adhesion properties of the tumor cells.

Glycobiology-based microarrays for novel therapeutic target identification

The steady-state expression of the oligosaccharides associated with a specific cell-surface glycoconjugate is the result of the concerted expression of all of the glycosyltransferases involved in its biosynthesis and the glycosidases, involved in its degradation (31). In a system as complex as brain tumorigenesis and invasion it seems as important to determine global context or pattern of these changes as it does to determine the aberrantly expressed, individual glyco-related gene changes found in such systems.

Microarray-based approaches have emerged as one of the cornerstone technologies in rapid throughput gene expression analyses and have made significant impact defining aberrant gene expression in a multitude of tumor systems. In order to begin to grasp the intrinsically complex regulation of genes responsible for the synthesis and degradation of specific carbohydrate structures that are essential for mediating glioma invasivity, we have created a microarray core facility to provide a comprehensive platform of glycoconjugate metabolism-associated oligonucleotides assuring the most up-to-date coverage of these gene families.
The 359 genes comprising our Human Glycobiology microarray are compiled from currently available NCBI/EMBL/TIGR human sequence databases and the Consortium for Functional Glycomics-CAZY databases. Unique sense 45-mer oligonucleotides corresponding to mRNAs of each gene used as probes are individually synthesized, purified and immobilized via a 5’-amino linker onto aldehyde-coated microarrays. Total RNA is reverse transcribed and used as the substrate for RNA amplification and labeling using the indirect aminoallyl methodology based on the Eberwine protocol (32). To circumvent the inherent biological heterogeneity of clinical GBM specimens, we employ a universal reference design (33) and comprehensive statistical analysis platforms to facilitate acquisition of expression profiles from a necessarily large number of biological and technical replicates. We have found that our high quality, application-specific, low density microarray platform provides an efficient strategy for such an endeavor.

Focused microarray analyses comparing a panel of Grade IV gliomas with a panel of age-matched normal brain specimens yielded 11 significant genes more highly expressed in gliomas compared to normal brain and 25 genes more highly expressed in normal brain compared to gliomas (34). Clearly there are many significant differences in expression of genes associated with glycoconjugate biosynthesis and degradation, many of them novel and all of them potential targets for the development of therapeutics for the treatment of brain tumors. The current and future direction of our program includes: (1) increasing the number of primary tumors analyzed by our microarrays, (2) evaluating human glioma cell lines by microarray analysis: Cell lines can provide powerful model systems to study the regulation of a given gene or associated gene family, (3) confirming and extending our microarray data with in situ hybridization studies and quantitative RT-PCR analyses, and (4) continuing to evaluate therapeutic candidates using viral vectors in preclinical mouse models.

Clinical significance
The data that we have obtained to date strongly suggest that a clinical trials program should be undertaken. Both the data that we have obtained in primary tumors and multiple tumor models together with the well established role that glycoconjugates play in modulating tumor metastasis and invasivity support this. However, significant challenges remain, for it will be difficult to find the right clinical setting to undertake a human Phase I gene-based clinical trial for malignant brain tumors and optimizing the delivery system to insure effective gene delivery to tumor cell targets will have to be addressed.

Acknowledgments
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References


Regulatory factors that are essential for proper T cell development are often common targets for mutation in T cell leukemia in humans and mice. Therefore, by studying the normal transcriptional regulation of T cell development, we can also learn about the molecular mechanisms that underlie T cell transformation. Recent studies in the Winandy laboratory have addressed the interacting roles of two essential regulators of T cell development, Ikaros and Notch, in controlling developmentally regulated gene expression and malignant transformation of thymocytes.

Ikaros is a sequence-specific DNA-binding protein that acts primarily as a transcriptional repressor in thymocytes [1, 2]. Ikaros expression is almost exclusively limited to hematopoietic lineages and is essential for normal T cell development [3]. When Ikaros levels are reduced or completely ablated in mice, thymocytes undergo leukemogenesis with 100% penetrance [3, 4]. This striking phenotype along with previous studies conducted in the Winandy laboratory with Ikaros null (Ik-/-) leukemia cell lines have demonstrated that Ikaros acts as a tumor suppressor for the T cell lineage [5]. Notch is a transmembrane glycoprotein that is expressed on thymocytes and regulates their differentiation, proliferation, and survival [6]. Notch is cleaved upon ligand binding to form intracellular cleaved Notch (ICN), which travels to the nucleus and activates transcription by interacting with the DNA-binding factor RBP-Jκ (RBPJ). Traditional models of Notch target
gene regulation have proposed a switch mechanism, whereby RBPJ is basally associated with transcriptional co-repressors and bound to regulatory regions of Notch target genes in the absence of ICN. When ICN enters the nucleus, it binds to RBPJ and displaces its associated co-repressors. ICN then nucleates a co-activator complex to activate transcription of Notch target genes (Fig 1). In lymphocyte progenitors, Notch expression is required for T cell lineage specification and the transition from the double negative (DN) to the double positive (DP) stage of thymocyte development [7-9]. Constitutive activation of Notch signaling induces T cell leukemia in mice, which indicates that Notch acts as an oncogene for the T cell lineage [10].

Deregulation of both Ikaros and Notch function have been implicated as important events in human acute lymphoblastic leukemia (ALL). Mutations in the Ikaros gene (IKZF1) are a strong predictor of increased likelihood of relapse in children with B-ALL and are a common genetic lesion in Philadelphia chromosome-positive adult ALL [11, 12]. Dominant negative non-DNA binding Ikaros proteins are abnormally expressed at high levels in leukemic cells from children with T- and B-ALL [13]. In addition, activating mutations in Notch have been observed in over 50% of human T-ALLs [14].

Notch deregulation is a common event in Ikaros null leukemia

In mice, several groups have postulated that loss-of-function mutations in Ikaros and gain-of-function mutations in Notch cooperate to promote T cell leukemia [15-17]. To further investigate this interaction, we surveyed a panel of four Ikaros-deficient T cell leukemia lines for aberrant activation of the Notch pathway. Our leukemia cell line panel represented two major types of Ikaros deficiency that are observed in patients with Ikaros mutations: 1) a complete lack of DNA-binding Ikaros expression (JE131, DO11, TU5), and 2) the simultaneous expression of DNA-binding Ikaros and non-DNA binding (dominant negative) Ikaros (D510). A majority of Ikaros deficient leukemia lines (3 out of 4) contained aberrant ligand-independent constitutive ICN expression [18]. However, abnormal ICN expression was only observed in leukemia cell lines that completely lacked DNA-binding Ikaros expression. Moreover, the ICN expressed in these cell lines was smaller than its expected mobility in two out of three cases (JE131, TU5), suggesting expression of a truncated protein similar to what is observed in human leukemia with activated Notch (Fig 2).

In humans, activating mutations in Notch are concentrated in two regions of the Notch protein. These mutations either potentiate
ligand-independent cleavage or lead to stabilization of ICN, which results in expression of a truncated ICN protein. (Please refer to diagram in Fig 2). Therefore, we performed sequence analyses of Notch cDNA in order to determine if such mutations contributed to the constitutive ICN expression observed in our Ikaros deficient cell lines. Mutations in these regions were identified in three of the Ikaros deficient cell lines, suggesting that Notch mutations that result in increased ICN expression are a common event in the transformation of Ikaros deficient T cell precursors.

Ikaros regulates the Notch pathway in a T cell leukemia cell line

Through analyses of gene expression using quantitative real-time PCR (qRT-PCR), we observed high-level expression of two canonical Notch target genes Hes1 and Deltex1 in the ICN-expressing Ikaros deficient leukemia cell lines [18]. Ikaros binds to regulatory elements within its target genes at the DNA consensus sequence, GGGAA [2], and the Notch-target gene activator RBPJ shares sequence specificity for this consensus site. Moreover, several groups have now demonstrated the ability of Ikaros and RBPJ to bind to the same regulatory elements within Notch target genes using in vitro and in vivo approaches [15, 16, 19-21]. Therefore, we hypothesized that Ikaros and ICN/RBPJ may function in opposing roles to promote regulated expression of Notch target genes. We tested this hypothesis using the Ikaros null T leukemia cell line, JE131. Re-introduction of Ikaros into the JE131 cell line using retroviral transduction demonstrated that Ikaros directly binds the Hes1 and Deltex1 promoters through its sequence-specific DNA binding zinc-fingers and this results in their repression [19]. Importantly, Ikaros-mediated repression of Notch target genes occurs without altering levels of ICN, demonstrating that Ikaros’ role in Notch target gene repression is downstream of ICN [18].

Retroviral transduction of Ikaros into the JE131 leukemia cell line results in a dramatic growth arrest [5]. If deregulated expression of ICN is required for leukemogenesis, we reasoned that abrogation of ICN expression, through treatment of the cells with a gamma-secretase inhibitor (GSI), would have a similar result. However, although GSI treatment of the ICN-expressing JE131 cell line was capable of preventing ICN formation, it was incapable of inducing growth arrest [19]. These data suggest that, although deregulated ICN expression is a common event in Ikaros-deficient leukemia, lack of Ikaros is the main contributor to the deregulated proliferation

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![Figure 2: Activating mutations in Notch occur in the HD and PEST regions in Ikaros-deficient T cell leukemia. Commonly mutated regions of the Notch protein observed in human leukemia were assayed for in Ikaros deficient T leukemia cell lines. The Notch HD (heterodimerization) domain is responsible for stable association of the intracellular and extracellular portions. Mutations in this region are thought to promote ligand-independent cleavage. The Notch PEST (Proline, Glutamine, Serine, Threonine-rich) domain negatively regulates protein stability. Frameshift mutations in this region introduce premature stop codons and increase Notch protein stability by removing PEST activity. The table indicates presence or absence of ICN, identity of mutations, and description of Ikaros activity in each T leukemia cell line.](image-url)
The lack of ICN expression in the D510 T leukemia cell line, which expresses high levels of dominant negative Ikaros proteins, also supports this hypothesis [18].

