“Do not go where the path may lead, go instead where there is no path and leave a trail.”

— Ralph Waldo Emerson
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The risk of losing the ability to have children due to cancer treatment is a source of concern for more than 135,000 children, adolescents and young adults (ages 15-39) diagnosed with cancer each year in the United States. Understanding the need to focus on the threat to fertility posed by cancer treatment, Lurie Cancer Center member Teresa Woodruff, PhD, the Thomas J. Watkins Memorial Professor of Obstetrics & Gynecology, established the Northwestern University Oncofertility Consortium in 2005. Woodruff was the recipient of a $21 million National Institute of Health (NIH) Roadmap Grant to support these efforts in 2007. She assembled a team of oncologists, fertility specialists, social scientists, educators and policy makers to collaborate and translate her research to the clinical care of women who will lose their fertility due to cancer treatment. To describe this effort, she coined the term oncofertility, a word that is now officially recognized as a new ‘slang’ term in the English language.

The Consortium’s goal in 2007 was to establish a handful of oncofertility centers throughout the United States. Today, the Oncofertility Consortium spans over 50 sites nationwide that use research and technology to integrate cancer treatment with fertility-sparing techniques at the time of diagnosis. The number of young cancer patients (men and woman) provided with a fertility preservation consultation has increased by a staggering 45% within the past five years, and these numbers are expected to double in 2011; evidence that there is a strong desire by the medical community as well as young cancer patients to have fertility preservation options available.

In addition to housing the Administrative Core and providing research space, materials, and support for five of the Consortium’s eleven research projects, Northwestern hosts the annual oncofertility Consortium Conference and the annual meeting to discuss Oncofertility from the perspective of the social sciences and humanities. The Consortium has partnered with Northwestern Memorial Hospital to provide oncofertility services to patients and their families in Chicago and across the United States.

Much of the Consortium’s success can be attributed to their commitment to educating scholars and patients on fertility-preservation options. They’ve developed a wide range of online resources, a hotline for patients and families, and most recently, an iPhone App--as well as making breakthroughs in basic science and clinical medicine research. Their efforts are paving the way for the many cancer survivors who dream of building a family.
A lifelong interest in science combined with the desire to make life better for others made the study of medicine compelling for Raymond Bergan, MD, Professor of Hematology / Oncology and Preventive Medicine at Northwestern University Feinberg School of Medicine. “I just wanted to take care of people who really needed my help,” he says.

Bergan grew up in Eden, New York, a small rural town outside of Buffalo. The youngest of three boys, Raymond and his siblings loved being outdoors and especially liked camping out in the woods near their home. “We practically lived in the woods,” he says. “It was great.”

His interest in science began in childhood and led him to pursue a degree in biochemistry at the State University of New York at Buffalo. He received his MD from SUNY Syracuse, where he also did his residency in internal medicine. Bergan’s fellowship in medical oncology at the National Institutes of Health was followed by a research fellowship there in drug discovery. He came to Northwestern University in 1998.

Research Work
A scientist and oncologist, Bergan is Director of Experimental Therapeutics at the Lurie Cancer Center. His laboratory is interested in understanding the molecular pharmacology of cancer chemopreventive agents and elucidating the pathways that determine how cells regulate processes such as metastasis. A current project involves a natural product called genistein, (a chemical found in soy), which may inhibit metastases in prostate cancer.

In the mid-1990’s, after observing the low incidence of metastatic disease in the Chinese population, Bergan and his team hypothesized that soy, which features prominently in Asian diets, might play a role. They devised a study to test genistein and have so far completed Phase I trials and a Phase II trial to determine efficacy. Another, larger Phase II study to determine whether genistein blocks cancer cells from moving out of the prostate gland to other parts...
of the body, is underway. While research is ongoing, Bergan says, “it looks promising.” Genistein has been shown to be safe, is well tolerated, and has good pharmacology.

Bergan is also co-Director, along with Karl Scheidt, PhD, of the Northwestern Center for Molecular Innovation and Drug Discovery (CMIDDD), which promotes an interdisciplinary approach that researchers hope will lead to effective new therapeutics to combat cancer, neurodegenerative diseases and a host of other medical conditions. “Drug discovery requires people from very different backgrounds,” says Bergan. “We need chemists to create new small molecules, biologists to analyze them in the laboratory, and physicians to understand the concepts related to humans.”

Bergan and Scheidt are working on developing a chemical agent they hope will prove to be more targeted, and perhaps more potent, against prostate cancer metastases. While natural compounds, such as genistein, have many advantages, they tend to do several things at once, Bergan explains. Over time, “those things can cause problems for patients.” Chemical agents, on the other hand, can be more specific.

Still in its early stages, their research is encouraging. “We’ve found the compound works on human prostate cancer cells in vitro, and stops the cells from metastasizing in mice,” Bergan says.

**Cancer Prevention Program**

In addition, Bergan is Co-Leader of the Lurie Cancer Center’s Cancer Prevention Program, a multidisciplinary effort focused on epidemiology, early detection, chemoprevention, and behavior modification. He works closely with Bonnie Spring, PhD, a psychologist and expert in behavioral risk factors who shares the leadership role with him, to encourage research on primary and secondary cancer prevention. The Cancer Prevention Program’s team spans a wide range of disciplines, and consists of 30 faculty members from 10 departments and three schools.

The chemoprevention aspect of the program involves pharmacologic intervention at the earliest stages of cancer, as well as before it presents in healthy individuals who are at high risk for certain cancers. Their research is focused primarily on discovering chemoprevention agents (such as genistein) for breast, prostate, skin, ovarian, and colorectal cancers.

Bergan emphasizes the importance of a comprehensive approach to developing strategies for a healthier life. He cites a virtual personal coaching program developed by Bonnie Spring that can be downloaded to a PDA as an example of a tool with potential for positive impact. “Chemoprevention and behavior modification go hand-in-hand,” he says.

Bergan lives with his wife, Gail, and their three children in the Chicago neighborhood of Edgewater, where they all take advantage of their proximity to bike paths and the beach!
Hard work and accomplishment come naturally to Bharat Mittal, MD, Professor and Chair of the Department of Radiation Oncology at Northwestern University Feinberg School of Medicine. In addition to his busy clinical practice at the Lurie Cancer Center where he treats patients for lymphomas, skin, and head and neck cancers, Dr. Mittal has an active research program and is deeply involved in educating and mentoring students. He thrives on the wide range of challenges, asserting the “variety keeps life interesting.”

Mittal grew up in the northern part of India, and became interested in medicine when his cousin, a general practitioner, invited the young high school student to accompany him on his rounds. Even then, he enjoyed the patient interaction and was inspired by his cousin’s ability to help others. “It just gave me a sense that medicine would allow me to do something for others, something worthwhile,” says Mittal. During his internal medicine rotation, he decided to specialize in radiation oncology. “I really enjoyed talking to the patients and their families and witnessing the difference we could make as physicians,” he says. “For this reason, the field of oncology seemed like the right fit for me.”

After earning his medical degree from Christian Medical College in Ludhiana, and completing his internship there, he completed his residency in radiation oncology at Northwestern University followed by a fellowship at the Mallinckrodt Institute of Radiology. He was a faculty member at Washington University School of Medicine in St. Louis and the University of Pittsburgh. In 1985 Mittal returned to Northwestern, joining the Division of Radiation Oncology in the Department of Radiology, becoming head of the division in 1993.

Mittal continued his leadership as founding chair when the Department of Radiation Oncology became the 25th department at the Feinberg School in 2006. He is justifiably proud of its growth. “We are treating many...
more patients, and our research funding has increased tremendously,” he says, nothing that the number of faculty and residents have nearly doubled.

Along with other members of the Radiation Oncology team, Mittal works closely with clinicians and researchers in a wide range of medical specialties, and believes that everyone benefits from these collaborations. “I agree with Isaac Newton—‘We build too many walls and not enough bridges’-- I definitely prefer building bridges,” he says.

He gets great satisfaction from all aspects of his work, but Mittal says he finds his clinical work especially gratifying. “I have lengthy relationships with most patients I treat. Some I’ve seen for as many as 15 years so we become close,” he says. Mittal often receives holiday cards and likes keeping up with his patients and their families. “I just enjoy being able to help them,” he says.

Mittal’s research focuses on using advanced radiation technologies in combination with chemotherapy to increase tumor control while decreasing toxicity. His work is international in scope and involves collaboration with experts at Northwestern and from around the world. Mittal says he may have been the first to study the effect of combined hyperthermia and 131 I-Labeled Anti-CEA monoclonal antibodies to control tumors and reduce the toxicity associated with conventional treatments. While this particular study was negative, radiolabeled antibodies are now routinely used in cancer treatment.

Mittal is currently Co-Principal Researcher for a study involving intensity modulated radiation therapy (IMRT) in the treatment of head and neck cancers. The aim of this NIH-funded study is to reduce the amount of radiation received by normal tissues surrounding the tumor, while simultaneously increasing the tumor’s absorbed dose. His clinical and translational research career covers nearly 30 years and includes over 200 articles, book chapters, and abstracts.

Teaching and mentoring is another aspect of Mittal’s career that he values. In addition to the medical students, residents, and fellows he has guided over the years, Mittal has mentored many high school and undergraduate students who have gone on to study medicine.

He is active in several professional organizations, including the American Society for Radiation Oncology (ASTRO), where he was recently elected to the board of directors, and the Society of Chairmen of Academic Radiation Oncology Programs (SCAROP), where he is president.

Mittal lives in Oak Brook with his wife, Raj, a medical oncologist in private practice. He has two children who have followed in their parents’ footsteps and become quite successful in their own careers. An avid golfer, skier and scuba diver, Dr. Mittal has gone heli-skiing in the mountains of British Columbia and recently explored the Great Barrier Reef.
“I feel fortunate that my job allows me to help others,” says Jeffrey Wayne, MD, FACS, an Associate Professor in the Department of Surgery and Chief of Melanoma and Sarcoma Surgical Oncology at Northwestern University Feinberg School of Medicine. “It makes every day a unique challenge and is incredibly satisfying on a personal level.”

Wayne’s busy clinical practice focuses primarily on cancers of the skin and the upper gastrointestinal tract. He says the combination provides a satisfying mix of smaller, relatively straightforward cases with larger, more complex ones. In addition, “these tend to be cases that are sent preferentially to referral and tertiary institutions,” says Wayne, who is also Associate Medical Director at the Lurie Cancer Center. “They are areas where the level of care we provide really benefits our patients.”

Research Efforts
In addition to his surgical practice at Northwestern Memorial Hospital, Wayne is actively involved in both clinical trials and outcomes research. As a member of the National Comprehensive Cancer Network (NCCN) Sarcoma Panel, for example, he examines how implementation of new practice guidelines, the introduction of new clinical agents, or approval of new drug regimens lead to changes in practice patterns — and what impact these changes have on patients. Patient outcomes are compared across facilities in an effort to provide the best possible care with the lowest morbidity and mortality rates.

Wayne is a member of the American College of Surgeons / Commission on Cancer Expert Panel that defined a set of formally developed quality indicators to study the care rendered to patients with melanoma in this country. “The ultimate goal is to feed the data back to individual hospitals so they can see how they’re doing against global benchmarks that have been identified by various professional organizations as quality measures,” he says. “We hope this effort will lead to better care nationwide.”
As an NCI-designated Comprehensive Cancer Center, Wayne adds that it is “part of the Lurie Cancer Center’s mission to educate physicians, as well as our patients, about what they should be looking for. It’s our hope that we can provide a model for other institutions across the country.”

“As we look at our ever-expanding health care system,” he says, “we want to make sure we are practicing not only in a cost-efficient manner, but, even more importantly, providing our patients with the most advanced treatment possible.”

A Team Effort
Wayne is committed to furthering increased collaboration among physicians. “I strive to provide the highest quality care to my patients and this, in my mind, clearly involves a multidisciplinary practice.”

When he arrived at Northwestern 10 years ago, Wayne says he found no unified effort for the treatment of melanoma and sarcoma patients. “My passion,” he says, has been “to create multidisciplinary teams for both disease sites.” To that end, he is working with his colleagues to establish a new skin care institute at the Lurie Cancer Center where surgeons, medical oncologists, dermatologists, and others will work closely together, providing exceptional care in one facility. “By being co-located, we will be able to provide our patients with instant consultation across specialties,” he says.

Family Life
Wayne grew up in Boston, earning his bachelor’s degree from Dartmouth College and his medical degree from Boston University. His residency at the University of Chicago was followed by a fellowship in surgical oncology at the University of Texas, MD Anderson Hospital. He arrived at Northwestern in 2001.

Wayne met his wife, Diane (a clinician and educator, who is Vice-Chair of Education and an Associate Professor in the Department of Medicine at the Feinberg School of Medicine) when they were both residents at the University of Chicago. They live in Wilmette with their two children, and enjoy skiing, traveling, and exploring Chicago’s restaurants and museums as a family.

They are all enthusiastic sports fans. “We like going to Cubs games, Bulls games, and Bears games, and we take the kids to watch Northwestern football, too.” This fall they will travel east to cheer on Northwestern, where they plan to tailgate with family and watch the Wildcats play Boston College.
INTERFACoE OF NANOcHNOLOGY
AND CANCER
Northwestern receives $12 million to improve cancer
diagnosis and treatment
Northwestern University, a leader in cancer
nanotechnology research, has received a five-
year, $12 million grant from the National
Cancer Institute to leverage the advantages of
nanotechnology to improve the way cancer is
diagnosed and treated. Results will be
disseminated to the wider research community
for ultimate translation to the clinic.