Ikaros null thymocytes exhibit deregulated Notch target gene expression prior to transformation since we demonstrated that Ikaros can repress Notch target gene expression in Ikaros deficient leukemias, we next wanted to test the relevance of these findings to developing, non-transformed thymocytes using an Ikaros null (Ik−/−) mouse model system. Therefore, expression levels of full-length Notch and ICN in thymuses from pre-leukemic 3-week old Ik−/− mice and their wild-type (Ik+/+) counterparts were examined using Western blot analyses. Equivalent levels of both were observed indicating that, unlike the situation observed in Ikaros-deficient leukemic cells, Notch expression is not deregulated in primary non-transformed Ik−/− thymocytes [18].

In contrast, analyses of Hes1 and Deltex1 expression in Ik−/− primary thymocytes revealed an abnormal pattern. Notch signaling is dynamically regulated in thymocytes throughout their development. ICN production and Notch target gene expression are robust in the early CD4^−CD8^− DN subset of thymocytes, but extinguished by the subsequent CD4^+CD8^+ DP stage [22]. DN and DP subsets from Ik−/− and Ik+/+ mice were sorted and assayed for expression of a panel of Notch target genes including Hes1, Deltex1, pTa, Gata3, and NRARP [21, 23-25]. Ik−/− and Ik+/+ DNs exhibit equivalent levels of Notch target gene expression, demonstrating that Notch target gene activation is not disrupted by lack of Ikaros. Conversely, analyses of Notch target gene expression in DPs demonstrated that Ik−/− DP thymocytes express abnormally high levels of Notch target genes as compared to their Ik+/+ counterparts. Since Notch target gene expression is downregulated when thymocytes transition from DN to DP, these data indicate that lack of Ikaros results in a failure to repress Notch target genes (Fig. 3). In support of this conclusion, further analyses of Ik−/− DP thymocytes revealed that deregulated Notch target gene expression did not stem from aberrant ICN expression in this population [18].

Deregulation of Notch signaling is not required for leukemogenesis in Ikaros null mice It has been postulated that constitutive activation of Notch target genes is a requirement for the leukemogenesis that occurs...
with 100% penetrance in Ik⁻/⁻ mice [15]. However, Notch target genes are derepressed in the absence of Ikaros prior to thymocyte transformation [18, 20]. Accordingly, this led us to hypothesize that deregulated Notch target gene activation, through aberrant ICN generation/stabilization, may not be required for leukemogenesis in Ik⁻/⁻ mice. To directly test this hypothesis, Notch target gene activation was abrogated in Ik⁻/⁻ DP thymocytes through conditional inactivation of the DNA-binding Notch target gene activator RBPJ. This was accomplished by breeding mice with floxed-RBPJ alleles (RBPJfl/fl) [26] onto the Ik⁻/⁻ genetic background. These mice were then bred to mice containing a Cre-recombinase transgene under the control of CD4 regulatory elements [27] so that RBPJ deletion would be achieved as thymocytes activate expression of CD4, which occurs as they transition from the DN to DP stage of development.

To assay for leukemia, we tested for the presence of clonally expanded populations in the thymus, which is the hallmark of leukemia in Ik⁻/⁻ mice, by examining the composition of the Vβ repertoire expressed by the TCRβ chain of the T cell receptor. Through flow cytometric analyses of thymocytes stained with a panel of Vβ-specific antibodies, clonally expanded Vβ populations were detected in thymuses of Ik⁻/⁻ mice, which either did or did not express RBPJ, as early as 5 weeks of age. In another measure of leukemogenesis, we assayed for the presence of clonally expanded T cell populations that escape the thymus and are found in the peripheral lymphoid organs such as the spleen. Importantly, the same predominant clonal population observed in the thymus was also present in the spleen of Ik⁻/⁻ mice with conditional inactivation of RBPJ (Fig 4). Clonal populations did not represent an outgrowth of cells that failed to delete RBPJ since there was no increase in RBPJ expression observed in leukemic cells as compared to their non-leukemic counterparts. Additionally, we demonstrated that Ik⁻/⁻ RBPJ- leukemic cells did not always display abnormal Notch expression, thereby eliminating the possibility that stabilizing/over-expressing mutations in Notch are required for their malignant transformation. Together, these studies definitively demonstrate that deregulated Notch signaling is not required for the transformation of Ik⁻/⁻ thymocytes. Future research will be aimed at defining the pathway of transformation in these cells, thereby defining the molecular mechanisms of leukemogenesis in the absence of Ikaros.

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Cancer cells have in their genomes mutated copies of normal cellular proto-oncogenes that cannot currently be removed or inactivated in the cancers. A major focus of our laboratory is the controlled induction of DNA cleavage through the use of titanium dioxide (TiO₂) nanoparticles. We anticipate that if this DNA cleavage by TiO₂ can be controlled, then it can be used to remove mutant DNA from cancer cells that have activated oncogenes. Titanium dioxide is a semiconductor material that can be formed into nanoparticles by various methods (for review see, Cozzoi et al, 2003; Kim et al, 2003; Ramakishna et al, 2003; Wu et al, 2002; Zhang et al, 2003; Zhao et al, 2007). Due to its semiconductor properties, illumination of TiO₂ leads to a charge separation and the
promotion of electrons from the valence band into the conduction band of TiO₂. In aqueous solution, this charge separation can lead to the production of both free electrons and free electropositive holes on the surface of TiO₂. The production of free electrons and electropositive holes, can result in the production of reactive oxygen species in the buffer in which nanoparticles have been dispersed at the time of illumination (Fujishima et al., 1972; Blake et al., 1999). In some of the publications investigating this event, cleavage of plasmid DNA was used as an indicator of this process. However, in TiO₂ nanoparticles, similar to the effect within quantum dots, electrons are confined to the nanoparticle and are believed to be unable to separate from it. Thus, only electropositive holes become available for charge recombination on the surface of the nanoparticle since they are still capable of physical separation from the TiO₂ (Rajh et al., 2001, 2002). Previous work by others has established that conjugation of electronic leads, such as dopamine, to the nanoparticle surface results in an extended charge separation where the electropositive holes move into the ligand—dopamine, and away from the nanoparticle itself (Rajh et al., 2001, 2002). In our laboratory we have investigated TiO₂ surface modification by Alizarin Red S (ARS) (Thurn et al., 2009) and found this conjugation to be as stable as the connection between Alizarin and TiO₂ (Rajh et al., 2002). Since the reaction between Alizarin and TiO₂ was described as having the same stability as the conjugation to dopamine, and since dopamine can be used to separate electropositive holes from the nanoparticles, we could infer that, likewise, ARS should aid in separation of electropositive holes from the TiO₂.

We proceeded to test the ability of TiO₂-Alizarin Red S complexes to introduce reactive oxygen species (ROS) into an aqueous solution. In the work presented here we particularly focused on the TiO₂-Alizarin Red S complex and on core-shell nanoparticles as a source of TiO₂. Core shell nanoparticles used for this purpose were composed of a core made of iron oxide (Fe₃O₄) coated with a TiO₂ shell with the final size of 6 nM. We have shown in previous studies that the Fe₃O₄ core will permit MR imaging of the nanoparticles and might be useful in diagnostic imaging. Similar to tests performed on TiO₂ nanoparticles, Fe₃O₄@TiO₂ nanoparticles were prepared and tested for their capacity to induce DNA cleavage by reactive oxygen species (Wu et al., manuscript in preparation).

Results
Conjugation of Alizarin Red S (ARS) to TiO₂-PNA nanoconjugates
To demonstrate that Alizarin Red S binds to TiO₂-peptide nucleic acid (PNA) nanoconjugates we have used UV-visible light spectroscopy (Thurn et al., 2009) as well as gel electrophoresis (Brown et al., 2008). TiO₂ nanoparticles are unable to migrate through a polyacrylamide gel (Paunesku et al., 2003, Brown et al., 2008). Thus, any molecule bound to the surface of a TiO₂ nanoparticle will also not be able to migrate through a polyacrylamide gel. When free ARS or nanoparticles with conjugated ARS were applied onto a 16% polyacrylamide gel and imaged for Alizarin Red S by either a digital camera or a fluoroimager at the appropriate setting (excitation laser 543nm, emission filter 560-615nm) ARS could be visualized (Figure 1). With visible light, the ARS-TiO₂ complex appears as a red band in those wells of the gel where it was conjugated to the TiO₂ nanoparticles (well 3, Figure 1). After additional electrophoresis and imaging of ARS, this dye is abundant only inside the gel and only in those lanes where ARS was not conjugated to the nanoparticles (lanes 1 and 2,
Figure 1). In the presence of TiO sub2 nanoparticles (lane 3) a small quantity of free Alizarin Red S is inside the gel, with most of the fluorescent signal still present in the well.

DMSO sequesters reactive oxygen species (ROS) reducing cleavage

Previous work by others with TiO sub2 release of ROS and plasmid DNA cleavage (Tachikawa et al., 2007) has shown that DMSO is an effective scavenger of TiO sub2 produced ROS. To determine if the production of reactive oxygen species by ARS coated nanoparticles could still be achieved using the same approach; DMSO was used to sequester reactive oxygen species from the samples (Figure 2). Since DMSO is a ROS scavenger, the addition of DMSO would prevent ROS from cleaving DNA. If the mechanism by which ARS and ARS-TiO sub2 are cleaving DNA is by the production of ROS, then the addition of DMSO would decrease cleavage. In our experimental design, samples were as follows: 119ng of pKaede plasmid alone, Alizarin Red S (equal to the same concentration at which the nanoparticles were coated) and plasmid, 6 nm TiO sub2 nanoparticles and plasmid, or plasmid and 6 nm TiO sub2 nanoparticles that were 60% coated with Alizarin Red S. These samples were then exposed to white light (7 minutes at an intensity of 75 watts by a Fiber-Lite® High Intensity Illuminator Series 180) or left untreated (control). Both of these treatments were with or without 0.1% DMSO. Samples were then run on a 1.2% agarose gel and stained with GelStar. Samples containing Alizarin Red S and plasmid showed a decrease in DNA cleavage upon addition of DMSO. Samples that contained uncoated TiO sub2 nanoparticles, however, showed no change in DNA cleavage upon the addition of DMSO.

The addition of DMSO to samples containing Alizarin Red S coated TiO sub2 nanoparticles showed a decrease in DNA cleavage as compared to the same sample without DMSO.

Cleavage of plasmid DNA with core-shell nanoparticles and ARS and an excess of ARS

In order to pursue new possible applications of TiO sub2 nanoparticles, we have decided to prepare core-shell nanoparticles that could be used for magnetic resonance imaging (MRI) as well as for DNA cleavage. To investigate the ability of Fe sub3O sub4@TiO sub2 nanoparticles coated with excess Alizarin Red S to result in cleavage of plasmid DNA in vitro we performed an agarose gel electrophoresis assay (Figure 3). In these studies 6nm Fe sub3O sub4@TiO sub2 nanoparticles (various concentrations, as indicated) were completely coated with Alizarin Red S (with free Alizarin Red S in excess in the solution, to a final total concentration of ARS at 1.5 mM). As an indicator of DNA cleavage induced by ROS production 14.65nM pKaede plasmid DNA was added to the Alizarin Red S coated nanoparticles and the samples were illuminated and then separated on a 1.2% agarose gel and stained with Gel Star. When the plasmid migrates through the gel its migration is dependent upon its conformation and the concentration of agarose in the gel. In a 1.2% agarose gel, the plasmid that is in a super-coiled conformation will migrate faster than a linearized or nicked (relaxed circular) conformation. When illuminated, and in the presence of excess Alizarin Red S and Fe sub3O sub4@TiO sub2, the plasmid is cleaved into nicked and linear conformations. A concentration of 56.4nM Fe sub3O sub4@TiO sub2 with 1.5 mM ARS is the minimal concentration of nanoparticles needed under these circumstances.
Cleavage of plasmid DNA with varying concentrations of Alizarin Red S

To determine the extent to which coating of Fe$_3$O$_4$@TiO$_2$ nanoparticles with Alizarin Red S, and having free ARS present in solution, enhance cleavage, Fe$_3$O$_4$@TiO$_2$ nanoparticles were either coated with excess Alizarin Red S (final concentration of 1.5mM) or left uncoated (Figure 3). The same procedure as in Figure 3 was performed. Samples containing Alizarin Red S coated Fe$_3$O$_4$@TiO$_2$ nanoparticles showed plasmid DNA to be in both a nicked and a linear conformation but it lacked a super-coiled conformation. Samples containing the same molarity of uncoated Fe$_3$O$_4$@TiO$_2$ nanoparticles, on the other hand, showed the presence of only super-coiled and nicked conformations but no linearized DNA. As can be seen from the lack of super-coiled plasmid DNA, and an increase in both nicked and linearized DNA, samples that contained Alizarin Red S showed more cleavage of plasmid DNA than samples that lacked Alizarin Red S.

Discussion

Recent advances in nanotechnology, have lead to the creation of nanomaterials that acquire novel properties at the nanoscale level. These novel properties may lead to the development of new nanoparticle formulations with applications in medical therapy and diagnostics. In our laboratory, we are working on the development of multi-purpose nanoparticles that can cause illumination dependent DNA cleavage and be used for magnetic resonance imaging as well. The ultimate goal of this work would be to develop a theranostic agent that would cleave DNA in an inducible and sequence specific manner that could be used for imaging and treating cancer cells simultaneously in the patient.

Currently our lab is evaluating the use of TiO$_2$ shell nanoparticles as a possible theranostic agent. Nano-scale TiO$_2$ exhibits unique properties: TiO$_2$ nanoparticles that are less than 20 nM in size can covalently bind bidentate enediol ligands such as dopamine and Alizarin Red S (Rajh et al., 2001, 2002). TiO$_2$ is also a wide gap semiconductor that exhibits photocatalytic activity. When TiO$_2$ is conjugated to a biological molecule such as DNA or PNA, via a dopamine linker, it still retains its photocatalytic activity. This property can be exploited for use in sequence specific DNA damage. When a TiO$_2$-PNA nanoconjugate is hybridized to a target sequence in a cell, the nanoconjugate can be induced with light energies to cause sequence specific cleavage of cellular DNA (Paunesku et al., 2003).

Therefore TiO$_2$ nanoconjugates can act as inducible endonucleases to target, in a sequence specific manner, genes of interest in a cell. This ability will allow for TiO$_2$-nanoconjugates to be used as a possible gene therapy to remove unwanted foreign DNA from cells without altering host DNA, such as one finds in cancer cells with mutated oncogenes.

To further extend the DNA cleavage capabilities of TiO$_2$ we designed experiments to determine if binding Alizarin Red S to TiO$_2$ nanoparticles increases ROS production in aqueous solution, thus increasing DNA damage. Alizarin Red S is a common textile dye that has been previously found to produce reactive oxygen species (ROS) when illuminated with white light, when in a solution of bulk TiO$_2$ (Guangming et al., 2000). The reactive oxygen species produced when Alizarin Red S undergoes photooxidation
with TiO$_2$ (bulk) are active oxygen radicals of super oxide (O$_2^-$) as well as hydroxyl radicals (·OH). Both of these reactive oxygen species can elicit DNA damage. Since Alizarin Red S is a bidentate endiol ligand, it can be covalently bound to the TiO$_2$ nanoparticle surface. In this paper we have discussed the potential use of coating TiO$_2$ and Fe$_3$O$_4$@TiO$_2$ nanoparticles and with Alizarin Red S to enhance DNA damage.

In a series of in vitro experiments we have shown that Alizarin Red S has the ability to bind to TiO$_2$ nanoparticles in solution and within cells (Thurn et al., 2009, Brown et al., 2008, Figures 1-4). In vitro cleavage assays done using Fe$_3$O$_4$@TiO$_2$ nanoparticles coated with excess Alizarin Red S show that not only does DNA cleavage occur upon illumination, but cleavage of plasmid DNA is enhanced by coating nanoparticles with Alizarin Red S. The proposed mechanism by which Alizarin Red S cleaves DNA is based on the production of reactive oxygen species. To re-confirm the production of ROS as the main cause of plasmid DNA cleavage, we added DMSO to samples containing Alizarin Red S coated TiO$_2$ nanoparticles and saw a decrease in DNA cleavage. In summary, we have shown through a series of in vitro experiments that the addition of Alizarin Red S to TiO$_2$ or Fe$_3$O$_4$@TiO$_2$ nanoparticles enhances the efficacy of TiO$_2$ and Fe$_3$O$_4$@TiO$_2$ nanoparticles to cause DNA damage in vitro.

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References


A multi-domain fold containing a bundle of helices and a beta sandwich… the mind races into overdrive, attempting to assign meaning to this seemingly random laundry list. Could this represent a futuristic fast food encounter, say a McDonald’s drive-through lane circa 2050? Alternatively, could this be a post-competition interview segment with a stoked California teenager, who is excitedly recapping the series of gravity-defying moves that earned him the extreme skateboarding crown? In reality, both these colorful scenarios miss the mark -- the italicized opening is actually a description of the protein structure shared by a family of proteins known as the serpins (an acronym derived from serine protease inhibitors, a characteristic common to virtually all members of the family.) In other words, the overwhelming bulk of the serpins function to block the actions of proteases, which are enzymes responsible for cleaving (i.e., breaking) proteins at key points to either activate or inactivate them. Recent studies emerging from the laboratory of cancer researcher Ming Zhang suggest that one of these serpins, an unassuming protein known simply as Maspin, may represent a potent new weapon in the cancer-fighting arsenal.

Serpin research has steadily matured from a sapling stage (i.e., during the 1960s and 70s, when the characterization of a scant handful of known serpins offered early glimpses of the importance that would one day be assigned to these proteins) to an ever-expanding family tree (currently consisting of over 40 proteins). Diversely present in humans, animals, plants, fungi, and viruses, the evolutionary success of the serpins can be attributed to a unique structural-functional characteristic – a common reactive region in the C-terminal portion of these proteins, known as the mobile reactive...
site loop, functions as “bait” for their target proteases. This loop has the innate capacity to radically alter its conformation, which in turn, enables the serpins to bind to their target proteases as a virtually irreversible complex. Most members of the serpin superfamily function as inhibitors of proteolytic enzymes but a subclass of serpins does not inhibit any proteases, and therefore are called non-inhibitory serpins. Inhibitory serpins block the actions of proteases, which are enzymes responsible for cleaving or “breaking” proteins at key points to either activate or inactivate them. As a consequence, the serpins function as indispensable regulatory molecules in complex biological processes such as blood coagulation (i.e., the thickening or congealing of the blood), fibrinolysis (i.e., the protein fibrin is the primary “building block” of a blood clot, providing the requisite scaffolding for the clot to form…fibrinolysis is the process by which the body breaks down this fibrin scaffolding and hence blood clots), phagocytosis (i.e., as the name implies (Greek: phago=eating; cyt=cell), this multi-step process involves phagocytes or “eating cells” (i.e., macrophages and their cousins the neutrophils) literally ingesting and digesting microorganisms, insoluble particles, damaged or dead host cells, cell debris, or activated clotting factors), inflammation, apoptosis (i.e., programmed cell death, which serves to eliminate unwanted cells during development, as well as cells damaged by external insults), cell migration, and connective tissue remodeling. The functions of Non-inhibitory serpins are more diversified. For example, ovalbumin is a storage protein, and pigment epithelial derived factor is a serpin involved in neuronal differentiation and angiogenesis. More importantly, not only serpins play diverse roles as a class, but also a single serpin molecule possesses multiple functions. One astute observer in the field summed up the ubiquitous nature of the serpins quite nicely when he remarked “they are evident in everyday life from the white of the breakfast egg (i.e., the non-inhibitory serpin ovalbumin), to the protein-rich head of foam atop the evening beer (i.e., the barley Z protease inhibitor).” One of the serpins, a relative newcomer to the family known as Maspin(Zou et al., 1994), has added tumor suppression to this impressive regulatory resumé. In the last few years, the Zhang group has discovered that maspin, although expressed in normal mammary epithelial cells, was downregulated (i.e., expressed at substantially lower levels or completely absent) in most mammary carcinoma cell lines. Furthermore, they found that maspin expression, when restored in these breast tumor cells, effectively inhibited their motility, invasiveness, and metastatic potential (i.e., maspin thwarted the ability of these cancer cells to migrate to distant sites in the body to colonize new sites of growth)(Zhang, 2004). These provocative findings served as the catalyst for a focused, international effort to further characterize maspin and dissect its associated genetic pathway.