The focus of the Northwestern University
Center of Cancer Nanotechnology
Excellence (NU-CCNE) is developing
nanomaterials and nanodevices primarily for
application in brain, breast and pancreatic
cancer diagnostics and therapeutics, with
potential for use in other forms of cancer.

Northwestern is one of only nine institutions
across the country, and the only one in the
Midwest, to receive a CCNE award in this
second funding phase of the NCI Alliance for
Nanotechnology in Cancer program.
(Northwestern’s first CCNE received NCI
support from 2005 to 2010.)

Augmenting the NCI CCNE grant, a $2.1
million award from the Chicago Biomedical
Consortium (CBC) will establish a new facility
enabling NU-CCNE discoveries to be shared
with CBC-affiliated biology laboratories at no
cost, broadening the impact of the center’s
research.

“The support from the National Cancer
Institute and the CBC will enable researchers to
continue to make significant cancer-relevant
discoveries that ultimately can be transferred to
the clinic,” said Steven T. Rosen, Co-Director
of the NU-CCNE, Genevieve Teuton Professor
of Medicine at the Feinberg School of Medicine
and Director of the Lurie Cancer Center.

The NU-CCNE combines the strengths and
resources of the Lurie Cancer Center and
Northwestern’s International Institute for
Nanotechnology (IIN). “Nanotechnology is a
key driver of advances in cancer detection and
treatment, and Northwestern has played a
major role in developing this field,” said Chad

A. Mirkin, Co-Director of the NU-CCNE,
George B. Rathmann Professor of Chemistry in
the Weinberg College of Arts and Sciences,
member of the Lurie Cancer Center and IIN
Director.

In making the CCNE award, the NCI cited
Northwestern for the leadership and
complementary expertise of Mirkin and Rosen,
the impressive record of accomplishments
developing nanotechnology-based therapeutics
(including several novel technologies
undergoing commercialization and clinical
trials) and the highly significant basic science
and clinical problems being pursued.

For more information about the Northwestern
University Center of Cancer Nanotechnology
Excellence, visit ccne.northwestern.edu.
CANCER SURVIVORS CAN’T SHAKE PAIN, FATIGUE, INSOMNIA, FOGGY BRAIN

Five years after cancer treatment ended, many survivors still suffer symptoms

One of the largest survivorship studies ever conducted reveals that many patients still suffer moderate to severe problems with pain, fatigue, sleep, memory and concentration three to five years after treatment has ended.

“We were surprised to see how prevalent these symptoms still are,” said study Co-Investigator Lynne Wagner, Associate Professor of Medical Social Sciences at Northwestern University Feinberg School of Medicine and a member of the Lurie Cancer Center. “This is one of the first looks at what’s really happening for survivors in terms of symptoms and treatment among community-based treatment settings across the U.S.”

The persistent pain in survivors who are cancer-free and no longer receiving any treatment is particularly puzzling, Wagner noted, because good treatment exists. “It seems we haven’t come a long way in managing pain despite a lot of medical advances,” she said. “This is eye opening. It tells us we need to be better in clinical practice about managing our survivors’ pain.”

Patients seem to slip through the cracks in healthcare in terms of getting help managing their pain and other symptoms. “Cancer survivors are left trying to put the pieces together to find optimal care. They ideally need to see someone who is knowledgeable about the long-term effects of treatment.” She pointed to the example of the Lurie Cancer Center’s STAR (Survivors Taking Action & Responsibility) Program, a comprehensive long-term follow-up program for survivors of pediatric cancer.

The study included a sample of 248 survivors of breast, colorectal, lung and prostate cancer. The survivors were primarily female and white, and most were more than five years post-diagnosis. They also had been treated in community settings -- where 80 percent of people with cancer are treated in the United States -- as opposed to academic medical centers. This group best represents the typical experience of cancer survivors around the country, Wagner said. The most common symptoms reported by survivors were fatigue (16 percent), disturbed sleep (15 percent), cognitive difficulties (13 percent) and pain (13 percent).

Read more about the study at http://www.cancer.northwestern.edu/press_releases/2011/06_june/survivors.cfm

NORTHWESTERN INVENTOR WINS HIGHEST PRESIDENTIAL AWARD

Researcher’s breast simulators will measure doctors’ ability to detect breast cancer

Carla Pugh, MD, PhD, a Northwestern Medicine researcher, inventor and surgeon, has been awarded the Presidential Early Career Award for Scientists and Engineers (PECASE) for her novel research to develop the first physical test that measures medical students’ and physicians’ ability to perform a clinical exam of breasts and diagnose cancer.

The PECASE is the highest honor bestowed by the United States government on science and engineering professionals in the early stages of their independent research careers.

Dr. Pugh is an Associate Professor of Surgery and Director of the Center for Advanced Surgical Education at Northwestern University Feinberg School of Medicine, a member of Lurie Cancer Center, and an acute care surgeon at Northwestern Memorial Hospital. She will be invited to the White House to meet President Barack Obama and attend an awards ceremony.
NEW PROSTATE CANCER TEST GIVES MORE ACCURATE DIAGNOSIS

In a large multi-center clinical trial, a new PSA test to screen for prostate cancer more accurately identified men with prostate cancer -- particularly the aggressive form of the disease -- and substantially reduced false positives compared to the two currently available commercial PSA tests. The only Food and Drug Administration-approved screening tests for prostate cancer currently available result in a high number of false positives and lead to unnecessary biopsies and possible over-detection and over-treatment of indolent cancer, which would not have caused suffering or death.

“This new test is more specific and accurate than the currently available blood tests for early prostate cancer detection,” said lead investigator William Catalona, MD, Professor of Urology at the Northwestern University Feinberg School of Medicine and Director of the Clinical Prostate Cancer Program at the Lurie Cancer Center. “This will focus on the detection of more life-threatening prostate cancers and reduce unnecessary biopsies in men 50 years of age and older.”

Read more about the study at http://www.cancer.northwestern.edu/press_releases/2011/04_april/catalona.cfm

JONATHAN WIDOM (1955-2011)

Jonathan Widom, the William Deering Professor of Molecular Biosciences in the Weinberg College of Arts and Sciences at Northwestern University, died July 18 of an apparent heart attack. He was 55.

Also the Principal Investigator of Northwestern’s Physical Sciences-Oncology Center, the internationally renowned scholar was admired by colleagues, students and friends alike for the creativity, humor and enthusiasm that he brought to all he endeavored. Northwestern’s PS-OC is one of 12 established nationwide in 2009 by the National Cancer Institute, bringing together physical scientists and cancer biologists to use non-traditional, physical sciences-based approaches to understand and control cancer. Widom was also a member of the Lurie Cancer Center.

In his research, Dr. Widom focused on how DNA is packaged into chromosomes -- and the location of nucleosomes specifically. The work has had profound implications for how genes are able to be read in the cell and how mutations outside of the regions that encode proteins can lead to errors and disease. The Department of Molecular Biosciences will be organizing a scientific symposium to celebrate Widom’s life and accomplishments. The event will be held during the upcoming academic year; details are forthcoming.
TERRANCE PEABODY JOINS
NORTHWESTERN AS CHAIR OF
ORTHOPAEDIC SURGERY
Terrance Peabody, MD, a renowned expert in the surgical treatment of bone and soft-tissue tumors, has joined Northwestern University Feinberg School of Medicine as the Edwin Warner Ryerson Professor of Orthopaedic Surgery and Chair of the Department of Orthopaedics. Peabody will serve as Chair of the Department of Orthopaedic Surgery at Northwestern Memorial Hospital and as a member of the Lurie Cancer Center. He will also provide care for patients at Children’s Memorial Hospital.

Peabody succeeds Michael F. Schaefer, MD, who is stepping down after serving as Ryerson Professor and Chair of the department for more than 30 years. Dr. Schafer will continue to practice and to teach, and will remain chair of the Communications Cabinet of the American Academy of Orthopaedic Surgeons.

“Dr. Peabody’s knowledge, experience and leadership in the field of orthopaedic surgery will benefit our patients and bring a new dimension to our programs,” said Steven Rosen, MD, Director of the Lurie Cancer Center.

Peabody’s research and clinical expertise focus on limb salvage surgery and functional restoration for adult and pediatric patients with bone and soft tissue tumors, metastatic diseases, and severe trauma or infection.

NEW iPHONE APP TELLS DOCTORS HOW TO SAVE CANCER PATIENTS’ FERTILITY
Northwestern launches app and micro-website for healthcare providers and patients
Cancer treatment can destroy a patient’s fertility, but not all physicians are familiar with the risks and options to preserve it. Now there’s an app for that.

An iPhone app launched in June gives oncologists a quick reference guide for preserving the fertility of children, women and men diagnosed with cancer. The information for physicians and patients also is available on the new micro-website savemyfertility.org

The app and website were created by the Oncofertility Consortium of Northwestern University, a national group of physicians and scientists dedicated to saving the fertility of cancer patients through research and education.

"Deciding how to best protect an adult’s or child’s fertility should be part of every physician’s discussion with a newly diagnosed cancer patient," said Teresa Woodruff, Director and Founder of the Northwestern Oncofertility Consortium. "We created the app and the website to help patients and their physicians have this vitally important discussion and make much more informed decisions about fertility preservation."

Woodruff also is Chief of Fertility Preservation at the Feinberg School of Medicine, and a member of the Lurie Cancer Center. "Oncologists are the gatekeepers to fertility preservation," Woodruff added. "Now a physician can quickly access this information when he or she is with a cancer patient. And it also allows a doctor to e-mail a fact sheet in English or Spanish to a patient."
Over a century ago, Paul Ehrlich coined the term ‘magic bullet’ to describe medicines that can selectively attack pathogens without harming the host organism. In modern days, this concept still figures prominently in our fight against cancer, where the ideal treatment is viewed as one that would selectively kill cancer tissue with little or no side effects to the patient. While this challenge has not yet been overcome to date, a promising solution came from nanoscience, where conventional small-molecule drugs can be “packaged” in nanoscale containers that can then be selectively absorbed by tumors. This selectivity is a consequence of the leaky vasculature in tumor tissues, which allows for the passive accumulation of nanoscale particles—a phenomenon known as ‘enhanced permeation and retention (EPR) effect’. As such, the nano-packaged drugs can exhibit many advantages over conventional small-molecule chemotherapy, including increased plasma solubility, prolonged circulation, improved local bioavailability at the tumor, and controlled drug release at the cancer sites. Such advantages contribute to both a reduction of the adverse side effects often associated with the parent small-molecule anticancer agents as well as improved efficacy.

Among the many emerging nanoscale drug delivery platforms, one of the most promising is based on liposomal vehicles. Liposomes with a broad range of diameters (0.2–5 μm) were first introduced in the mid 1960s as a model system to evaluate the diffusion property of ions...
through biological membrane.\textsuperscript{3,4} In recent years, through the substantial improvements in particle size tunability, particle size uniformity, surface charge control, and particle stability,\textsuperscript{5,6} liposome has emerged as a highly promising platform for drug delivery, with several liposomal drug formulations having been approved for clinical treatments of a wide range of diseases, including cancers.\textsuperscript{7} The advantages of liposomal formulations in cancer therapy arise from the encapsulation of cytotoxic drugs in biocompatible lipid components that can protect these drugs from both enzymatic degradations and renal clearance, in addition to reducing their harmful effects toward normal tissue.

As prepared, bare liposomes (BLs) are often unstable for long periods in biological environments due to the non-covalent nature of their self-assembled lipid membranes, which eventually caused membrane fusion and cargo leakage in serum\textsuperscript{6} (Figure 2A). Additionally, BLs are susceptible to being captured by the reticulo-endothelial system (RES), primarily those components that reside in the liver, and have shortened circulation half life.\textsuperscript{6} Thus, liposomes with biocompatible poly[ethylene glycol] (PEG) surface groups was developed where the PEG groups can both sterically stabilize the lipid membrane and reduce opsonin binding, thus minimizing RES-based capture\textsuperscript{6} (Figure 2B). Such sterically stabilized liposomes (SSLs) have been used for the clinical treatment of several types of solid tumors, with the most often-cited example being Doxil\textsuperscript{TM} (trade name of Ortho Biotech Products, L.P., Horsham, PA, Figure 2, C and D).\textsuperscript{8,9} The nanoscale size (80–100 nm, Figure 2D) of the particles in Doxil\textsuperscript{TM} allows it to take advantages

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure.png}
\caption{Schematic illustration of the ‘enhanced permeation and retention (EPR)’ effect in solid tumor tissue that generally exhibits more leaky vasculature, poor lymphatic drainage, and more acidic interstitial environment in comparison to normal tissue.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{(A) A schematic illustration of cargo leakages from bare liposomes during membrane fusion process. (B) Membrane fusion and cargo leakage is greatly reduced in sterically stabilized liposomes (SSLs). (C) Schematic illustration of a doxorubicin-encapsulated SSL (Doxil\textsuperscript{TM}). (D) A cryogenic transmission electron microscope image of Doxil\textsuperscript{TM} particles. Image is adapted from the website of U S Food and Drug Administration.\textsuperscript{11}}
\end{figure}
of the EPR effect, with dramatically increased plasma half-life in humans (~55 h) for the encapsulated doxorubicin (DXR) compared to the free drug (10 h).\textsuperscript{9,10}

The Doxil\textsuperscript{TM} formulation also benefited from the ion gradient-mediated (IGM) protocol, where the drug is remotely loaded post SSL synthesis,\textsuperscript{6} allowing for a high bolus of DXR (>20k drug molecules per particles) to be encapsulated.\textsuperscript{9} The loaded DXR forms a striated gel precipitates\textsuperscript{11} inside the SSL particle (Figures 2C and 2D), making the particle more mechanically robust and slowing down the drug leakage during circulation. The solid encapsulation of DXR in this manner allows for the eventual delivery of the encapsulated drug in more concentrated forms, beyond its native aqueous solubility.\textsuperscript{12} Post-liposome-formation drug loading protocols such as IGM allows for the facile encapsulation of a wide range of drugs from DXR to vincristine,\textsuperscript{13} and temepamine.\textsuperscript{14} In the case of the inorganic drug arsenic trioxide\textsuperscript{15} (Trisenox\textsuperscript{TM}, Cephalon Inc., Frazer, PA) an IGM-loaded arsenate formulation with Ni\textsuperscript{2+} counterions has been shown by the O’Halloran group (RHLCCC, Basic Sciences Division) to have enhanced \textit{in vivo} tumor growth suppression over the free drug in murine xenograft model.\textsuperscript{16}

The synthesis of PCN.