Despite tremendous strides in this regard, however, maspin’s target(s) and mechanism(s) of action remain frustratingly elusive. Maspin researchers are a determined lot, however, and from their dogged pursuit of these issues a much clearer picture is beginning to emerge. Studies by the Zhang lab and others have established that maspin is expressed in the cytoplasm, nucleus, and, in certain instances, outside the membrane. But what role(s) might it play intracellularly? The Zhang lab postulates that maspin may in fact play an active role in apoptosis. This assertion is largely based upon observations from a transgenic mouse model the group developed to overexpress maspin in the mammary gland under the whey acidic protein (WAP) promoter. In other words, the group selected a gene known to be abundantly expressed in the mammary gland (i.e., the WAP gene) and fused its promoter (i.e., a group of DNA fragments that would trigger the expression of WAP) to the maspin gene. This WAP-driven expression enabled the group to examine the impact of above-normal levels of maspin on mammary gland development in these mice. Surprisingly, the group witnessed stunted development in these animals during pregnancy and lactation (i.e., developmental time points when mammary gland development should be at its peak). Upon closer inspection, the group identified a significant increase (as compared to wildtype animals) in the number of apoptotic cells present, which implied that overexpression of maspin during pregnancy and lactation increased the number of apoptotic cells in the mammary gland(Zhang et al., 1999). Carrying the analysis even further, the group sought to determine whether elevated levels of maspin might hinder tumor progression in vivo (i.e., in living cells). They investigated this issue by crossing the WAP-
maspin transgenic mice described above with mice derived from the WAP-TAg transgenic model of tumor progression. Exploiting similar genetic engineering, creation of the latter model involved fusing the WAP promoter (i.e., a group of DNA fragments that would trigger the expression of WAP) to a known cancer-causing gene called the SV40 T antigen (TAg)(Zhang et al., 2000a). Subsequently, during the course of mammary development, this WAP-driven TAg expression caused these mice to develop tumors of the breast. When the group examined the mice resulting from this marriage of models, they found that maspin overexpression markedly reduced mammary tumor growth through a combination of decreased angiogenesis (maspin’s impact on angiogenesis will be touched upon in greater detail below) and increased apoptosis (witnessed in both precancerous cells and migrating tumor cells). These early findings lead to the multi-year efforts to uncover the mechanism through which maspin controls cell apoptosis (Latha et al., 2005). It is now clear that maspin migrates from cytoplasm to mitochondria, a key organelle in cell death signaling, causing a drastic change in mitochondria membrane potential and the release of cytochrome c and inducing cell apoptosis. Zhang lab is now working diligently to identify molecules that promote the translocation of maspin from cytoplasm to the mitochondria.

Numerous other functions of maspin have been identified by Zhang lab in the last several years. In vivo functional insights have emerged from the group’s maspin studies, including potential roles in angiogenesis (i.e., the biological manufacture of blood vessels) and organ development (embryonic, breast, and prostate development). Regarding the former, several members of the serpin family, including plasminogen activated inhibitor-1 and -2 (PAI-1 and -2) and the urokinase plasminogen activated (uPA) receptor, have been implicated in angiogenesis. Cognizant of this fact, Zhang collaborated with Drs. Noel Bouck and Olga Volpert at the Northwestern University to explore the possibility that maspin may also be involved in this process(Zhang et al., 2000b). Rather than participating in the development of blood vessels, however, the groups’ studies suggested the converse was true (i.e., maspin appeared to function as an angiogenic inhibitor) – and this was witnessed both in vivo and in vitro in a rat cornea assay (i.e., in cell populations in both live rats and in a culture medium). In the in vivo scenario, the team induced angiogenesis in these animals and discovered that it was subsequently blocked by maspin expression. In the in vitro scenario, they showed that maspin effectively blocked the migration and motility of endothelial cells lining vessel walls. Although quantifying maspin’s effectiveness as an angiogenic inhibitor and determining whether this effect might be highly specific (i.e., unique to this particular population of cells in the eye) are the topics of ongoing studies, the above findings may have enormous implications for the treatment of cancer (i.e., tumors must induce the growth of new blood vessels to sustain themselves and maspin may offer a means of severing this vital lifeline). Maspin’s potential involvement in mouse organ development first came to light when the Zhang lab engineered a maspin knockout mouse. Interestingly, the absence of functional maspin was homozygote lethal in this model (i.e., chromosomes are arranged in pairs, one male and one female, with a copy of the maspin gene present on each – when the group disrupted the normal function of both copies of the maspin gene in these mice, their offspring died at day 5.5 stage of development, which represented an extremely early time point in embryonic development)(Gao et al., 2004). This outcome was quite surprising in that no other members of the serpin family had ever displayed this lethality when their functionality was disrupted. This early lethality, coupled with the subsequent finding that maspin was highly expressed in blastocyst outgrowths (i.e., tiny, spherical clusters of cells representing one of the earliest time points in the developmental program), suggested that maspin may play an essential role in early embryonic development. Interestingly, heterozygotes (i.e., mice in which the normal function of only one copy of the maspin gene was disrupted, thereby enabling production of the protein in diminished amount) in this same model also displayed a severe phenotype relating to mouse mammary gland and prostate development. For example, loss of one copy of maspin gene in Mp+/– heterozygous knockout mice leads to the development of prostate hyperplastic lesions in neonatal and aged mice, and this effect was mediated through decreased level of CDK inhibitors p21 and p27(Shao et al., 2008). A recent report by Dr. Michael Karin’s group at UCSD demonstrated that maspin is a key...
tumor suppressor of prostate cancer metastasis (Luo et al., 2007).

Maspin’s ability to inhibit tumor growth and metastasis via the three-pronged attack of increased apoptosis, decreased angiogenesis, and inhibition of tumor cell migration offers newfound hope that a cure for certain cancers (particularly those of the breast and prostate) may be well within their reach. Zhang lab, along with other maspin researchers are currently devising strategies to restore maspin expression in tumor cells via pharmacological intervention, which could represent a promising new therapeutic option in the treatment of cancer. Several studies have hinted that the antiestrogen drug tamoxifen, which is currently used in the treatment of certain breast cancer patients, may be particularly effective in this regard. Importantly, these studies have shown that tamoxifen decreases or slows breast cancer progression, and that it’s function may be mediated by the up-regulation of the maspin gene (Liu et al., 2004). Approaching the problem from more than one angle, the Zhang lab is also pinning considerable hope on an alternative means of restoring maspin expression through upstream transcription factors that control maspin gene expression. The list includes tumor suppressor genes p53, PTEN, and an Ets transcription factor named PDEF. Zhang lab discovered that PDEF transactivates maspin and a cell cycle inhibitor p21. Both in vivo and in vitro experiments showed that PDEF inhibits breast cancer progression. Zhang lab has extended the study along the line of tumor suppressor genes to investigate the role of PDEF in normal development, and breast and prostate cancer progression.

Immune Control of Cancer Metastasis
Another focus of research in Zhang lab is to identify immune components that control breast cancer metastasis. While current standards of treatment for breast cancer have greatly improved patient morbidity and mortality, the fact that 40% of patients still succumb to the disease underscores the need for improved treatment strategies that limit toxicity and achieve lasting tumor regression. Since Ehrlich first proposed the idea of immune surveillance and eradication of nascent tumor cells in 1909, the goal of eradication of breast cancer through bolstering the immune system’s ability to recognize and eliminate cancerous cells remains a promising therapeutic direction. However, despite renewed interest in the field over the past two decades, clinical immunotherapeutics have proven largely ineffective and overcoming inhibition of anti-tumor immunity remains a formidable obstacle. Further insights into the mechanisms of immune regulation of cancer are required in order to design more effective treatment strategies that overcome tumor tolerance and promote immune cell-mediated tumor rejection. Just as viruses and bacteria have evolved ways to evade the immune system over time, cancerous cells that escape initial detection and elimination continue to proliferate and evolve similar strategies to avoid further immune system eradication. However, while poorly immunogenic tumor cells evolve to evade the immune system, other more immunogenic cells continue to succumb to inflammation-mediated destruction. Chronic activation of inflammation and subsequent tissue destruction creates toxic bioactive by-products that further increase neoplastic transformation and drive tumor evolution. Many recent studies have demonstrated a strong correlation between tumor progression and the presence of cancer-associated inflammation. Chronic inflammation and tissue destruction eventually lead to inhibition of the immune response in a protective negative-feedback mechanism. Suppressive immune cells are recruited to the sites of inflammation and function to inhibit both innate and adaptive immune responses, enabling tumor tolerance and unmitigated tumor progression.

To study the interplay between tumor and immune cells, Zhang lab has developed a unique animal model of breast cancer that reproduces different stages of breast cancer bone metastasis. The model consists of a number of closely related breast epithelial cell lines. These include parent non-tumorigenic cell line FSK4, and primary tumor TM6 (non-metastatic), TM40D (low bone metastatic), TM40D-MB (aggressive metastatic to bone). The differential metastatic potential of the cell lines is in part determined by their immunogenicity. While TM40D cells express CD1d1, TM40D-MB tumors have lost expression of this molecule; transfection of CD1d1 renders TM40D-MB immunogenic and non-metastatic. Recent data from Zhang lab, in collaboration with several Northwestern University immunologists Drs. Paul Stein and
Chyung-Ru Wang, demonstrate an important role of CD1d1 in breast cancer metastasis. Following the line of immune control of cancer metastasis, Zhang lab has also collaborated with Dr. Khashayarsha Khashaie, a gastrointestinal biologist and immunologist at Northwestern University to show that expansion of the tumor cells in the bone corresponds with a drop in the frequency of lymphocytes in the bone marrow at the cost of larger granular leukocytes, typical of the myeloid lineage. In addition, Zhang lab has also obtained evidence that tumor-derived factors in promoting immunosuppression and tumor tolerance using their mouse model. For example, tumors are known to constitutively secrete a number of immunosuppressive cytokines such as TGF-β and IL-10 that promote immune cell recruitment and conversion to a suppressive phenotype. Many tumors have also been shown to upregulate the pro-inflammatory enzyme cyclooxygenase-2 (COX2), which synthesizes and secretes prostaglandin E2 (PGE2). PGE2 secretion by tumors has been demonstrated to directly recruit myeloid-derived suppressor cells (MDSCs) from the bone marrow and activate them at the tumor site to promote immunosuppression and tumor progression. These cells recruit and convert other suppressive immune cells, such as tumor-associated macrophages (TAMs) and regulatory T cells (Tregs), driving chronic inflammation and immunosuppression. Tumors have also been demonstrated to constitutively secrete the enzyme indoleamine-2,3-dioxegenase (IDO) through IFN-γ/STAT1 activation, further inducing immunosuppression and tumor progression. In the analysis of gene microarrays comparing early vs. late stage mammary tumors, Zhang lab has identified changes in many pro-inflammatory genes, including COX2 and the IFN-γ-activated transcription factor STAT1 (Li et al., 2007). They are currently studying roles of these genes in tumor-driven evolution that control chronic inflammation and immunosuppression. They hypothesize that these key pro-inflammatory genes are upregulated in highly metastatic breast cancers, which function synergistically to recruit and activate suppressive MDSCs, TAMs and Tregs, inducing chronic inflammation and an immunosuppressive tumor microenvironment conducive to metastatic breast cancer progression.

Summary
The Zhang laboratory is well positioned to address key questions relating to the function and involvement of maspin and other tumor suppressor genes in tumor progression and the immune components in the control of tumor metastasis. Understanding these questions is important for basic biology and may lead to a therapy for cancer and metastasis disease.

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References
The Robert H. Lurie Comprehensive Cancer Center of Northwestern University funds shared facilities and resources that provide services, equipment and expertise that assist researchers in understanding the basic biology and clinical manifestations of cancer. These facilities and resources are accessible to all of the members of the Cancer Center and support the Cancer Center’s mission to foster basic and translational research in the mechanisms and treatment of cancer.

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The Clinical Research Office (CRO) provides a centralized resource to facilitate the development, conduct, quality assurance monitoring, compliance with regulatory agency requirements, and evaluation of clinical research/trials at the Robert H. Lurie Comprehensive Cancer Center of Northwestern University. As such, the office coordinates the majority of clinical research conducted in medical oncology, malignant hematology, gynecologic-oncology, neuro-oncology, radiation oncology, surgical oncology, and chemoprevention.

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The genomics core at the Center for Genetic Medicine is a shared resource facility that provides a wide range of services to Cancer Center members and the Northwestern University research community. Our goal is to provide services using the state-of-the-art technologies at an affordable price. Currently, we provide expression analysis and SNP analysis using both Affymetrix and Illumina platforms, RT-PCR and low density SNP analysis using 7900HT from ABI, RNA quality control using the Agilent 2100, DNA sequencing using 3730 from ABI, custom array fabrication using MicroGridII and high through put DNA extraction by Autopure LS from Gentra.

Keck Biophysics Facility
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The Keck Biophysics Facility is a unique resource that provides researchers with 24-hour access to state of the art instruments. The facility is designed to facilitate biophysical and biochemical characterization of macromolecules. Services include use of fluorometers, spectrometers, calorimeters, imagers, fermentors, a light scattering instrument, an HPLC and a real-time PCR machine.

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The Monoclonal Antibody Facility provides investigators access to the technology for the efficient creation of hybridoma cell lines and the production of monoclonal antibodies from these cell lines. These services include immunization of animals, somatic cell fusions, cloning and screening of hybridomas, subcloning and establishment of antibody producing cell lines, and production of active antibodies from hybridoma lines. In addition to providing these services, the facility provides consultation and training for investigators.
interested in establishing any of these activities in their own research laboratory or using monoclonal antibodies in their research.

**Mouse Phenotyping Core Facility**  
*Director: Warren G. Tourtellotte, MD*  
*Facility Manager: Donna Emge*  
312.503.2679

The purpose of the facility is to assist investigators with gross and histological characterization of genetically modified murine models. Studies can be performed on individual organs or involve a systemic overview of all major organ systems to identify new target organs for genes. Pathologist consultation will allow the development of strategies to elucidate the phenotype and gain mechanistic insight regarding the biologic actions of the targeted molecule. Investigators can be trained in dissection techniques, as well.

**Outcomes Measurement and Survey Core**  
*Director: Elizabeth Hahn, PhD*  
224.364.7373 or e-hahn@northwestern.edu

The mission of this core facility is to provide consultation and support for research that involves collecting, analyzing or interpreting self-report data, and to promote the understanding of measurement fundamentals and the improvement of research practice. The facility provides consultative and analytic expertise on the best ways to measure outcomes derived by self-report, serve as a central resource for state-of-the-art instruments and measurement methods, and provides in-house research support services for the collection of outcomes data.

**Pathology Core Facility**  
*Director: Ximing Yang, MD, PhD*  
*Facility Manager: Adekunle Raji*  
312.908.9595 or xyang@northwestern.edu  
a-raji@northwestern.edu

The Pathology Core Facility has three main components: research histology, specimen procurement and protocol review. The research histology component provides all of the tissue processing and histology services typically performed in a clinical laboratory but it is specifically dedicated to the needs of the Northwestern University research community in general and the Cancer Center research community in particular. The Pathology Core Facility is unique in that it has the capability and flexibility to address specific research protocol needs. The tissue procurement component of the Pathology Core Facility has two main functions: (1) human tissue and fluid procurement, storage and distribution and (2) quality assurance and protection of research subjects. The tissue procurement component addresses the growing need for human tissue and serves as an “honest broker” with HIPAA-covered entities in an effort to expedite research activities, particularly in the use of human biological materials and associated data.

**Structural Biology Facility**  
*Director: Alfonso Mondragon, PhD*  
*Facility Manager: Pamela Focia, PhD*  
312.503.0848 or a-mondragon@northwestern.edu  
focia@northwestern.edu

The facility is essential for the research programs of investigators who are studying the relationship between macromolecular structure and function or who are using protein structure as the starting point for structure-based drug design. The Structural Biology Facility is a unique resource at Northwestern University that capitalizes on the extensive expertise of a large group of users and regular access to the synchrotron radiation X-ray source at the DND-CAT beamline at the Advanced Photon Source at Argonne National Laboratories. This resource also serves to nucleate the development of a local community with expertise in structural and computational biology.

**Transgenic and Targeted Mutagenesis Laboratory**  
*Director: Warren Tourtellotte, MD, PhD*  
*Director of Core Operations: Lynn T. Doglio, PhD*  
312.503.0088 or warren@northwestern.edu  
l-doglio@northwestern.edu

The Transgenic and Targeted Mutagenesis Core Facility is a university-wide shared resource dedicated to generating genetically-modified animals for investigators within the research community at Northwestern University and its affiliate institutions. Transgenic and gene targeting technologies are used to generate animal models in which the complexities of gene function and regulation can be studied. The ability to either express or functionally inactivate, in genetically modified animals, defined genes in a developmentally- and tissue-specific manner has lead to significant insights into and the understanding of the role genes play under both normal and abnormal conditions in many different and diverse fields of scientific study.
Sankar, N; Baluchamy, S; Kadeppagari, R-K; Singhal, G; **Weitzman, S; Thimmapaya, B**

p300 provides a corepressor function by cooperating with YY1 and HDAC3 to repress c-Myc.


**Abstract**

We showed earlier that p300/CBP plays an important role in G1 progression by negatively regulating c-Myc and thereby preventing premature G1 exit. Here, we have studied the mechanism by which p300 represses c-Myc and show that in quiescent cells p300 cooperates with histone deacetylase 3 (HDAC3) to repress transcription. p300 and HDAC3 are recruited to the upstream YY1-binding site of the c-Myc promoter resulting in chromatin deacetylation and repression of c-Myc transcription. Consistent with this, ablation of p300, YY1 or HDAC3 expression results in chromatin acetylation and induction of c-Myc. These three proteins exist as a complex in vivo and form a multiprotein complex with the YY1-binding site in vitro. The C-terminal region of p300 is both necessary and sufficient for the repression of c-Myc. These and other results suggest that in quiescent cells the C-terminal region of p300 provides corepressor function and facilitates the recruitment of p300 and HDAC3 to the YY1-binding site and represses the c-Myc promoter. This corepressor function of p300 prevents the inappropriate induction of c-Myc and S phase.

Salabat, Mohammad R; Melstrom, Laleh G; Strouch, Matthew J; Ding, Xian-Zhong; Milam, Benjamin M; Ujiki, Michael B; Chen, Catherine; **Pelling, Jill C**; Rao, Sambasiva; Gripp, Paul J; McGarry, Thomas J; Bentrem, David J

Geminin is overexpressed in human pancreatic cancer and downregulated by the bioflavonoid apigenin in pancreatic cancer cell lines.


**Abstract**

Pancreatic adenocarcinoma is among the deadliest of human cancers. Apigenin, an antitumor flavonoid, inhibits pancreatic cancer cell proliferation in vitro. Geminin is a recently identified novel protein that plays a critical role in preventing abnormal DNA replication by binding to and inhibiting the essential replication factor Cdt1. Microarray analysis identified geminin to be downregulated in pancreatic cancer cells treated with apigenin. Therefore, we investigated the effects of apigenin on geminin expression and other proteins involved in replication (Cdc6, Cdt1, and MCM7) in pancreatic cancer cell lines CD18 and S2013. Real time RT-PCR and western blotting analysis showed that geminin expression is downregulated by apigenin at both mRNA and protein levels. Furthermore, treatment of cells with proteosome inhibitor MG132 reversed the downregulation of geminin by apigenin, supporting our hypothesis that the degradation pathway is another mechanism by which apigenin affects geminin expression. Apigenin treatment also resulted in
downregulation of Cdc6 at both mRNA and protein levels. However, Cdt1 and MCM7 expression was not affected in apigenin-treated cells. The effect of apigenin treatment on geminin promoter activity was measured by transient transfection of Hela cells with a reporter gene, demonstrating that apigenin inhibited geminin promoter activity. Geminin expression was also evaluated in human pancreatic tissue (n = 15) by immunohistochemistry and showed that geminin is overexpressed in human pancreatic cancer compared to normal adjacent pancreatic tissue. In conclusion, our studies demonstrated that geminin is overexpressed in human pancreatic cancer and downregulated by apigenin which may contribute to the antitumor effect of this natural flavonoid

Subramanian, Hariharan; Pradhan, Prabhakar; Liu, Yang; Capoglu, Ilker R; Li, Xu; Rogers, Jeremy D; Heifetz, Alexander; Kunte, Dhananjay; Roy, Hemant K; Taflove, Allen; Backman, Vadim

Optical methodology for detecting histologically unapparent nanoscale consequences of genetic alterations in biological cells.