Although drug-loaded SSLs have shown improved efficacy in many cancer treatments, these formulations lack the ability to actively target cancer tissues and specific triggers that release the encapsulated drug at these target sites. While there have been many attempts to design liposomes with built-in handles for targeting or triggered release,\textsuperscript{17} all of these systems rely on the synthesis of specialized lipids making them synthetically cumbersome. Additionally, the PEG-functionalized lipids can eventually dissociate from the surface of SSLs, returning them to the unstable state.\textsuperscript{18} In 2005, under a Center of Cancer Nanotechnology Excellence (CCNE) grant, we collaborated with the O’Halloran group to engineer liposome into a more versatile drug delivery platform. We designed a drop-in modification strategy that is synthetically easy to implement into liposome synthesis and can be tuned in a modular fashion to include not only targeting ligands but also triggered release. To this end, we took advantage of the vast amount of knowledge known in liposome fabrication to graft a cholesterol-terminated poly(acrylic acid) (Chol-PAA) modifier (MW ~5.1 kDa) to the lipid bilayer of drug-encapsulated liposome templates that are ~100 nm in diameter. At 10 mol% insertion, the resulting polymer-grafted
liposomes possess ~600,000 acrylate groups on the surface, which can be cross-linked with alkyne-modified telechelic diamine linkers to afford a core-shell structure that we called Polymer-Caged Nanobins (PCN, Figure 3, Top). At 50% cross-links, empty PCNs can be freeze-dried and redispersed readily without any significant membrane rupture of the liposomal core. The steric stabilization by the polymer cage also greatly reduced the amount of cargo leakages from drug-loaded PCNs in serum compared to the parent BLs. Remarkably, the cross-linked polymer cage can quickly collapse the liposomal core under acidic conditions, enabling PCN to release drugs selectively at low-pH target sites such as tumor interstitium and cellular endosomal vesicles (Figure 3, Bottom).

The broad versatility of our PCN strategy can be demonstrated through the ease in which the PCN polymer cage can subsequently be modified, with additional drug molecules, targeting groups, or imaging agents. Not only can the alkyne groups on the cross-linkers serve as a handle for the attachment of the aforementioned agents, the uncrosslinked surface acrylate groups (~300,000 after 50% cross-linking) can also be used for functionalization in an orthogonal fashion. As a model test for the attachment of tumor-targeting ligands, we conjugated folic acid to the alkyne groups on the polymer shell of PCN via copper-catalyzed reactions. The resulting folate-conjugated, DXR-loaded PCNs (f-PCN_{DXR}) demonstrated enhanced potency to folate receptor (FR)-positive tumor cells, such as KB and OvCa432, over FR-negative MCF7 cells. When incubated with KB cells that overexpress folate receptors, f-PCN_{DXR} are fifty-times more potent than the untargeted doxorubicin-loaded PCNs (PCN_{DXR}). More impressively, f-PCN_{DXR} can readily discriminate FR-positive KB and OvCa432 tumor cells as a function of the level of cellular FR-expression, showing different degrees of potentiation in each cell types. This system is now ready to be tested with other targeting ligands such as monoclonal antibodies and peptides.

Drug delivery with PCNs leads to enhanced uptake, enhanced anticancer efficacy at higher dosages, and attenuated side effects. As mentioned above, the modularity of the IGM loading methodology allows us to load PCNs with very high density of diverse drugs ranging from organic agents such as doxorubicin (DXR) and gemcitabine (GMC) to inorganic agents like arsenic trioxide. Such “nano-packaging” of drugs inside the PCN can potentially increases its cytotoxicity through enhanced cellular uptake (via endocytosis), followed by pH-triggered cytosolic drug release in acidic endosomal environments. Indeed, GMC-loaded PCN (PCNGMC) shows higher cytotoxicity against HeLa cells than the free drug, presumably because PCNGMC can bypass the membrane transporter proteins, a common internalization pathway for nucleoside analog drugs, resulting in a highly efficient cellular internalization of GMC.

In collaboration with the Cryns group (RHLCCC, Clinical Sciences Research Division), we and the O’Halloran group have evaluated the efficacy of PCN_{DXR} in a murine...
xenograft model of triple-negative breast cancer and found it to be more effective than the free drug in inhibiting tumor growth. More importantly, PCN DXR was well-tolerated by the treated mice without significant weight loss, suggesting the attenuated systemic toxicity. Taken together, these results suggest that toxic drugs can potentially be used in higher doses for treatments without significant side effects if they can be delivered via the PCN platform. If this strategy can be demonstrated beyond murine models, better efficacy may be achieved for cancer treatments with low impacts on the quality of life.

Enhanced drug synergism in combination chemotherapy with a dual-drug PCN. As mentioned above, the modular nature of our PCN platform also allows for the addition of a second type of drug to the polymer shell, yielding a ‘core-shell’ type dual-drug platform with multi-modal release profiles. As an example, we have immobilized a high amount of a modified cisplatin (PtII) prodrug in the polymer cage of PCN DXR to create a dual-drug PCN platform (PtII-PCN DXR). The resulting PtII-PCN DXR, having near-neutral surface charge, can be easily uptaken via endocytosis to deliver a predetermined drug combination to cancer cells. The concurrent delivery of both PtII-prodrug and DXR via this platform shows enhanced cytotoxicity against partially PtII-resistant MDA-MB-231 cancer cells over both the free drug combination and the separately nano-packaged drug combination (Figure 4), clearly showing synergistic drug potency for this drug combination when being co-delivered on a single nanoparticle. To the best of our knowledge, this is the first demonstration of drug synergy in a nanoscale drug delivery system, suggesting that the PCN platform can provide a new means for achieving highly synergistic combinational chemotherapies while reducing the chance for drug resistance to develop.

A PCN-based theranostic platform. As a further demonstration of the versatility of the PCN platform, we and the O’Halloran group have collaborated with the Meade group (RHLCCC, Basic Sciences Research Division) to tag PCN GMC with a GdIII-based MRI contrasting agent (Figure 5A). The resulting GdIII-PCN GMC theranostic (‘therapeutic + diagnostic’) platform exhibited significantly enhanced MR relaxivity per GdIII ion, resulting in a very high relaxivity per particle (715 500 mM⁻¹ s⁻¹ per particle at 60 MHz at ~45,000 GdIII ions per particle). In addition, conjugation of GdIII to PCN GMC leads to significant enhancement in uptake into HeLa cell comparing to free GdIII (Figure 5B), suggesting that delivery of GdIII agent via the PCN platform can greatly reduce the amount of GdIII agents currently used in conventional imaging sessions. Theranostic platform such as GdIII-PCN GMC can eventually be used to simultaneously deliver both drugs and imaging agents to a disease site, allowing the treated tissue to be monitored in real time and enabling a timely adjustment of clinical treatment decisions. This is an emerging concept in the future of personalized medicine, especially concerning cancer treatments, where
it is advantageous to know if a therapy is working in real time. If this concept can be extended beyond the laboratory, the prescribed regimen can be adjusted in a timely fashion from ongoing feedback information and can allow for more successful treatments.

Conclusion.
In conclusion, we have demonstrated that the PCN design possesses many merits as a delivery system, including ease of synthesis, biocompatibility, pH-sensitive release trigger, and a highly versatile modularity that allows for the incorporation of a wide range of enhancing agents that include additional drugs, targeting ligands, and imaging agents. Results from benchtop and in vitro evaluations thus far have uniformly highlighted the tremendous potential offered by PCNs for improving cancer chemotherapy. Indeed, the PCN platform has been recognized in a recent perspective article in *ACS Nano* as including all the characteristics of a near-ideal system for drug delivery. While we hope to see the development of PCNs into a practical drug delivery system soon, extensive in vivo evaluation and engineering development must be carried out before PCN can be taken into the realm of clinical applications. As in the case of Doxil™, such process will require extensive investments from a major institution and collaborations by a large team of chemists, material scientists, biologists, and clinicians.

**Acknowledgments**
The work reported herein could not have been accomplished within the five short years of the CCNE grant without the valuable contributions and hard works of a top-notch team of collaborators. Thus, we would like to indicate our gratitude to the following scientists who have helped us with the investigation of the PCN platform: Dr. Richard W. Ahn, Dr. Feng Chen, Dr. Haimei Chen, Prof. Vincent Cryns, Ms. Angela J. Fought, Dr. Bong Jin Hong, Dr. One-Sun Lee, Dr. Keith W. MacRenaris, Mr. Daniel J. Mastarone, Prof. Thomas J. Meade, Prof. Thomas V. O’Halloran, Prof. George C. Schatz, and Dr. Ying Song. This work was financially supported by the NIH (NCI Center of Cancer Nanotechnology Excellence Grant U54CA119341 Project 4, Cancer Nanotechnology Platform Partnerships Grant U01CA151461, and Core Grant P30CA060553 to the Robert H. Lurie Comprehensive Cancer Center of Northwestern University). Currently, Sang-Min Lee is an NRC postdoctoral fellow at the National Institute of Standards and Technology (Gaithersburg, MD 20899-6520) and the National Institute of Health (Bethesda, MD 20892)

**References**


The development of targeted therapies for the treatment of cancer has followed a seemingly simple paradigm: look for overexpression or increased activation/activity of the relevant target then try to target or inhibit it. However, like most things in drug development, this has turned out to be more challenging in practice than initially postulated. Small molecular inhibitors of intracellular targets such as kinases and epigenetic targets such as histone deacetylases (HDACs), HSP90, and possibly mTOR have not only faced the standard challenges of bioavailability, acceptable plasma pharmacokinetics and tumor tissue distribution but have also revealed new mechanisms for drug resistance such as compensating pathways that to date have limited the clinical effectiveness of these drugs. Further, extracellular targets can now be targeted using monoclonal antibodies that have fairly predictable plasma pharmacokinetics but suffer from a poor understanding of the biology of the target and a lack of selective tissue expression. Differential display approaches and genomic data produced a wealth of possible targets but most of these have not worked out for the discovery and development of targeted therapeutics. Monoclonal antibodies have certainly not lived up to their press releases touting little or no toxicity—rather, the toxicities are different than for conventional cytotoxic cancer drugs and have presented new challenges on how to manage patients receiving some of these treatments.
It is with these challenges in mind that the discovery and development of a monoclonal antibody targeting the urokinase plasminogen activator receptor (uPAR) was initiated. There is extensive data in the literature “validating” uPAR as a target for the treatment of a broad spectrum of solid tumors and uPAR expression has been reported in breast, prostate, ovarian, pancreatic, lung, liver, kidney, and colon cancer as well as in glioma, soft tissue sarcomas and a few hematologic malignancies including acute leukemia and myeloma. The term “validated” simply refers to pre-clinical data showing uPAR expression in a number of different solid tumor types and the therapeutic benefit of inhibiting uPAR activity either genetically or pharmacologically on tumor progression in mouse cancer models. A number of important observations that have informed the discovery and development process came out of these validation studies. For example, uPAR is expressed not only on the tumor cells themselves but also on tumor associated cells such as angiogenic endothelium and macrophages, both of which contribute to tumor progression. In fact, uPAR expression by tumor cells or by tumor associated cells has prognostic significance in multiple cancer types.

Figure 1. uPA/uPAR: A key tumor progression and angiogenesis check-point. The urokinase plasminogen activator receptor (uPAR) is a three-domain protein anchored to the outer via a glycosylphosphatidyl inositol (glycolipid) anchor. All three domains are required to bind to urokinase plasminogen activator (uPA). uPA binding to uPAR regulates extracellular proteolysis mediated by plasmin by increasing the efficiency of activation of plasminogen to plasmin. Plasmin is a promiscuous protease that can activate other zymogen proteases such as matrix metalloproteases (MMPs) and can lead to matrix remodeling and growth factor release from the ECM. uPA can be inactivated by a specific inhibitor, PAI-1, and the uPA-PAI-1-uPAR complex can be internalized and free uPAR recycled to the cell surface. uPAR also interacts with other ligands (adaptor molecules) in addition to uPA such as integrins and can signal via these complexes.

uPAR expression is also greater in high-grade disease and metastasis and is often associated with more aggressive cancer. uPAR expression also appears to be selectively increased in tumor as compared to normal quiescent tissue based on adjacent normal tissue analysis in tumor sections although no systematic analysis of uPAR expression in normal human tissue had ever been reported. Finally, uPAR has been implicated in the signaling of a diverse set of pathways, many of which have already been implicated as contributing to tumor progression including VEGF, FGF-2, HGF, IGF-1, Akt, FAK, MAPK, and RAS. These observations have been confirmed across multiple cell types and lines although how and if these various signaling pathways integrate with each other with respect to the role of uPAR in cancer progression remains to be elucidated. Nevertheless, these results suggest that targeting uPAR may have pleiotropic effects on tumor growth and dissemination and fits with our current hypothesis that robust antitumor effects (except in the rare cases where a tumor is pathway addicted to a single signaling effector) will require co-ordinated inhibition of multiple signaling pathways involved in tumor progression.
The role of uPAR in tumor progression—then and now

The understanding of uPAR function and its role in normal and pathological cell biology seemed fairly simple for many years. uPAR is a three-domain extracellular protein that is covalently linked to the outer leaf of the cell membrane via a glycolipid anchor and therefore has no transmembrane domain (Figure 1). uPAR is the only specific binding site on the cell surface for the serine protease, urokinase plasminogen activator (uPA), and regulates the activation and deactivation of uPA through the binding of plasminogen activator inhibitor-1 (PAI-1), which inhibits the catalytic activity of uPA, followed by internalization and recycling of free uPA to the cell surface. uPA activates a cascade of extracellular proteolysis by activating plasminogen to plasmin, which leads to activation of other proteases such as the matrix metalloproteases (MMPs), remodeling of extracellular matrix (ECM) and release and/or activation of growth factors residing within the ECM such as VEGF, HGF, and IGF. uPA bound to uPAR activates plasminogen to plasmin much more efficiently than when uPA is free and thus, it was concluded that the role of uPAR was to act as a simple cell surface binding molecule for uPA, which in cancer led to enhancement and modulation of extracellular proteolysis resulting in tumor growth, angiogenesis and metastasis. However, attempts by many groups (including our own) to interfere with this proteolytic process pharmacologically by inhibiting the uPA-uPAR interaction generally led to modest effects on metastasis and little or no effect on tumor growth in mouse tumor models. In parallel, a number of groups were publishing data that suggested that despite lacking a transmembrane domain, uPAR could also mediate cell signaling, independent of its regulation of the proteolytic activity of uPA. Studies from our laboratory as well as others demonstrated that uPAR could mediate tumor and endothelial cell proliferation, differentiation, and survival and that these effects were cell-type specific and involved different signaling intermediates. uPAR complexes with a number of signaling adapters were demonstrated providing a structural basis for how a glycolipid anchored receptor with no transmembrane domain could mediate cell signaling. uPAR appears to interact either directly or indirectly with integrins, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptor (PDGFR), the internalizing receptor

lipoprotein receptor-related protein (LRP), caveolin, the G-protein-coupled receptor FPRL-1, a homologue to the fMLP receptor, and vitronectin. Thus, from the perspective of pharmacologically targeting uPAR, the challenge went from trying to inhibit a well-defined interaction (uPA binding to uPAR) that had little effect on tumor progression to potentially trying to inhibit one or more other interactions of uPAR that could have some effect on tumor growth. New rationale for targeting uPAR interactions that were downstream of uPA binding also came from uPAR knockdown studies using antisense and siRNA approaches, which demonstrated that uPAR knockdown could have very profound effects on tumor progression and could induce apoptosis and significant inhibition of tumor growth in vivo, in contrast to what had been observed previously when targeting only the effects of uPAR on uPA-mediated proteolysis. This disconnect between the pharmacological inhibition of uPA dependent proteolysis and the effects of knocking down uPAR expression informed our pursuit of a uPAR targeted therapeutic that could mimic the effects of the uPAR knockdown and prompted us to focus on generating uPAR targeted monoclonal antibodies that could more easily and selectively block the protein-protein interactions that we speculated were important for uPAR function in cancer.

Discovery and development of ATN-658, a novel uPAR targeted monoclonal antibody for the treatment of cancer

In order to maximize the probability of obtaining a uPAR antibody which could inhibit tumor progression, we devised a multi-tiered approach to immunization and antibody screening that took advantage of the extensive literature on uPAR biology. One of the challenges to screening a potential uPAR targeted monoclonal was that it was still not clear which molecular interaction of uPAR should be targeted. Obviously, one could attempt to tease out the contribution of each uPAR interaction to tumor growth and then target the most relevant one(s) but this approach could take decades. Thus, several assumptions were made and a “cast the wide net” approach was utilized. Although uPAR is a highly glycosylated protein with 5 potential N-linked glycosylation sites, uPAR antibodies developed in the 1990’s mostly targeted glycosylated epitopes of uPAR and did not
have much biological activity in tumor models. Thus, in order to maximize the probability that any antibodies generated would target protein and not carbohydrate epitopes, minimally glycosylated uPAR was expressed and used for immunization. Second, uPAR can reside on the cell surface as either a fully intact protein or a proteolytic fragment (D2D3) that lacks domain 1, no longer binds to uPA, but retains other biological activities. From a targeting perspective, we hypothesized that targeting both forms of uPAR would be beneficial. Since cryptic epitopes with chemotactic activity are exposed in the D2D3 fragment, we utilized a highly purified D2D3 fragment for immunization. Finally, screening of clones consisted of a multi-tiered approach that involved uPAR binding, inhibition of uPA binding to uPAR, inhibition of cell migration, fibronectin matrix assembly and in vivo screening in an A549 lung adenocarcinoma model. This approach yielded four monoclonal antibodies: two (ATN-616 and ATN-617) that inhibited uPA binding to uPAR (Figure 2A) and two (ATN-615 and ATN-658) that did not (Figure 2A and B). Of these clones, ATN-658 inhibited cell migration in vitro (Figure 3), subcutaneous (SC) A549 tumor growth in vivo, and could bind to all forms of uPAR (full size or D2D3 fragment) regardless of whether uPA was bound or not (data not shown). Initial comparison of ATN-658 with ATN-617, which inhibited uPA binding to uPAR, in several SC models of tumor growth (A549, A2780) confirmed that ATN-658 had superior antitumor activity. Thus, ATN-658 was advanced for further evaluation in additional animal tumor model testing.

Initially, the species cross-reactivity of ATN-658 was evaluated. Since uPAR in cancer may be

<table>
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<th>Antibody</th>
<th>ELISA (K_D, nM)</th>
<th>HeLa Cell Assay (K_D, nM)</th>
<th>Competition of/by uPA (HeLa)</th>
<th>Anti-tumor Activity</th>
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<tr>
<td>ATN-615</td>
<td>0.6</td>
<td>1.3</td>
<td>No</td>
<td>None</td>
</tr>
<tr>
<td>ATN-658</td>
<td>0.7</td>
<td>5.4</td>
<td>No</td>
<td>Strong</td>
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Figure 2. Identification and characterization of uPAR targeted mouse monoclonal antibodies. A: (above) Characterization of clones for binding to uPAR using solid phase ELISA (immobilized uPAR) and uPAR expressing HeLa cells as well as competition of binding to HeLa by uPA. Anti-tumor activity was evaluated in A549 xenograft models in vivo. B: (left) Competition of 125I-uPA binding to HeLa cells.

Figure 3. ATN-658 inhibits IGF-1 stimulated invasion of KM12L4 colon carcinoma cells in Matrigel. Invasion assays were carried out as described. ATN-658 was tested at 10 ng/mL (figure adapted from ref. 30).
expressed in multiple tumor compartments and cells, we wanted to understand exactly what was being targeted in our tumor models. ATN-658 did not cross-react with mouse uPAR or in fact, any non-human uPAR with the exception of chimpanzee. This observation significantly altered the development plan for ATN-658. First, any antitumor activity observed with ATN-658 would be due to direct antitumor cell effects in the xenografts and not due to targeting angiogenic endothelium or tumor-associated macrophages. Since angiogenic endothelial cells and macrophages would both be targeted in humans, it is likely that any antitumor activity observed in mouse xenografts would underrepresent what might be expected in human cancer patients. Second, conventional pre-clinical animal toxicology would not be scientifically reasonable because of the lack of cross-reactivity with uPAR expressed in typical toxicology species. Thus, before committing time and resources to this project, we decided to explore the expression of uPAR in normal human tissues. Clearly, broad expression of uPAR in normal tissues would present a significant challenge to the development of ATN-658 and would raise concerns about the potential toxicity of the drug once it went into human patients. However, immunostaining analysis of 30 normal tissues from each of three human donors using ATN-658 demonstrated that uPAR expression is actually very selective for tumor and that most normal, quiescent tissues do not express uPAR (Figure 4). This analysis has now been extended to an additional 8 donors using both formalin fixed and frozen tissue with essentially the same type of results. The major normal cells that express uPAR are monocytes, macrophages and neutrophils with rare expression observed in the occasional endothelial or epithelial cell. This high selectivity provided further rationale for developing ATN-658 as a therapeutic agent and mitigated some of the concern that ATN-658 would have broad spectrum toxicity in patients although this remains to be evaluated in actual cancer patients.

ATN-658 has now been evaluated in a number of different orthotopic tumor models. In addition to recapitulating the tumor environment more accurately than a SC tumor, orthotopic models are often spontaneously metastatic allowing evaluation of both tumor growth and metastasis in the same model. ATN-658 inhibited tumor growth, invasion and metastasis in the L3.6pl pancreatic carcinoma model and enhanced the antitumor effects of gemcitabine when used in combination with this agent (Figure 5A and B). ATN-658 monotherapy also inhibited L3.6pl tumor proliferation, which to our knowledge was the first such demonstration for a uPAR targeted pharmacological agent in vivo (Figure 5B). Further, the effect of ATN-658 on uPAR signaling as well as tumor progression has been evaluated in several additional orthotopic tumor model studies. In a model of metastatic outgrowth of prostate tumor cells (PC-3)
inoculated directly into bone, ATN-658 significantly inhibited tumor growth (Figure 6A) as well as activation of FAK, AKT(Figure 6B) and MAPK in vivo. In that same study, a modest inhibitory effect of ATN-658 on SC growth of the same tumor cell line (PC-3) was also observed and this effect was consistent with the more modest effect on AKT and MAPK activation in the SC tumors. Recently, ATN-658 was also demonstrated to inhibit the migration and invasion of several ovarian cancer cell lines in vitro as well as their growth and dissemination (Figure 7A) when inoculated intraperitoneally (IP) in vivo. Gene expression was evaluated in this study using Affymetrix gene chip analysis and ATN-658 inhibited the expression of a number of genes associated with tumor progression including FGFR1, αvβ3, c- 

<table>
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<tr>
<th>Treatment Group</th>
<th>Control</th>
<th>Gemcitabine</th>
<th>ATN-658</th>
<th>Gemcitabine + ATN-658</th>
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<td>Tumor Volume (mm³)</td>
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<td>1019 ± 138 *</td>
<td>817 ± 105 *</td>
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<td>0/11 *</td>
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<tr>
<td>Incidence of Liver Metastases</td>
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<td>1/11 *</td>
<td>1/13 *</td>
</tr>
<tr>
<td>Proliferation Index (# BrdU + cells/HPF)</td>
<td>392 ± 26</td>
<td>379 ± 19</td>
<td>108 ± 16 * ^</td>
<td>59 ± 9 * ^</td>
</tr>
</tbody>
</table>

* p<0.05 vs. Control; ^ p<0.005 vs. Gemcitabine.
Met, HGF, Rho, CD44, JAK1 and 3 and others. These results were confirmed in several cell lines (CaOV3, SKOV3ip) *in vitro* and *in vivo*\(^28\). Tumors from ATN-658 treated mice also displayed lower expression of both uPA and uPAR (Figure 7B) as well as a decreased co-localization of uPAR with \(\alpha_5\beta_1\). Previously published studies had demonstrated that inhibiting the \(\alpha_5\beta_1\)-uPAR interaction induced tumor cell dormancy\(^29\). Thus, our current hypothesis is that the \(\alpha_5\beta_1\)-uPAR interaction may be the molecular target for ATN-658, leading not only to inhibition of proliferation and induction of dormancy but also apoptosis in models of ovarian cancer (Figure 7C)\(^28\). We are now pursuing whether inhibiting the \(\alpha_5\beta_1\)-uPAR interaction is the basis for the antitumor effects observed in all of our models to date as the expression of uPAR and \(\alpha_5\beta_1\) would provide a basis for patient selection in the clinic.

**Path to the clinic**

Based on this data, ATN-658 was humanized to create the clinical candidate huATN-658. huATN-658 is an IgG\(_1\),\(\kappa\) just like its mouse monoclonal counterpart and is approximately 96% human IgG sequence. Functionally, ATN-658 and huATN-658 are indistinguishable. huATN-658 binds to pure uPAR with the same affinity as ATN-658 and detects uPAR expression in tumor sections. It has been compared to an ATN-658 positive control in several of the tumor models described above and shown to be equivalent to mouse ATN-

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**Figure 6B. ATN-658 inhibits PC-3 tumor progression in an orthotopic mouse model of prostate cancer metastasis**\(^2\). Immunohistochemical analysis for activated (phosphorylated) focal adhesion kinase (FAK) and AKT as described in ref. 27. Analysis was carried out on tumors grown in bone and SC.