Abstract

Recently, there has been a major thrust to understand biological processes at the nanoscale. Optical microscopy has been exceedingly useful in imaging cell microarchitecture. Characterization of cell organization at the nanoscale, however, has been stymied by the lack of practical means of cell analysis at these small scales. To address this need, we developed a microscopic spectroscopy technique, single-cell partial-wave spectroscopy (PWS), which provides insights into the statistical properties of the nanoscale architecture of biological cells beyond what conventional microscopy reveals. Coupled with the mesoscopic light transport theory, PWS quantifies the disorder strength of intracellular architecture. As an illustration of the potential of the technique, in the experiments with cell lines and an animal model of colon carcinogenesis we show that increase in the degree of disorder in cell nanoarchitecture parallels genetic events in the early stages of carcinogenesis in otherwise microscopically/histologically normal-appearing cells. These data indicate that this advance in single-cell optics represented by PWS may have significant biomedical applications.

Ibrahim, Saad M; Mulcahy, Mary F; Lewandowski, Robert J; Sato, Kent T; Ryu, Robert K; Masterson, Elizabeth J; Newman, Steven B; Benson, Al III; Omary, Reed A; Salem, Riad

Treatment of unresectable cholangiocarcinoma using yttrium-90 microspheres: results from a pilot study.


Abstract

BACKGROUND: The objective of this report was to present data from an open-label cohort study in which patients with intrahepatic cholangiocarcinoma (ICC) underwent radioembolization with yttrium-90 (90Y) microspheres. METHODS: Twenty-four patients with histologically proven ICC were treated. The planned target dose was 120 Gray. Patients were stratified according to Eastern Cooperation Oncology Group (ECOG) performance status, tumor morphology (infiltrative vs peripheral), tumor distribution (solitary vs multifocal), and the presence or absence of portal vein thrombosis (PVT). Before and after the procedure, the following variables were assessed: 1) biochemical and clinical toxicity, 2) imaging (computed tomography/magnetic resonance imaging) response according to World Health Organization and European Association for the Study of Liver Disease (EASL) criteria, and 3) median survival after the first treatment using Kaplan-Meier methodology. RESULTS: In total, 48 (90)Y treatments were administered to hepatic segments or lobes. Fatigue and transient abdominal pain were reported in 18 patients (75%) and 10 patients (42%), respectively. One patient (4%) developed grade 3 bilirubin toxicity. One patient (4%) developed a treatment-related gastroduodenal ulcer. On imaging follow-up of 22 patients, tumors demonstrated a partial response in 6 patients (27%), stable disease in 15 patients (68%), and progressive disease in 1 patient (5%). By using
EA SL guidelines, 17 patients (77%) showed >50% tumor necrosis on imaging follow-up. Two patients (9%) demonstrated 100% tumor necrosis. The median overall survival for the entire cohort (n = 24) was 14.9 months. The median survival for patients with an ECOG performance status of 0, 1, and 2 was 31.8 months, 6.1 months, and 1 month, respectively (P < .0001); the median survival for patients without and with PVT was 31.8 months and 5.7 months, respectively (P = .0003); and the median survival for patients with peripheral versus periductal-infiltrative tumors was 31.8 months and 5.7 months, respectively (P = .0005). CONCLUSIONS: Radioembolization with (90)Y may be a therapeutic option for the treatment of unresectable ICC. Cancer 2008.

Edwards, Beatrice J; Gounder, Mrinal; McKoy, June M; Boyd, Ian; Farrugia, Mathew; Migliorati, Cesar; Marx, Robert; Ruggiero, Salvatore; Dimopoulos, Meletios; Raisch, Dennis W; Singhal, Seema; Carson, Ken; Obadina, Eniola; Trifilio, Steve; West, Dennis; Mehta, Jayesh; Bennett, Charles L

Pharmacovigilance and reporting oversight in US FDA fast-track process: bisphosphonates and osteonecrosis of the jaw.


Abstract
More than half of all serious adverse reactions are identified 7 or more years after a drug receives approval from the US Food and Drug Administration (FDA). In 2002, 9 months after the intravenous bisphosphonate zoledronic acid received regulatory approval for marketing, the FDA received reports of nine patients with cancer, who were treated with zoledronic acid, who unexpectedly developed osteonecrosis of the jaw. During the next 2 years, three oral surgeons described 104 patients with cancer with osteonecrosis of the jaw in the medical literature and identified intravenous bisphosphonate therapy as being common to the care of these patients. In subspecialty medical, radiology, and dental journals, case reports and case series described clinical features of osteonecrosis of the jaw in patients with cancer who were treated with bisphosphonates. Manufacturer-sponsored epidemiological studies reported the first estimates of the incidence of this toxic effect, ranging from 0.1% to 1.8%. By contrast, independent epidemiological efforts from clinicians and the International Myeloma Foundation reported incidence estimates between 5% and 10%. Between 2003 and 2005, warnings about the risks of bisphosphonate-associated osteonecrosis were disseminated by national regulatory agencies, the manufacturers of bisphosphonates, and the International Myeloma Foundation. From 2006, independent clinical recommendations for diagnosis, prevention, and treatment of this toxic effect have been disseminated by manufacturers, national regulatory authorities, the International Myeloma Foundation, and medical specialty organisations. Furthermore, independent efforts by pharmaceutical manufacturers, dental and medical professionals, a non-profit organisation (the International Myeloma Foundation), patients, and regulatory authorities has led to the rapid identification and dissemination of safety information for this serious adverse reaction. Better coordination of safety-related pharmacovigilance initiatives is now needed.

Cella, David; Li, Jim Z; Cappelleri, Joseph C; Bushmakin, Andrew; Charbonneau, Claudie; Kim, Sindy T; Chen, Isan; Motzer, Robert J

Quality of life in patients with metastatic renal cell carcinoma treated with sunitinib or interferon alfa: results from a phase III randomized trial.


Abstract
PURPOSE: In an international, randomized phase III trial, sunitinib demonstrated statistically significant efficacy over interferon alfa (IFN-alpha) as first-line therapy in patients with metastatic renal cell carcinoma (mRCC) (progression-free survival time, 11 v 5 months, respectively; P < .001; objective response rate, 31% v 6%, respectively; P < .001). We report health-related quality-of-life (QOL) results from this trial. PATIENTS AND METHODS: Seven hundred fifty mRCC patients were randomly assigned to sunitinib (6-week cycles: 50 mg orally once daily for 4 weeks, followed by 2 weeks off) or IFN-alpha (9 million units subcutaneous injections, three times weekly). QOL measures included the Functional...
Assessment of Cancer Therapy-General (FACT-G), the FACT-Kidney Symptom Index-15 item (FKSI-15), and the EuroQoL-5D’s utility score (EQ-5D Index) and its visual analog scale (EQ-VAS). The primary QOL end point was the FKSI Disease-Related Symptoms (FKSI-DRS) subscale. Higher scores indicated better outcomes (better QOL or fewer symptoms). Data were analyzed for the intent-to-treat population using mixed-effects models, supplemented with pattern-mixture models.

RESULTS: Patients receiving sunitinib reported higher FKSI-15 and FKSI-DRS scores at each cycle than those receiving IFN-alpha, with a significant difference in the overall least squares means (3.27 and 1.98, respectively; P < .0001). Similarly, differences in least squares means for FACT-G (and all subscales), EQ-5D Index, and EQ-VAS were all significantly favorable for sunitinib (P < .01). Per pre-established thresholds, between-treatment differences in the mean scores were clinically meaningful after cycle 4 for FKSI-DRS and at all assessments for FKSI-15, FACT-G, and the FACT-G functional well-being subscale. CONCLUSION: Sunitinib provides superior QOL compared with IFN-alpha in mRCC patients.

Kaklamani, Virginia G; Wisinski, Kari B; Sadim, Maureen; Gulden, Cassandra; Do, Albert; Offit, Kenneth; Baron, John A; Ahsan, Habibul; Mantzoros, Christos; Pasche, Boris

Variants of the adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) genes and colorectal cancer risk.


Abstract

CONTEXT: Current epidemiological evidence suggests an association between obesity, hyperinsulinemia, and colorectal cancer risk. Adiponectin is a hormone secreted by the adipose tissue, and serum levels are inversely correlated with obesity and hyperinsulinemia. While there is evidence of an association between circulating adiponectin levels and colorectal cancer risk, no association between genes of the adiponectin pathway and colorectal cancer have been reported to date.

OBJECTIVE: To determine the association of 10 haplotype-tagging single-nucleotide polymorphisms (SNPs) of the adiponectin (ADIPOQ) and adiponectin receptor 1 (ADIPOR1) genes with colorectal cancer risk.

**DESIGN, SETTING, AND PATIENTS:** Two case-control studies including patients with a diagnosis of colorectal cancer and controls were recruited between 2000 and 2007. Case-control study 1 included a total of 441 patients with a diagnosis of colorectal cancer and 658 controls; both groups were of Ashkenazi Jewish ancestry and from New York, New York. Case-control study 2 included 199 patients with a diagnosis of colorectal cancer and 199 controls from Chicago, Illinois, matched 1:1 for sex, age, and ethnicity. MAIN OUTCOME MEASURES: ADIPOQ and ADIPOR1 SNP frequency among cases and controls.

RESULTS: In study 1, after adjustment for age, sex, and SNPs from the same gene, 3 ADIPOQ SNPs and 1 ADIPOR1 SNP were associated with colorectal cancer risk: rs266729 (adjusted odds ratio [AOR], 0.72; 95% confidence interval [CI], 0.55-0.95) and rs822396 (AOR, 0.37; 95% CI, 0.14-1.00) were associated with decreased risk whereas rs822395 (AOR, 1.76; 95% CI, 1.09-2.84) and rs1342387 (AOR, 1.79; 95% CI, 1.18-2.72) were associated with increased risk. In study 2, after adjustment for age, sex, race, and SNPs from the same gene, the ADIPOQ SNP rs266729 was associated with a decreased colorectal cancer risk of similar magnitude as in study 1 (AOR, 0.52; 95% CI, 0.34-0.78). Combined analysis of both studies shows an association of rs266729 with decreased colorectal cancer risk (AOR, 0.73; 95% CI, 0.53-0.99). CONCLUSION: The SNP rs266729, which tags the 5’ flanking region of the ADIPOQ gene, is associated with decreased colorectal cancer risk.