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**Figure 7A. ATN-658 inhibits ovarian cancer growth and dissemination in the CaOV3 IP model**\(^3\). Tumors were inoculated and mice treated as described in ref. 28. ATN-658 (10 mg/kg) was administered 3X/week and paclitaxel (5 mg/kg) was administered 2X/week; ***, p<0.001
Pre-clinical development of huATN-658, including in vitro toxicology studies using human cells due to lack of cross-reactivity of huATN-658 with non-human uPAR, has nearly been completed. huATN-658 is also able to mediate ADCC in vitro suggesting that ADCC may also contribute to any antitumor activity in patients. Approved cancer therapeutic antibodies such as Rituxan and Herceptin are thought to act through both direct antagonism of target and ADCC and a similar mechanism is proposed for huATN-658. We are currently collaborating with the NCI NExT program to complete pre-clinical development of huATN-658 and plan to file an Exploratory IND and initiate a phase 0 trial in 2011. Thus, huATN-658 may represent the first uPAR targeted therapeutic agent to be advanced into phase for evaluation in cancer patients.

Acknowledgments
We thank Phenopath Laboratories (Seattle, WA) for TMA analysis of uPAR expression in normal human tissues (Figure 4).

References


Micro (mi)RNAs are small 19-22 nucleotide (nt) long non-coding RNAs that inhibit gene expression at the posttranscriptional level. They are first transcribed as parts of longer molecules, up to several kilobases in length (pri-miRNA), which are processed in the nucleus into hairpin RNAs of 70-100 nt by the double-stranded RNA-specific ribonuclease Drosha. The hairpin pre-miRNAs are then transported to the cytoplasm by exportin 5 where they undergo further processing by a second, double-strand specific ribonuclease called Dicer. In animals, single-stranded miRNAs are incorporated into RNA-induced silencing complexes (RISC) and primarily bind specific messenger RNA (mRNA) at specific sequence motifs (seed sequences) predominantly within the 3' untranslated region (3'UTR) of the transcript. Given the frequency with which miRNA seed sequence motifs are conserved within 3'UTRs, it has been estimated that almost all human genes are targets of miRNAs. It has also been estimated that for each miRNA there are approximately 200 genes carrying the cognate seed sequence within the 3'UTR.

A strong link between miRNA and human cancers has been established. A comparison of miRNA expression in normal and tumor tissues revealed global changes in miRNA expression associated with various human malignancies. miRNAs have been demonstrated to act either as oncogenes (e.g. miR-155, miR-17-5p, miR-21) or tumor suppressors (e.g. miR-34, miR-15a, miR-16-1, let-7)\(^6,5\). The ubiquitously...
expressed let-7/miR-98 family was one of the first mammalian miRNAs to be identified. In many tissues let-7 comprises more than 50% of the total cellular miRNA content.

**Let-7 and miR-200.**

The let-7 family is composed of 12 family members (let-7a-1, a-2, a-3, b, c, d, e, f-1, f-2, g, i, and miR-98) located on 8 different chromosomes. These 12 family members represent 9 distinct let-7 sequences with identical seed sequences and, very likely, overlapping sets of targets. Let-7 is expressed late in mammalian embryonic development and plays an evolutionarily conserved role from Caenorhabditis elegans to Drosophila to mammals. The let-7 targets that have been identified include cell cycle regulators such as CDC25A and CDK6, promoters of growth including RAS and c-myc, and a number of early embryonic genes including HMGA2, Mlin-41 and IMP-1.

The miR-200 family is composed of 5 members (miR-200a, b, c, miR-141, and miR-429). They are located within two clusters on separate chromosomes. Interestingly, the 5 members can be subdivided into two subgroups according to their seed sequences (miR-200a/141 and miR-200b/c/429). Although target prediction algorithms predicted little overlap in the targets of these groups, experimental approaches suggested that their sets of targets are highly overlapping.

We had recognized earlier that the 60 cell lines of the so called drug screening panel maintained by the National Cancer Institute (NCI60) could be subdivided into two major superclusters (SC), one of which, SC2, was reported to express an epithelial gene signature, while the other one, SC1, expressed a mesenchymal gene signature. We postulated, therefore, that SC1 cells represented more advanced and less differentiated forms of cancer. To identify key regulators of SC1 and SC2 cells, we subjected 10 SC1 and 10 SC2 cell lines to a miRNA array analysis which allowed quantification of more than 450 miRNAs. We found that a highly significant marker for SC2 cells was the let-7 family of miRNAs.

From database screening using miRNA target prediction algorithms, HMGA2 was identified as the number one let-7 target. Subsequently, HMGA2 was experimentally validated as a genuine target of let-7. HMG proteins are a class of low molecular weight, non-histone, nuclear proteins that bind DNA and function as co-factors involved in the regulation of chromatin conformation and gene transcription. Consistent with HMGA2’s role as a major target of let-7, the expression levels of let-7 and HMGA2 during mouse embryonic development are inversely related. Inactivation of the HMGA2 gene prevents cell transformation in experimental tumor models. HMGA2 has, therefore, been described as an oncogene.

HMGA2 was undetectable in normal ovarian surface epithelium or benign ovarian tumors, but was expressed in cancerous tissue from patients with ovarian cancer. While normal ovarian epithelium did not express HMGA2, early stage tumor cells, represented by *in situ* cancers, clearly expressed the HMGA2 protein. HMGA2 expression was further increased in full-blown carcinoma. These data suggest that HMGA2 expression is upregulated early upon transformation of ovarian epithelial cells. We showed that high expression of HMGA2 combined with low expression of let-7d correlated significantly with an adverse prognosis both for progression free and overall survival of patients with advanced ovarian cancer.

The connection between let-7 and HMGA2 we found in the ovarian cancer likely extends to various other forms of cancer as evidenced by the number of reports that either linked high HMGA2 expression or lack of let-7 expression to advanced tumors and poor prognosis—let-7 family members are frequently downregulated in advanced lung and colon cancer, and expression of let-7 correlates with overall survival of patients with NSCLC and adenocarcinoma.

miRNAs are expected to regulate multiple mRNAs. After we identified HMGA2 as a let-7 target we used an unbiased, genome-wide bioinformatics approach to identify other cancer-relevant let-7 targets. We identified a set of 12 let-7-regulated oncofetal genes (LOGs), eight of which were known to be upregulated during progression of various human cancers (Fig. 1). The second most relevant LOG after HMGA2 was IMP-1 (IGFB2BP1/CRD-BP) which stabilizes the mRNAs of c-myc and other genes. IMP-1 has a classical oncofetal expression pattern.
Another relevant LOG, LOG3/LIN28B, is a structural and functional homolog of LIN2818. LIN28, together with SOX2, NANOG, and OCT4, was demonstrated to be sufficient to reprogram somatic cells to become pluripotent stem cells19. LOG3/LIN28B as a stem cell factor was, therefore, a good candidate gene to become upregulated in cancer cells when let-7 expression was lost. Consistent with this prediction, LOG3/LIN28B upregulation has been reported for human hepatocarcinoma cells18. Recent reports suggest that, in addition to LOG3/LIN28B, LOG1/HMGA2 and LOG2/IMP-1 are also upregulated in stem cells20,21. Moreover, let-7b-regulated HMGA2 plays a vital role in maintaining young stem cells21. Increased expression of LOGs could, therefore, have two tumorigenic effects: First, increased LOG expression could increase the tumorigenicity of cancer cells. Consistent with this prediction are data that demonstrate that let-7 is, indeed, a factor that regulates self-renewal and tumorigenicity of breast cancer22. Second, increased LOG expression could affect sensitivity to chemotherapeutic drugs through regulating let-722, which is consistent with our finding that let-7 low cells are less sensitive to Taxanes than let-7 high cells23.

Identification of the miR-200 family as a marker for E-cadherin positive and Vimentin negative tumor cells.

Loss of the epithelial nature of cancer in the initial stages of metastasis is seen as an important aspect of cancer progression often referred to as epithelial-to-mesenchymal transition (EMT). EMT-like processes occur as part of embryonic development, wound healing24, and during carcinogenesis25 when tumor cells undergo a change from a differentiated to a more invasive, dedifferentiated tumor24. After EMT induction, cells lose epithelial features and acquire mesenchymal characteristics, including Vimentin filaments and a flattened phenotype.

To identify miRNAs that correlated with the expression of epithelial or mesenchymal markers in the NCI60 cell lines, we first determined the expression levels of E-cadherin and Vimentin using Western blot analysis. We then sorted all NCI60 cell lines from highest to lowest E-cadherin/Vimentin ratio7. We used a data set of 207 miRNAs (obtained by real-time PCR) expressed in the set of NCI60 cell lines that we had used to confirm expression of let-7 in SC2 cells8. We found 4 miRNAs that were selectively expressed in epithelial cells, all with extraordinary levels of significance (p = 10^{-12} to 10^{-20})7. These four miRNAs (miR-200a, miR-200b, miR-200c, and miR-141) belong to the miR-200 family of miRNAs, which also includes miR-429 (miR-429 was not part of the initial data set, but was later confirmed also to be expressed in all epithelial cells [unpublished data]).

The E-box binding transcription factors ZEB1 and ZEB2 are both upregulated in advanced human cancers26. Both proteins are the top predicted targets of the miR-200 family27,28, possessing 8 and 7 predicted miR-200 seed matches in their 3’UTR, respectively7. We then went on to validate both ZEB1 and ZEB2 as genuine miR-200 targets7 and showed that introduction of miR-200 into mesenchymal...
cells induced MET; and its inhibition in epithelial cells induced EMT\(^7\). Our work was complemented by a study published in parallel, which identified members of the miR-200 family as extraordinary regulators of TGF\(\beta\)-induced EMT in MDCK cells\(^9\). Other groups have confirmed the connection between miR-200, EMT, and the ZEB proteins\(^{30,31}\). These data have identified miR-200 as a powerful master regulator of EMT in cancer cells and suggest that introducing miR-200 into cancer cells could provide a novel means of reversing tumor progression.

Positive feedback loops ensure switch-like regulation by let-7 and miR-200. Both let-7 and miR-200 are almost absent in SC1 cells, consistent with the assumption that these cells represent more advanced forms of cancer\(^7\). Interestingly, the regulation of expression of both miRNA families involves double negative (\(=\) positive) feedback loops (Fig. 2). The amount of let-7 is regulated at a posttranscriptional level\(^{32}\). Three studies showed that the let-7 targets, LIN28 and LIN28B, are both inhibitors of let-7 processing, and their expression is restricted to early embryonic development. Both proteins were shown to bind to the loop region of let-7 precursors, which results in blocking of processing of let-7 at either the Drosha\(^{33,34}\) or the Dicer level\(^{35}\) (Fig. 2A) followed by terminal uridylation of let-7 precursors resulting in their degradation\(^{36}\). Hence, the two proteins would seem to suppress let-7 expression by acting at multiple levels. In contrast to let-7, miR-200 family members are regulated mostly at the transcriptional level. miR-200 negatively regulates the expression of the E-box binding transcription factors, ZEB1 and ZEB2\(^{7,8}\) (Fig. 2B). Interestingly however, the promoters of both miR-200 clusters, one on chr 1 and the other on chr 12, contain E-boxes and are negatively regulated by ZEB1 or ZEB2\(^{31,37}\). Both the let-7 and the miR-200 miRNA families, are composed of multiple members, and each member is predicted to regulate a largely overlapping set of targets. In order to alter the expression of these targets without having to regulate expression of each miRNA individually, regulatory mechanisms are in place such that reducing the amount of only one abundant member of each family is thought to result in the reduction of the total amount of miRNA below a threshold at which suppression of expression of the inhibitors is abrogated. This results in the expression of inhibitors and the consequent shutting down of the expression of all miRNA family members. These kinds of positive feedback regulatory mechanisms ensure that the differentiation processes regulated by entire families of miRNAs behave in a switch-like fashion.

**Figure 2. Expression of let-7 and miR-200 Is Regulated by Negative Feedback Loops.** A: Regulation of let-7 processing by the let-7 targets, Lin28 and Lin28B. B: Regulation of miR-200 expression by the miR-200 targets, ZEB1 and ZEB2.\(^{38}\)

**Future directions.**

Clearly, let-7 and miR-200 are not the only miRNA families deregulated during tumor progression. However, in the context of carcinomas derived from epithelial cell layers, miR-200 will be frequently downregulated.
Let-7 expression increases with tissue differentiation, but during tumor progression, when cells dedifferentiate, let-7 is lost. Replacement therapy with let-7 and/or miR-200 may, therefore, be a viable treatment option for solid tumors, assuming that normal tissues can tolerate an increase in these miRNAs. Future experiments are aimed at testing this possibility.