Loeb, Stacy; Sutherland, Douglas E; D’Amico, Anthony V; Roehl, Kimberly A; Catalona, William J

PSA velocity is associated with gleason score in radical prostatectomy specimen: marker for prostate cancer aggressiveness.


Abstract

OBJECTIVES: Conflicting evidence has been reported on the association of prostate-specific antigen velocity (PSAV) with Gleason score in prostate needle biopsy specimens. The Gleason score is an important prognostic indicator for
men with prostate cancer, and, in modern practice, it frequently affects treatment decisions. To our knowledge, the relationship between preoperative PSAV and Gleason score in the radical prostatectomy specimen has not been formally demonstrated. METHODS: A total of 1049 men treated with radical prostatectomy had data on PSAV and Gleason score. Statistical analysis was performed to examine the relationship between the preoperative PSAV and the prostatectomy Gleason score and other adverse tumor features. RESULTS: The median preoperative PSAV was 0.84, 0.97, and 1.39 ng/mL/y in men with a Gleason score of 6, 7, and 8-10, respectively (P = .05). A PSAV greater than 2 ng/mL/y was significantly associated with a prostatectomy Gleason score of 7 or greater on univariate and multivariate analysis. In addition, the preoperative PSAV was significantly lower in men with organ-confined disease (0.82 vs 1.17 ng/mL/y, respectively, P = .002). CONCLUSIONS: Our results have further validated PSAV as a marker for prostate cancer aggressiveness. The preoperative PSAV was a significant independent predictor of the Gleason score and non-organ-confined disease in the radical prostatectomy specimen. Thus, PSAV could be useful in treatment decision-making and in assessing the likelihood of long-term cancer control in men with prostate cancer.

Woodard, Jennifer; Sassano, Antonella; Hay, Nissim; Platanias, Leonidas C

Statin-dependent suppression of the Akt/mammalian target of rapamycin signaling cascade and programmed cell death 4 up-regulation in renal cell carcinoma.


Abstract

PURPOSE: Statins are pharmacologic inhibitors of the 3-hydroxy-3-methylglutaryl-coenzyme A reductase with potent regulatory effects on cholesterol biosynthesis in vitro and in vivo. There is accumulating evidence that, beyond their cholesterol-lowering properties, statins inhibit cell proliferation and promote apoptosis of malignant cells in vitro, but the mechanisms by which they generate such responses remain to be defined.

EXPERIMENTAL DESIGN: Combinations of experimental approaches were used, including immunoblotting and cell proliferation and apoptosis assays. RESULTS: We provide evidence that fluvastatin is a potent inducer of apoptosis and suppresses proliferation of renal cell carcinoma (RCC) cells in vitro. Such effects are mediated by direct targeting of the Akt/mammalian target of rapamycin (mTOR) pathway, as evidenced by the suppression of phosphorylation/activation of Akt, resulting in inhibition of its downstream effectors, mTOR and p70 S6 kinase. In addition, fluvastatin blocks the mTOR-dependent phosphorylation/deactivation of the translational repressor eukaryotic initiation factor 4E (eIF4E)-binding protein, leading to the formation of eIF4E-binding protein-eIF4E complexes that suppress initiation of cap-dependent mRNA translation. Importantly, inhibition of p70 S6 kinase activity by fluvastatin results in the up-regulation of expression of programmed cell death 4 (PDCD4), a tumor suppressor protein with inhibitory effects on the translation initiation factor eIF4A, suggesting a mechanism for the generation of antitumor responses.

CONCLUSIONS: Altogether, our findings establish that fluvastatin exhibits potent anti-RCC activities via inhibitory effects on the Akt/mTOR pathway and raise the possibility that combinations of statins and Akt inhibitors may be of future therapeutic value in the treatment of RCC.

Kelemen, Katalin; Peterson, Loann C; Helenowski, Irene; Goolsby, Charles L; Jovanovic, Borko; Miyata, Sarah; Aranha, Olivia; Rosen, Steven T; Winter, Jane N; Nelson, Beverly P; Gordon, Leo I; Evens, Andrew M

CD23+ mantle cell lymphoma: a clinical pathologic entity associated with superior outcome compared with CD23- disease.


Abstract

Mantle cell lymphoma (MCL) commonly lacks expression of CD23. However, a significant minority of MCLs express CD23, as assessed by flow cytometric immunophenotyping (FCIP).
The aims of our study were to investigate the expression of CD23 by FCIP in patients with MCL and to correlate CD23 expression with pathologic and clinical parameters, including outcome. We studied 53 patients with untreated MCL who had CD23 expression determined by FCIP. At diagnosis, 14 MCLs (26%) were CD23+ at all tissue sites, whereas 33 (62%) were CD23-, and 6 (11%) had discordant CD23 expression among different tissue sites. Patients with CD23- MCL had extranodal disease more commonly compared with patients with CD23+ MCL. Moreover, with 57-month median follow-up, the 4-year event-free and overall survival rates for CD23+ MCL were 45% and 75%, respectively, compared with 19% and 51% for CD23- MCL. In multivariate Cox regression analysis, CD23 status and leukemic-phase MCL were the most important factors predicting outcome.

Gerami, Pedram; Wickless, Scott C; Rosen, Steve; Kuzel, Timothy M; Ciurea, Ana; Havey, Jilian; Guitart, Joan

Applying the new TNM classification system for primary cutaneous lymphomas other than mycosis fungoides and Sezary syndrome in primary cutaneous marginal zone lymphoma.


Abstract
BACKGROUND: Primary cutaneous marginal zone lymphoma is recognized as a unique subset of low-grade cutaneous B-cell lymphoma with indolent course in the current World Health Organization-European Organization on Research and Treatment of Cancer classification system. However, few large series on this entity have been reported, including the new TNM (tumor, (lymph) node, metastasis) classification for non-mycosis fungoides cutaneous lymphomas. OBJECTIVE: We aimed to characterize the clinical features including new TNM classification for non-mycosis fungoides cutaneous lymphomas, as well as outcomes and responses to therapy in 30 patients with primary cutaneous marginal zone lymphoma. RESULTS: Primary cutaneous marginal zone lymphoma typically presents with deep-seated nodular or papular lesions on the upper extremities or trunk (25/30). Disease course is indolent and none of 30 patients died of disease. Sustainable complete remissions were obtained only in patients with T1a (n = 3) and T2a (n = 1) disease. Most patients have persistent stable disease independent of treatment. Two patients developed systemic disease and 5 developed large cell transformation. LIMITATIONS: The average follow-up time was 63 months (range, 3-204 months). Longer follow-up time is needed to determine whether patients with untreated persistent stable disease are at greater risk relative to patients treated aggressively early in the disease course. CONCLUSIONS: Primary cutaneous marginal zone lymphoma is a distinct subtype of marginal zone lymphoma with an indolent disease course. Patients with T1a or T2a disease (ie, single lesions or a localized cluster of lesions) may achieve sustained complete remission, whereas patients with multiple nonlocalized lesions are unlikely to maintain complete remission independent of treatment modality. Systemic involvement is typically preceded by large cell transformation and may be an indication for more systemic therapy. Death from disease is rare.

Demou, Zoe N; Hendrix, Mary J C

Microgenomics profile the endogenous angiogenic phenotype in subpopulations of aggressive melanoma.


Abstract
Beyond the elemental role of blood vessels in tumor growth, fluid conducting networks lacking endothelium (termed vasculogenic mimicry) were identified previously in metastatic melanoma and other cancer types. The etiology remains unclear, though it appears to involve dysregulation of the tumor-specific phenotype and transdifferentiation. Instigating the molecular deciphering of this phenomenon, we established a novel technique for microdissecting the spontaneously formed vascular-like networks and the randomly arranged cells (nests) from living 3D cultures of melanoma and performed microgenomics analysis. For the first time we show that despite the shared genotype, transcription was differentially regulated among the phenotypically distinct melanoma structures in vasculogenenic mimicry. Several angiogenesis-
specific genes were differentially expressed in higher levels in network cells of both uveal and cutaneous melanoma with intriguing representation of the ephrin family of angiogenesis factors, which was confirmed with immunocytochemistry. Interestingly, the adjacent nest-cells over-expressed ECM-related genes. Moreover, expression of angiogenesis-specific genes in melanoma resembled that of normal microvascular cells and was enhanced in melanoma disseminating hematogenously. The findings suggest that melanoma plasticity could enable autopoiesis of vascular-mimicking elements within the tumor infrastructure with significant clinical implications, such as response to anti-angiogenic treatments. Identifying factors regulating tumor plasticity and heterogeneity at the molecular level is essential in designing effective anti-cancer therapies.

Fushimi, Kazuo; Ray, Payal; Kar, Amar; Wang, Lei; Sutherland, Leslie C; Wu, Jane Y

Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5.


Abstract

Similar to many genes involved in programmed cell death (PCD), the caspase 2 (casp-2) gene generates both proapoptotic and antiapoptotic isoforms by alternative splicing. Using a yeast RNA-protein interaction assay, we identified RBM5 (also known as LUCA-15) as a protein that binds to casp-2 pre-mRNA. In both transfected cells and in vitro splicing assay, RBM5 enhances the formation of proapoptotic Casp-2L. RBM5 binds to a U/C-rich sequence immediately upstream of the previously identified In100 splicing repressor element. Our mutagenesis experiments demonstrate that RBM5 binding to this intronic sequence regulates the ratio of proapoptotic/antiapoptotic casp-2 splicing isoforms, suggesting that casp-2 splicing regulation by RBM5 may contribute to its tumor suppressor activity. Our work has uncovered a player in casp-2 alternative splicing regulation and revealed a link between the alternative splicing regulator and the candidate tumor suppressor gene. Together with previous studies, our work suggests that splicing control of cell death genes may be an important aspect in tumorigenesis. Enhancing the expression or activities of splicing regulators that promote the production of proapoptotic splicing isoforms might provide a therapeutic approach to cancer.
Polo, Jose M; Ci, Weimin; Licht, Jonathan D; Melnick, Ari

Reversible disruption of BCL6 repression complexes by CD40 signaling in normal and malignant B cells.


Abstract

Germinal center (GC) B cells undergo somatic hypermutation, class switch recombination, and rapid clonal expansion to produce high-affinity antibodies. The BCL6 transcriptional repressor facilitates this phenotype because it can repress DNA damage checkpoint genes. GC B and T cells can make transient direct physical contact; T cells were observed to be associated with dead B-cell fragments. We thus hypothesized that one function of CD40 signaling from T cells within this timeframe could be to modulate BCL6 activity. CD40 signaling rapidly disrupts the ability of BCL6 to recruit the SMRT corepressor complex by excluding it from the nucleus, leading to histone acetylation, RNA polymerase II processivity, and activation of BCL6 target genes, such as CD23b, ATR, and TP53. Washout of CD40 to emulate transient T-cell contact permitted BCL6 target gene mRNA levels to return to their repressed levels, demonstrating that this is a reversible process, which could allow centroblasts that pass quality control to either continue proliferation or undergo terminal differentiation. These data suggest that transient CD40 signaling in the GC might allow T cells to weed out heavily damaged centroblasts while at the same time promoting survival of intact B cells, which could undergo differentiation or additional rounds of proliferation.

Zheng, Jiamao; Koblinski, Jennifer E; Dutson, Laura V; Feeney, Yvonne B; Clevenger, Charles V

Prolyl isomerase cyclophilin A regulation of Janus-activated kinase 2 and the progression of human breast cancer.


Abstract

The activation of the Janus-activated kinase 2 (Jak2) tyrosine kinase following ligand binding has remained incompletely characterized at the mechanistic level. We report that the peptidyl-prolyl isomerase (PPI) cyclophilin A (CypA), which is implicated in the regulation of protein conformation, is necessary for the prolactin (PRL)-induced activation of Jak2 and the progression of human breast cancer. A direct correlation was observed between the levels or activity of CypA and the extent of PRL-induced signaling and gene expression. Loss of PRLr-CypA binding, following treatment with the PPI inhibitor cyclosporine A (CsA), or overexpression of a dominant-negative PRLr mutant (P334A) resulted in a loss of PRLr/Jak2-mediated signaling. In vitro, CsA treatment of breast cancer cells inhibited their growth, motility, invasion, and soft agar colony formation. In vivo, CsA treatment of nude mice xenografted with breast cancer cells induced tumor necrosis and completely inhibited metastasis. These studies reveal that a CypA-mediated conformational change within the PRLr/Jak2 complex is required for PRL-induced transduction and function and indicate that the inhibition of prolyl isomerases may be a novel therapeutic strategy in the treatment of human breast cancer.

Gaba, Ron C; Wang, Dingxin; Lewandowski, Robert J; Ryu, Robert K; Sato, Kent T; Kulik, Laura M; Mulcahy, Mary F; Larson, Andrew C; Salem, Riad; Omary, Reed A

Four-dimensional transcatheter intraarterial perfusion MR imaging for monitoring chemoembolization of hepatocellular carcinoma: preliminary results.


Abstract

Purpose: Angiographic endpoints for chemoembolization of hepatocellular carcinoma (HCC) are subjective, and optimal endpoints remain unknown. Transcatheter intraarterial perfusion (TRIP) magnetic resonance (MR) imaging, when performed in a combined MR/interventional radiology (MR-IR) suite, offers an objective method to quantify intraprocedural tumor perfusion changes, but was previously limited to two spatial dimensions. This study prospectively tested the hypothesis that a new volumetric acquisition
over time, four-dimensional TRIP MR imaging, can measure HCC perfusion changes during chemoembolization. MATERIALS AND METHODS: Seven men (mean age, 53 years; range, 42-65 y) with eight tumors (mean size, 2.5 x 2.4 cm(2); diameter range, 1.5-5.2 cm) underwent chemoembolization in an MR-IR suite between February and December 2007, with intraprocedural tumor perfusion reductions monitored with four-dimensional TRIP MR imaging. Microcatheter chemoembolization was performed with a 1:1 mixture of chemotherapy agent and emulsifying contrast agent, followed by the administration of gelatin microspheres. Pre- and post-chemoembolization time-intensity curves were generated for each tumor. Semiquantitative measures of tumor perfusion, including area under the curve (AUC), peak signal intensity (SI), time to peak SI, and maximum upslope (MUS), were calculated, and mean differences before and after chemoembolization were compared with paired t tests. RESULTS: Four-dimensional TRIP MR imaging-monitored chemoembolization was successful in all cases. Calculated AUCs before and after chemoembolization (439 vs 221, P = .004, 50% reduction), peak SI (32 vs 19, P = .012, 41% reduction), and MUS (11 vs 3, P = .028, 73% reduction) showed significant reductions after chemoembolization. Time to peak SI did not significantly change (23 sec vs 36 sec, P = .235, 57% increase). CONCLUSIONS: Four-dimensional TRIP MR imaging can successfully measure semiquantitative changes in HCC perfusion during MR-IR-monitored chemoembolization. Future studies may correlate changes in these objective functional parameters with tumor response.
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  Executive Associate Dean for Clinical and Translational Research  
  Feinberg School of Medicine

- **Mary Hendrix, PhD**  
  President & Scientific Director, Children’s Memorial Research Center

- **J. Larry Jameson, MD, PhD**  
  Vice President for Medical Affairs and Lewis Landsberg Dean  
  Feinberg School of Medicine

- **Peter Kopp, MD**  
  Interim Director, Center for Genetic Medicine  
  Feinberg School of Medicine

- **Alicia Löffler, PhD**  
  Director, Kellogg Center for Biotechnology  
  Kellogg School of Management

- **Jeffrey Miller**  
  Vice Dean and Chief Operating Officer  
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- **Bharat Mittal, MD**  
  Chairman, Dept. of Radiation Oncology  
  Feinberg School of Medicine

- **William Muller, MD, PhD**  
  Chairman, Department of Pathology  
  Feinberg School of Medicine

- **Leonidas C. Platanias, MD, PhD**  
  Deputy Director, Lurie Cancer Center  
  Feinberg School of Medicine

- **Steven T. Rosen, MD**  
  Director, Lurie Cancer Center  
  Feinberg School of Medicine

- **Eric Russell, MD**  
  Chairman, Department of Radiology  
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- **Douglas Vaughan, MD**  
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- **Tim Volpe**  
  Associate Director, Administration  
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  Feinberg School of Medicine

- **Joseph (Jay) Walsh, PhD**  
  Vice President for Research  
  Northwestern University

- **Teresa Woodruff, PhD**  
  Executive Director, Institute for Women’s Health Research  
  Feinberg School of Medicine
CONTINUING MEDICAL EDUCATION PROGRAMS
Throughout the year, the Lurie Cancer Center offers Continuing Medical Education (CME) programs on various cancer specialties. Below is a list of programs for 2009. For registration and additional information, visit cancer.northwestern.edu/education or 312.695.1204.

11th Annual Lynn Sage Breast Cancer Symposium
October 1-4, 2009
Fairmont Hotel
Chair: William Gradishar, MD
lynn sage breast cancer.org

NCCN Clinical Practice Guidelines in Oncology Symposium: Kidney Cancer
October 20, 2009
The Westin Michigan Avenue Chicago

Brain Tumor Symposium
November 6, 2009
Prentice Women’s Hospital, 3rd Floor
Chairs: Kenji Muro, MD and Sean Grimm, MD

International Conference on Differentiation Therapy
November 11-14, 2009
Hyatt Regency Chicago
Chairs: Martin Tallman, MD and Jonathan Licht, MD

12th Annual Oncology Nursing Conference
December 4, 2009
Prentice Women’s Hospital, 3rd Floor
Chairs: Barbara Gobel, RN, MS, AOCN and Lynda Chick, RN
COMMUNITY EVENTS / PATIENT PROGRAMS

The Lurie Cancer Center is committed to educating the public about cancer prevention and treatment, and offers a wide range of community events and patient programs throughout the year. For more information about these programs, visit cancer.northwestern.edu/events or call 312.695.1304.

Gynecologic Cancer Foundation Ovarian Cancer Survivors Course

*September 12, 2009*
*Robert H. Lurie Medical Research Center, Hughes Auditorium*
*Chair: Julian Schink, MD*

**Lynn Sage Breast Cancer Town Hall Meeting**

*October 4, 2009*
*Fairmont Hotel*
*Chair: William Gradishar, MD*

**Brain Tumor Patient and Caregiver Seminar**

*November 3, 2009*
*Lurie Medical Research Center, Baldwin Auditorium*
*Chair: Laurie Rice, RN, MSN, ANP-BC*

**Chronic Lymphocytic Leukemia Patient Education Forum**

*November 4, 2009*
*Robert H. Lurie Medical Research Center, Hughes Auditorium*
*Chair: Steven Rosen, MD*

**Cutaneous T-cell Lymphoma Patient Education Symposium**

*November 19, 2009*
*Prentice Women’s Hospital, 3rd Floor*
The Robert H. Lurie Comprehensive Cancer Center of Northwestern University is the focus of cancer research, treatment and education at Northwestern University. The Lurie Cancer Center coordinates and integrates the University’s cancer and cancer-related activities and unites scientists, clinicians and educators in the fight against cancer. The Lurie Cancer Center’s administrative offices and many of its basic science research activities are at Northwestern University’s Feinberg School of Medicine on the Chicago campus. Additional offices and basic science research labs are located on the Evanston campus. Clinical research is conducted at the Feinberg School of Medicine’s various affiliated teaching hospitals: Northwestern Memorial Hospital, Children’s Memorial Hospital, the Rehabilitation Institute of Chicago and Jesse Brown VA Medical Center.
First established at Northwestern University in 1974, the Cancer Center was invigorated in 1989 when Ann Lurie and Robert H. Lurie made a commitment to endow an institution dedicated to research and advancement in the battle against cancer. In 1991, the Cancer Center was dedicated as the Robert H. Lurie Cancer Center of Northwestern University. This title was modified in 1998, when the National Cancer Institute (NCI) awarded the Cancer Center the highly competitive “comprehensive” designation. Today, the Robert H. Lurie Comprehensive Cancer Center of Northwestern University stands among the country’s leaders as one of only 41 cancer centers in the nation to hold this NCI distinction.

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