References
29 Gregory, P.A., Bert, A.G., Paterson, E.L., Barry, S.C.,


With longer duration of immunosuppression in kidney transplant recipients (KTRs), squamous cell carcinoma (SCC) develops predominantly in sun exposed areas of the body.1 After 20 years of immunosuppression, cumulative SCC incidences as high as 70%-82% have been noted in KTRs.2,3 Skin cancer incidence among KTRs is greater in areas with high solar exposure, such as Australia3,4, than in areas with low solar exposure, such as the Netherlands.5 This geographic difference in skin cancer incidence among KTRs as well as the predilection for SCC to occur on commonly sun-exposed areas of the body suggests that sun protection after transplantation may reduce the risk of developing skin cancer.
Nationally, delivery of sun protection and early detection education to KTTRs is variable. The absence of uniform guidelines as to when transplant centers transfer care of KTTRs to community nephrologists may be one of the reasons for the lack of systematic patient education. In the first year after transplantation, clinicians in tertiary care centers have the best access to patients to provide counseling. However, the value that KTTRs place on sun protection and early detection information was not known. The number of KTTRs and the quality of care that they receive in the nationally recognized Comprehensive Transplant Center at Northwestern under the direction of Dr. Michael Abecassis provided a unique opportunity to explore KTTRs’ beliefs and self-care practices regarding sun protection and early detection.

In May 2006, Drs. Murad Alam and Simon Yoo of the Department of Dermatology at Northwestern Feinberg School of Medicine had the vision to start a dermatology database of organ transplant patients to identify patients with skin cancer and identify their risks of developing skin cancer. In February 2007, Dr. Robinson joined the dermatology team. From 2007 to 2010, vigorous recruitment of participants by medical students during summer research experiences in dermatology resulted in a database with over 700 solid organ transplant recipients. Then, in 2009 the research effort was broadened by collaboration with Elisa Gordon, PhD, MPH, Research Associate Professor, Institute for Healthcare Studies & Department of Surgery, Comprehensive Transplant Center, and Dr. John Friedewald, the Director of Clinical Research of the Comprehensive Transplant Center.

Our research with this multiracial population confirmed prior reports that skin cancer was associated with patients having skin types 1, 2 (lighter skin) and being older; however, in our population 5% of those with skin types 3-5 (darker skin types) developed skin cancer compared to 19% of those with skin types 1,2. In our KTTRs, the mean duration of immunosuppression prior to developing skin cancer was greater in those with darker skin tones [mean 8.9 (± 5.2)] than those with lighter skin tones [mean 7.5 (± 3.2)]. For our patients with both light and dark skin tones the average time to the development of skin cancer was within the range of 4 to 9 years after organ transplantation, as reported by others.

Focus groups with KTTRs identified that one year after transplantation was the optimal time for SCC early detection education. Furthermore, during cognitive interviews with KTTRs, the optimal time for sun protection education was identified as 4-6 months after transplantation. As KTTRs are initially faced with many stressors that can affect their adherence to the medical regimen, it is important to present the skin cancer prevention and early educational intervention when a KTTR is ready to receive and possibly act upon it. Because the process of assimilating information and acting upon it may extend over a period of time, patient education is a repetitive process. Patients participating in our focus groups and cognitive interviews obtained skin cancer information from the following sources: Internet, newsletters, family and friends, and physicians and nurses, with the Internet the most frequent source.

Since the Internet was an important source of information for KTTRs, we performed a systematic review of Internet websites for KTTRs endorsed by transplant physicians and dermatologists, and convened an expert panel of 8 dermatologists and one social scientist (EJG) to assess the 40 websites identified for reading grade level, inclusion of people with skin of color, sufficient content to support effective sun protection, and description of sun protection strategies and skin self-examination. Of the 40 sites identified in 2009, 11 contained information about sun protection or increased risk of any type of cancer. The websites had a ninth-grade median reading level (range 7th grade to college senior). Skin cancer risk was presented as relevant to those with fair skin. Sites recommended regular use of sunscreen with sun protection factor ≥ 15 (n=3) to reduce the risk of developing skin cancer (n=4). Few sites recommended using protective clothing (n=5), seeking shade (n=4), avoiding deliberate tanning with indoor or outdoor light (n=1). The websites provided incomplete recommendations. Since many patients seek self-management information from the Internet, websites need to provide more thorough educational information about skin cancer prevention and health promotion at a lower reading grade level. In addition, sun-protection messages, which often target those
with fair skin that sunburns easily, need to become culturally sensitive for KTRs to include those with skin of color, who may not perceive that sun protection is needed.

Our hypothesis is that in the first year after transplantation, a two-staged sequential approach to skin cancer primary (sun protection at 4-6 months) and secondary (early detection at one year) prevention will promote self-management with effective sun protection and skin self-examination (SSE) for early detection of SCC, and may lessen the psychological impact for the KTR of being at risk to develop skin cancer. (see Figure 1) To test this hypothesis, we developed a color workbook for the early detection of SCC and a brochure on sun protection strategies. To date, our pilot research with the early detection educational workbook shows promise in promoting skin self-examination one month after reading it. Manageable steps that build self-confidence with explanations of the benefit of the behavior are the hallmark of the educational intervention. Those KTRs receiving the early detection workbook demonstrated a significant change in knowledge, concern about developing a SCC, and the importance of SSE, and confidence in recognizing a SCC in comparison with the control group of KTRs (p<.001). One month after education, 89% of those KTRs reading the workbook checked their skin in comparison to 22% of the control KTRs (p<.001). While the short duration of this pilot research made it impossible to examine the potential reinforcement of SSE by finding a suspicious lesion, our pilot research found support for the belief of the KTRs who participated in our focus groups and cognitive interviews that one year after transplantation was an appropriate time for education. Future research will evaluate the effectiveness of the sun protection brochure, and explore the use of the Internet to deliver the educational interventions.
Systematic educational efforts are needed to bridge the gap between tertiary care provided in transplant centers and delivery of care in the community for KTRs. Printed educational materials can be initiated at the end of the first year and serve as a reference for KTRs across a continuum of time during which KTRs may be transferred from the tertiary care center to community nephrologists. The scope and intensity of the repetitive education is challenging. Further, print materials do not accommodate limited literacy levels of the KTR patient population. A promising avenue for delivering health education on skin cancer prevention and protection is through the use of the Internet, which is increasingly used as an effective, low-cost approach for reaching many individuals while enabling consumer-centric health and patient empowerment. Internet-based education is valuable for its convenience, timeliness, reduction of stigma, and control over the learning environment. Future research is needed to develop and evaluate culturally competent, Internet educational resources for KTR on SCC.

References
A number of studies over the years have suggested that African–American women have more adverse overall breast cancer prognosis than white women. This difference is particularly evident among younger women diagnosed before the age of 50\(^1,2\). While socioeconomic and health care access factors certainly play a role in worse survival rates in African-American women, unique biologic characteristics in this patient population may also be contributing in the observed differences in patient outcome.

Invasive breast carcinomas are known to consist of a heterogeneous group of tumors with different biologic behavior, prognosis and response to treatment. The classic pathologic classification of breast carcinomas was recently redefined by gene microarray studies that identified distinct subtypes of breast carcinomas as follows: luminal A, luminal B, HER-2 overexpressing and basal\(^3,6\). These subtypes of breast carcinomas display different gene expression signatures and are associated with different clinical outcomes\(^4,6\). The tumors of the basal subtype of breast carcinomas express one or more of the basal cytokeratins (such as CK5/6, 14 or 17) and are histologically poorly differentiated, ER-negative, HER-2 negative ductal carcinomas with minimal or absent in situ component\(^7-10\). Basal subtype breast carcinomas comprise the majority of “interval” breast cancers presenting between mammograms and are associated with high proliferation rates and poor prognosis\(^3,5,11\). In
addition, the majority of the BRCA1 associated breast carcinomas are thought to belong to the basal subtype of breast tumors\textsuperscript{12-14}. We, and others, recently reported that ER-negative/PR-negative/HER-2 negative (so called “triple negative”) breast tumors express epidermal growth factor receptor (EGFR), a member of the human epidermal growth factor family of transmembrane tyrosine kinases that activate a number of signaling pathways involved in cell proliferation and carcinogenesis\textsuperscript{15-19}. Furthermore, a number of studies including our own, have suggested that the majority of these “triple negative”, EGFR positive tumors belong to the basal subtype and hypothesized that these tumors may be the subtype of breast carcinomas that could potentially benefit from EGFR-targeted therapeutic approaches\textsuperscript{7, 18, 20-23}.

Against this background, the objective of the current study was to evaluate possible racial differences in the incidence of these triple-negative/EGFR-positive/basal subtype tumors in a large cohort of consecutive breast cancer patients.

Materials and Methods
Our study population consisted of 306 consecutive breast cancer patients. The age and race of patients and the histologic parameters of tumor type, grade and size at the time of diagnosis were recorded. The following breast cancer marker profile was obtained immunohistochemically on all patients (ER, Dako; PR, Dako; MIB-1, Dako, EGFR, Dako,

<table>
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<tr>
<th></th>
<th>African-American (n=61)</th>
<th>Caucasian (n=213)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (&lt;40 yo)</strong></td>
<td>10</td>
<td>15</td>
<td>&lt;0.01</td>
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<tr>
<td><strong>ER negative</strong></td>
<td>30</td>
<td>50</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td><strong>PR negative</strong></td>
<td>41</td>
<td>84</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td><strong>Unfavorable MIB-1 (&gt;20%)</strong></td>
<td>36</td>
<td>68</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>High histologic grade (III)</strong></td>
<td>41</td>
<td>81</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>EGFR expression</strong></td>
<td>23</td>
<td>46</td>
<td>&lt;0.03</td>
</tr>
<tr>
<td><strong>CK5/6 expression</strong></td>
<td>12</td>
<td>11</td>
<td>&lt;0.01</td>
</tr>
</tbody>
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Table 1: Correlation between Race and Breast Tumor Marker Profile. HER-2 amplification, Tumor size, Histologic Subtype: NS.

<table>
<thead>
<tr>
<th></th>
<th>Phenotype Present</th>
<th>Phenotype Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>16 (26%)</td>
<td>45 (74%)</td>
<td>61</td>
</tr>
<tr>
<td>Caucasian</td>
<td>22 (10%)</td>
<td>191 (90%)</td>
<td>213</td>
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</tbody>
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Table 2: Correlation between Race and Triple Negative/EGFR-positive phenotype. P<0.02

<table>
<thead>
<tr>
<th></th>
<th>Phenotype Present</th>
<th>Phenotype Absent</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>African-American</td>
<td>12 (20%)</td>
<td>49 (80%)</td>
<td>61</td>
</tr>
<tr>
<td>Caucasian</td>
<td>17 (8%)</td>
<td>196 (92%)</td>
<td>213</td>
</tr>
</tbody>
</table>

Table 3: Correlation between Race and Triple Negative/CK5/6(basal)-positive phenotype. P<0.007
CK5/6, Zyomed) and analyzed by quantitative image analysis (Chromavision, ACIS system). HER-2 amplification status was evaluated by FISH (PathVysion, Vysis).

Results
1. Correlation between race and breast tumor marker profile
Overall 61 African-American, 213 Caucasian, 5 Asian, 9 Hispanic and 18 other patients were recorded. African-American patients tended to be younger; 10/61 (16.4%) versus 15/213 (7%) were less than 40 years of age (p<0.01). African-American patients had ER-negative (30/61 versus 50/213, p<0.0005), PR-negative (41/61 versus 84/213, p<0.0005) tumors that were highly proliferative (36/61 versus 68/213 had MIB-1 values of >20%, p<0.001). In addition, most African-American patients had grade III tumors (67% versus 38%, p<0.01). No association with HER-2 gene amplification, tumor size or histologic subtype was observed. Furthermore, a much higher percentage of African-American patients expressed EGFR, 23/61 (37.7%) versus 46/213 (21.5%), p<0.03. Of interest, cytokeratin CK5/6 expression was high as well (12/57 versus 11/186, p<0.01), thus defined by immunohistochemistry to belong to the basal subtype of breast carcinomas. (Table 1).

2. Correlation between race and triple negative/EGFR-positive phenotype
When patients were grouped by their ER-negative/PR-negative/HER-2-negative (=triple negative)/EGFR-positive phenotype, more than double (16/61, 26%) of the African-American patients expressed this phenotype compared to only 22/213 (10.3%) of the Caucasian patients (p<0.02). (Table 2).

3. Correlation between race and triple negative/ basal phenotype
Of interest, when the incidence of the basal phenotype was examined, 19.6% (12/61) African-American patients expressed the ER-negative/PR-negative/HER-2-negative (=triple negative)/CK5/6-positive basal subtype phenotype compared to only 8% (17/213) of the Caucasian patients (p<0.007). (Table 3 and Figure 1).

Conclusions
In this study we report that:
1. The majority of African-American patients have ER-negative/PR-negative, highly proliferative, poorly differentiated (grade III/III) breast carcinomas.
2. In addition, these tumors tend to express EGFR and belong to the basal subtype of breast carcinomas that are traditionally associated with poor prognosis.
3. Based on the high incidence of basal subtype, as well as EGFR expression in ER-

Figure 1: ER-negative/PR-negative/HER-2-negative infiltrating ductal carcinoma, grade III/III, exhibiting strong membranous staining for EGFR and strong positivity for cytokeratin CK5/6.
negative/PR-negative/HER-2-negative patients, we propose that triple negative patients are routinely tested for CK5/6 and EGFR expression and, within the appropriate medical context, informed decisions are made regarding follow-up genetic testing, given the known association of basal tumors with BRCA1 mutations.

Our findings support the hypothesis, as other groups have suggested,

\[1\]

that underlying biologic differences may account for the worse overall prognosis of breast carcinomas in African-American patients. A clinical trial of the EGFR-targeting inhibitor erlotinib in a metastatic setting in triple negative/EGFR positive African-American and caucasian patients, coupled with pathology studies of molecular correlates of a possible effective response, are currently underway and the results are awaited with great interest.

**References**


Inflammation has been considered for a long time as being of limited duration and beneficial to any immune response. However, it is now widely accepted that smoldering chronic inflammation is a significant force driving chronic disease, including cancer, where it responds to tissue injury to promote repair through cell remodeling, proliferation and angiogenesis. Thus, chronic inflammation considerably contributes to most stages of tumorigenesis, including tumor initiation, promotion, malignant differentiation, invasion and metastasis. About 18% of all cancers have a chronic infectious etiology. This close correlation between infection, chronic local inflammation and tumorigenesis is well established for several cancers: bladder cancer is associated with Schistosoma and Gram-negative uropathogen infections; cervical cancer with Papillomavirus infection and cervicitis; ovarian cancer with Pelvic inflammatory disease; pancreatic cancer with pancreatitis; gastric cancer and MALT lymphoma with Helicobacter pylori infection and gastritis; esophageal cancer with Oesophagitis and Barrett’s metaplasia; colorectal cancer with inflammatory bowel disease and and Bacteroides infections; hepatocellular cancer with Hepatitis virus B and C infections and hepatitis; bronchial cancer with silicosis, asbestosis, and bronchitis; mesothelioma with asbestosis; Karposi’s sarcoma with Human herpes virus type 8 infection; Burkitt’s lymphoma and Hodgkin’s disease with Mononucleosis and Epstein-Barr virus infection; skin cancer with Papillomavirus.
infection and warts; breast cancer with inflammation; and gall bladder cancer with Cholecystitis and bacterial infection. While acute inflammatory responses can be beneficial for cancer treatment, chronic inflammation frequently caused by tumorigenic pathogens and autoimmunity, significantly increases one’s cancer risk. In addition, solid tumors promote a pro-inflammatory microenvironment. Cancer cells recruit leukocytes, which produce tumor promoting chemokines and cytokines. Furthermore, solid tumors ultimately outgrow all available blood supply resulting in oxygen deprivation and necrosis, which is pro-inflammatory through release of inflammatory mediators, including IL-1, which perpetuate the inflammatory response.

Tumor-associated macrophages.
There is continuous interplay between immune cells and cancer cells. Macrophages are abundantly present in the tumor microenvironment, and are referred to as tumor-associated macrophages (TAMs), which are alternative activated M2 macrophages and therefore promote cancer growth, survival, metastasis and impair immunosurveillance by the adaptive immune system. Many tumor cells produce chemokines, including M-CSF, VEGF or CCL2, to attract circulating blood monocytes to infiltrate tumor tissues and to differentiate into macrophages. While macrophages recognize and are able to eliminate cancer cells, they can be exploited to locally produce pro-angiogenic factors, growth factors or matrix metalloproteases to aid tumor cell proliferation, invasion and metastasis. TAMs promote proliferation through release of growth factors, such as EGF, M-CSF, PDGF, FGF or TGFβ. They promote angiogenesis to increase the local blood supply for tumors through the release of pro-angiogenic factors, including VEGF and chemokines, including CCL2, CCL5, CXCL1, CXCL8, CXCL13 and CXCL12. TAMs release matrix metalloproteases (MMPs), including MMP-2, MMP-7, MMP-9 and MMP-12, which also aid in neovascularization. Thus, highly vascularized tumors usually contain large numbers of TAMs. Finally, TAMs promote metastasis through production of cytokines, such as TNFα, IL-1β and the release of MMPs to aid in the dissemination of cancer cells, which occurs most frequently close to TAMs and thus high TAM infiltration and presence in tumor tissue correlates frequently with a bad patient prognosis.

Interleukin-1β.
There is ample evidence for a tumor promoting role of local low level chronic inflammation and the infiltration of macrophages into the tumor microenvironment. One of the early on produced and highly potent pro-inflammatory cytokine is interleukin (IL)-1β, which is primarily produced by monocytes and macrophages. IL-1 was the first cytokine discovered. It mediates diverse biological functions and had been known by various identifiers, including endogenous pyrogen, pyrexin, catabolin, lymphocyte activating factor, leukocytic endogenous mediator, mononuclear cell factor, osteoclast activating factor or hemopoietin. Subsequently it was shown that these responses were actually mediated by two related cytokines IL-1α and IL-1β, which have very related function and signal through the same receptor complex. Two IL-1 receptors exist, IL-1RI and the decoy IL-1RII, which lacks most of the cytosolic signaling domain. IL-1 signaling is further regulated through soluble receptors and the requirement of the IL-1R accessory protein (IL-1Rαc) for conversion into the high affinity, signaling competent receptor complex. The naturally occurring IL-1R antagonist (IL-1Ra) competes with IL-1 for receptor binding, but does not initiate signal transduction. However, while IL-1α remains primarily intracellular, IL-1β is released from cells by an unconventional mechanism into the circulation. IL-1β is a potent pro-inflammatory cytokine and essential for the inflammatory host response to infection and tissue damage, it is now also firmly linked to the development of chronic inflammatory and immune diseases, including cancer.

Regulation of IL-1β production is unique among cytokines and only shared with the related IL-1 family cytokine IL-18. Most inflammatory cytokines, including IL-1β, IL-6, IL-8 or TNFα, are regulated through inducible transcription. Accordingly, IL-1β is absent under normal conditions, but inflammation and stress cause rapid up regulation of its transcription, but also translation is uncoupled from transcription and regulated independently. However, in contrast to most inflammatory cytokines, transcription and translation of IL-1β produces only an inactive precursor (pro-IL-1β), and the generation of the biologically active, mature IL-1β requires
additional proteolytical processing to remove its pro-domain. Mature IL-1β is then released by an atypical, leader peptide-independent mechanism, which is still controversial. Maturation of IL-1β is accomplished by the cysteine-aspartic protease caspase-1, which cleaves the precursor following Asp to liberate the 17 kDa mature cytokine. Also caspase-1 is initially produced as a zymogen, pro-caspase-1, and requires activation, which occurs in an inducible protein complex, termed inflammasome. Caspase-1 together with caspases-4 and -5 (and their mouse parologue caspase-11), and caspase-12 form the inflammatory caspase subfamily, which are initiator caspases and contain a caspase recruitment domain (CARD). The CARD is essential for the clustering of pro-caspases required for their autocatalytic transactivation by the induced proximity mechanism, and this close proximity occurs in the inflammasomes.

Inflammasome.
The inflammasome represents a host defense system, which is part of the innate immune system based on the recognition of infection and tissue damage by germ-line encoded cytosolic pattern recognition receptors (PRRs). The PRRs that activate inflammasomes belong to the Nod-like receptors (NLRs) and AIM2-like receptors (ALRs), though also the cytosolic RNA-sensor RIG-I activates inflammasomes. NLRs display a tripartite domain architecture, consisting of a C-terminal leucine rich region (LRR), a central nucleotide binding NACHT domain, and an N-terminal effector domain crucial for downstream signaling, which in the majority of NLRs is a CARD (NLRCs) or a PYRIN domain (PYD) (NLRPs). Both are protein-protein interaction domains and required for assembly of inflammasomes. The LRR is the putative ligand recognition domain and responsible for NLR activation, and accordingly, in vivo deletion of the LRR of NLRP3 renders the protein unresponsive. The NACHT contains a NTPase domain as demonstrated for NLRP1, NLRP3 and NLRP12. Although 22 human NLR and 33 mouse Nlr genes exist, the function of most NLRs has yet to be elucidated and only a few NLRs have been linked to inflammasome activation. Little is known about the nature of most NLR ligands. NLRP1 is involved in the recognition of the peptidoglycan component muramyl-dipeptide (MDP) and the lethal toxin (LeTx) of Bacillus anthracis in a complex with Nod2. NLRP2 assembles an inflammasome in vitro, but its ligand and in vivo relevance is still elusive. NLRP3, perhaps the best studied NLR, senses a large number of agonists by multiple mechanisms that include potassium efflux, lysosomal damage and generation of reactive oxygen species, suggesting an indirect mechanism for agonist sensing. NLRC4 is activated by intracellular bacteria via recognition of cytosolic flagellin. In addition, NLRC4 recognizes the rod structure of bacterial type III secretion systems from several pathogens. Increasing evidence supports that NLRs also heterodimerize. NLRP1/Nod2 complexes mediate MDP and LeTx recognition. Similarly, flagellin is recognized by NLRC4 in concert with NLRB1. As one can expect, there is increasing evidence that multiple NLRs synergistically recognize microbial infections, likely via distinct pathogen associated molecular patterns (PAMPs). For example, Listeria monocytogenes infection is recognized by NLRP3, NLRC4, and AIM2. The ALR AIM2 is the PRR that directly recognizes cytosolic DNA to mediate the host response through activation of inflammasomes. AIM2 belongs to the HIN-200 family of interferon-response genes and consists of an N-terminal PYD and a HIN-200 domain, and AIM2 recognizes cytosolic dsDNA, including synthetic DNA, viruses and bacterial DNA and genomic DNA. Infection of AIM2 deficient macrophages with the bacterial pathogens Francisella tularensis and L. monocytogenes, or the DNA viruses vaccinia virus, HSV-1 and CMV results in impaired caspase-1 activation, IL-1β release and

![Figure 1: Formation of the inflammasome by NLRs.](image)

Activated NLRs recruit the adaptor ASC by PYD-mediated interaction, which then brings pro-caspase-1 into the complex by CARD-mediated interaction.
pathogen clearance. The PYD in NLRs and AIM2 is the protein interaction domain required for recruitment of the adaptor protein ASC, which in turn recruits pro-caspase-1 to form the caspase-1-activating inflammasome (Figure 1, 2). Besides responding to PAMPs, inflammasomes also respond to tissue damage and sterile inflammation through released danger associated molecular patterns (DAMPs). NLRP3 is activated by exogenous ATP, uric acid crystals, Amyloid beta, Cholesterol crystals and hyaluronan, while AIM2 is activated by cytosolic DNA released by cell damage. Accordingly, activation of inflammasomes have been linked to numerous inflammatory diseases, which are commonly characterized by excessive production of IL-1β, including periodic fever syndromes, gouty arthritis, systemic onset juvenile idiopathic arthritis, Still’s disease, System lupus erythematosus, multiple sclerosis, asthma, Chron’s disease, atherosclerosis, Alzheimer’s disease or type 2 diabetes.

While macrophages require inflammasomes and thus caspase-1 for IL-1β release, several other cell types can release IL-1β, which often is pro-IL-1β, such as IL-1β released from short lived dying or damaged cells. For example, neutrophils and mast cells release serine proteases that are frequently present in inflammatory fluids. These proteases, including proteinase-3, elastase, chymase, matrix metalloproteinases and others can also mature pro-IL-1β.

Interleukin-1β and cancer. Since IL-1β is a pleiotropic pro-inflammatory mediator and most cancers have an inflammatory component, IL-1β has been linked to tumorigenesis. Although, IL-1β is primarily produced by TAMs, some cancer cells also acquired this capability, and thus its cancer promoting effects have been attributed to autocrine and paracrine-mediated up regulation of pro-angiogenic factors to promote neovascularization, such as VEGF, IL-8, TNFα, CXCL2, HGF and TGFβ and metastasis, including MMPs, adhesion molecules and growth factors (Figure 3). Several studies clearly demonstrated a correlation between high expression of IL-1β in cancer and a poor prognosis for patients, including head and neck cancer, colon cancer, lung cancer, melanoma, breast cancer, leukemia and myeloma. IL-1β gene polymorphisms causing increased expression of IL-1β have been linked to an increased risk of gastric cancer following H. pylori infection.
*pylori* infection. Similar results were obtained in numerous studies for different ethnic populations, but not for all populations analyzed. Further correlation was detected to cervical cancer, gallbladder cancer, breast cancer and esophageal cancer. Metastatic pancreatic cancer cell lines exhibit higher expression of the IL-RI than non-metastatic lines. Ectopic expression of IL-1β in lung cancer cells did not show any changes in proliferation in vitro, but causes a significant increase in tumor growth in vivo and tumors showed significant increase in vascularization, supporting a paracrine mechanism. Parietal cell-specific expression of IL-1β in transgenic mice caused spontaneous gastric inflammation and adenocarcinomas. Support for the metastatic function of IL-1β is emphasized by a study showing that administering IL-1β before the i.v. injection of melanoma cells augmented lung metastasis. Compelling evidence came finally from Voronov and colleagues, who demonstrated in xenograft tumor models that IL-1β is essential for tumor neovascularization, thus growth, and invasiveness using IL-1β-deficient mice, thus IL-1β-deficient mice showed no metastasis and survived, while wild type mice succumbed to lung metastasis, and co-cultured IL-1β-deficient TAMs with melanoma cells released reduced levels of VEGF and TNFα. Similar results were also obtained in models of metastatic breast and prostate cancer.

As discussed above, IL-1β signaling is regulated by the naturally occurring inhibitory protein IL-1Ra. Since in healthy individuals IL-1Ra is present in excess compared to IL-1β, the concept is that in inflammatory disease the local and circulating levels of IL-1Ra are insufficient to block IL-1β signaling. Thus, in cancer low levels of IL-1Ra might fail to prevent the angiogenic and metastatic properties of IL-1β. This scenario is observed in several forms of hematopoietic tumors, including lymphomas, multiple myeloma and leukemia, which show constitutively elevated levels of IL-1β and reduced levels of IL-1Ra, which however is not a unified model for all cancers, and elevated levels of IL-1Ra exist in tumors that are associated with more severe forms. However, since IL-1Ra is expressed as a consequence of IL-1β signaling, it might merely indicate excessive production of IL-1β. Based on the convincing studies that IL-1β promotes angiogenesis and metastasis, several studies investigated application of the recombinant IL-1Ra in tumor mouse models and demonstrated the efficacy of IL-1Ra for cancer therapy. First, similar to *IL-1B* gene polymorphisms, also polymorphisms in the *IL-1RN* gene, which encodes IL-1Ra, are linked to an increased risk for gastric, colorectal, cervical, gallbladder, esophageal, lung and prostate cancer. IL-1Ra-deficient mice develop tumors following exposure to the chemical carcinogen 3-MCA, while only some IL-1β-deficient mice developed tumors, which are smaller and lacked neutrophil and macrophage infiltration. Fibrosarcoma cell lines derived from the 3-MCA primary tumors from IL-1Ra-deficient mice were most aggressive and metastatic in an experimental metastasis model, while cell lines from IL-1β-deficient mice showed the lowest number of lung metastases. Along these lines, expression of IL-1Ra in skin carcinoma cells significantly reduced tumor growth in xenograft mouse models. Melanoma xenograft growth and metastasis in nude mice was strongly reduced, when cells were transduced with IL-1Ra. Those and similar studies strongly support a role of IL-1Ra in preventing tumorigenesis.

Melanoma xenograft growth is inhibited in vivo, when IL-1Ra is administered at the same time as melanoma cells and also improved efficacy of chemotherapy. Administering IL-1Ra in mouse melanoma models reduced lung and liver metastases, which has been attributed to the impaired expression of adhesion molecules. Yet another study demonstrated that administering IL-1Ra shows efficacy as either preventative or as a treatment regimen, which reduced the liver metastasis burden by 80% caused by intrasplenic injection of melanoma cells. Voronov and colleagues treated wild type mice with IL-1Ra, which resulted in significantly reduced tumor vascularization in a melanoma mouse model. A xenograft mouse study evaluating the primary tumor growth also demonstrated a beneficial effect from IL-1Ra treatment of melanoma, colon adenocarcinoma and lung adenocarcinoma cell lines that produce IL-1β, but not that of a melanoma and squamous cell carcinoma cell line lacking IL-1β expression. As discussed above, the stomach-specific expression of IL-1β caused spontaneous gastric cancer, which could be prevented by a 6-week treatment regimen using IL-1Ra.
Several mouse studies also explored novel continuous delivery methods for IL-1Ra to treat fibrosarcoma with microencapsulated genetically engineered cells that secrete IL-1Ra, or melanoma xenograft growth with continuous release of IL-1Ra from biodegradable microspheres, providing evidence for the successful long-term application of IL-Ra\textsuperscript{58,59}. Anakinra is a recombinant, non-glycosylated form of the IL-1Ra, which is a well established and tolerated approach to interfere with excessive circulating IL-1β in several autoinflammatory and autoimmune diseases, such as Rheumatoid Arthritis, Cryopyrinopathies, Familial Mediterranean fever, and others\textsuperscript{60}. Due to some disadvantages, such as a short half life of 4 hours that require daily injections and injection site irritations, novel anti-IL-1β therapeutics have been recently developed, including humanized monoclonal anti-IL-1β antibody (Canakinumab), which only requires bi-monthly administration and which is approved for Cryopyrinopathies. Rilanocept (IL-1 Trap) is a fusion protein between the extracellular domains of the IL-1Rαc and IL-1RI with the Fc portion of human IgG1, which allows high affinity interaction with, and sequestration of, IL-1β, and is also approved for the treatment of Cryopyrinopathies. Of note is that anakinra has been used for the long-term treatment of arthritis patients without adverse side effects, emphasizing the excellent safety profile of this drug\textsuperscript{69}.

**Anti-IL-1β clinical trials.**

Based on such promising results from mouse models, 2 clinical trials have been initiated to test the application of IL-1Ra in cancer patients. A phase I study from the National Cancer Institute (NCI) investigates anakinra for treating patients with metastatic cancer expressing the IL-1β gene (NCT00072111), which is still ongoing. A phase II study from the Mayo Clinic investigates patients with smoldering or indolent multiple myeloma (NCT00635154)\textsuperscript{61}. Multiple myeloma, a B-cell malignancy has benign preconditions, including smoldering multiple myeloma (SMM) and indolent multiple myeloma (IMM). SMM patients have a median progression to active myeloma of 26 months and IMM patients of only 8-10 months. 47 SMM/IMM patients were enrolled and received anakinra (100 mg/wk) for 6 months and non-responders and patients with stable disease received then in addition a low dose dexamethasone (20 mg/wk), which induces myeloma cell death in vitro when combined with IL-1β inhibition\textsuperscript{61}. Initial results from this study indicate that anti-IL-1β therapy can significantly increase progression free survival (PFS) of high risk patients. The median PFS was 37.5 months and disease stability was achieved in 8 patients for 4 years while receiving therapy, which is still ongoing\textsuperscript{61}. This first clinical trial provides a compelling argument for application of anti-IL-1β therapies in cancer patients to achieve the conversion into a manageable chronic disease, and thus warrants further studies in metastatic disease and highly vascularized tumors.

**Our research focus.**

Our laboratory studies the molecular mechanisms that control the maturation step of pro-IL-1β in macrophages and we are focusing on 2 aspects: 1) we investigate the signals that activate several novel NLRs and test their contribution to inflammasome activation. 2) we identified a family of inflammasome inhibitors, which we termed PYRIN domain-only proteins (POPs)\textsuperscript{62}. POPs regulate the recruitment of the adaptor ASC to NLRs/ALRs in vitro and we are currently testing their function in vivo using novel macrophage-specific mouse models as well as targeting the inflammasome using recombinant POps.

**Acknowledgements**

I thank the talented members of my laboratory for their work and dedication and I am grateful for the collaboration with Drs. Andrea Dorfleutner and Harris Perlman, and for the support of our work by the National Institutes of Health, the American Heart Association, the Arthritis Foundation, the Concern Foundation and the Save the Ta-tas Foundation.

**References**


interleukin-1 blockade?


Pesticides are widely used and pervasive in our environment. Our dependence upon pesticides is increasing. Although pesticides sold in the US have passed the Environmental Protection Agency (EPA) screening procedures for carcinogenicity based on genotoxicity and mutagenicity tests, in vitro and animal studies have shown that pesticides induced carcinogenicity. A number of pesticides have also been repeatedly associated with various cancers in epidemiological investigations of farmers and pesticide manufacturing workers. The elevated cancer risk following exposure to pesticides indicates a gap in the current knowledge of pesticide carcinogenicity, and provides evidence that pesticide may cause cancer through alternative mechanisms, such as epigenetic changes.

Methylation of 5'CpG islands in gene promoter region has been consistently found in malignant tissues from a variety of cancer sites and indicated as a critical early change in the development of human cancers. Alterations in gene promoter DNA methylation have also been increasingly found in relation to exposure to various environmental chemicals, including several pesticides. Animal studies have shown that exposure to pesticides, such as vinclozolin, methoxycyclor and dichlorvos induced promoter DNA methylation alterations of multiple genes, including GPR33, KCNE2, ANXA1, etc. Genomic DNA methylation content in blood leukocyte DNA was inversely associated with plasma levels of pesticide residues in an Arctic...
population\textsuperscript{12}, and a similar observation was made in a Korean population\textsuperscript{13}. However, the scientific evidence on the effect of pesticide exposure on DNA methylation alteration is still limited, and DNA methylation has not yet been included in carcinogenicity testing by the EPA or other agencies. Previous studies have been limited to the evaluation of small sets of candidate methylation markers. To the best of our knowledge, no genome-scale investigation has been conducted to identify epigenomic loci that are sensitive to pesticides. Recently, in vitro system has been used to determine if chemical exposure alters DNA methylation\textsuperscript{14}. The purpose of the present study is to conduct genome-wide investigations to comprehensively examine whether exposure to three commonly used organophosphate pesticides (OPs) that have been associated with several cancers in human studies, induces DNA methylation alterations in in vitro.

Materials and Methods

Exposure of human K562 cells to three OPs.
The human chronic myelogenous leukemia cell line K562 was exposed to individual pesticide (i.e., fonofos, parathion, and terbufos) at dose of 0.001, 0.01, and 0.1 µM, or ethanol (control) for different time period (6, 12, 24, 48, 72 hours). DNA was prepared with a Wizard Genomic DNA purification kit and normalized to 25ng/µl for the genome-wide DNA methylation analysis.

Genome-wide examination of DNA methylation alterations.
We performed genome-wide DNA methylation examination on DNA samples obtained from cells exposed to each of the three OPs at 0.1 µM for 12 hours using Illuma Infinium Human Methylation27 BeadChip, which contains 27,578 individual CpG sites covering 14,000 genes. BeadChips were scanned with an Illumina iScan and then analyzed by the Illumina GenomeStudio software. All experiments were conducted following the manufacturer’s protocols in the Genomic Core Facility of the Center for Genetic Medicine at Northwestern University.

Bioinformatic/biostatistics analysis.
The Illuma Infinium methylation microarray data was processed by Bioconductor lumi package\textsuperscript{15}. The relative level of methylation was calculated as the ratio of signal from a methylated probe relative to an unmethylated probe. Bayesian-adjusted t-tests were used to identify differentially methylated sites. A cut-off of False Discovery Rate (FDR)-adjusted p-value (q-value) < 0.05 and fold change > 2 was used to identify candidate CpG sites. The significantly differentially methylated CpG-sites were then mapped to the closest downstream genes. Furthermore, we performed functional enrichment analysis for these overlapped genes based on Gene Ontology (GO) using Bioconductor GeneAnswers package\textsuperscript{16}.

Results
An overview of the sample relations based on Principal Component Analysis (PCA) plot of genome-wide DNA methylation profiles (Figure 1) and a heatmap of all differentially methylated genes (Figure 2A) showed distinct
Figure 2. All differentially methylated genes (panel A) and common genes with similar DNA methylation changes among three OPs (panel B, C). Panel A. DNA methylation heatmap of all differentially methylated genes in fonofos, parathion, and terbufos-treated cells compared with other OPs-treated or control cells. The heatmap color corresponds to the Beta-value of the measured CpG-sites. The Beta-value is in the range of 0 (shown in dark grey) and 1 (shown in light grey) with 0 representing purely unmethylated and 1 representing purely methylated. The color bar above the heatmap represents the sample types, in which the dark grey color represents three concordant pesticides including fonofos, parathion, and terbufos; black corresponds to the control samples and light grey represents all other pesticide-treated samples. Panel B and C. Venn diagram showing genes with comparable levels of methylation changes in all three OPs, including 625 hypermethylated genes (B) and 87 hypomethylated genes (C).
methylation patterns of fonofos, parathion, and terbufos-treated cells in comparison with other OPs-treated or control cells. We identified 1759, 1746, and 1580 CpG sites with significant methylation changes in cells exposed to fonofos, parathion and terbufos respectively. Out of these genes, 712 genes were seen for all three OPs with comparable levels of methylation changes, including 625 hyper-(Figure 2B) and 87 hypo-methylated genes (Figure 2C). Our further analysis on gene functions by GO analysis showed that some of these genes are implicated in cancer development or in the related biological pathways, and some are functionally unknown.

**Pesticide-specific genes with DNA methylation changes.**

We identified genes with methylation alteration in response to each specific OP. For example, fonofos-treated cells exhibited increased DNA methylation levels in genes of mutS homolog 2 (MSH2), a DNA repair-related gene, 1-myc-1 proto-oncogene isoform 2 (MYCL1) and cyclin-dependent kinase inhibitor 1B (CDKN1B). In parathion-treated cells, DNA methylation levels of CD1D antigen; d polypeptide (CD1D) and BRCA1 associated protein-1 (BAP1) increased 4.0-fold and 2.3-fold, while melanoma antigen family C; 2 (MAGEC2) decreased 2.0-fold respectively. The genes with increased methylation in terbufos-treated cell include insulin induced gene 1 isoform 1 (INSIG1), RAS-like; family 12 protein (RASL12) and cytokine induced apoptosis inhibitor 1 (CIAPIN1) (Table 1).

**Genes with similar DNA methylation changes among the three OPs examined.**

We observed 712 genes with similar DNA methylation changes for all three examined OPs. Some of these genes have been implicated in carcinogenesis. For example, tumor protein p53 inducible protein 11 (TP53I11) exhibited increased DNA methylation for all three OPs. Other genes with increased DNA methylation in all three OP-exposed cell lines include aristless-like homeobox 4 (ALX4), pancreatic and duodenal homeobox 1 (PDX1) and WNT1 inducible signaling pathway protein 3 (WISP3). Some genes with biological functions related to cancer etiology, such as immune response, DNA repair, and telomere length maintenance, were also identified to have altered methylation levels for all three OPs. For example, growth arrest and DNA-damage-inducible gamma (GADD45G) showed an increase of methylation in response to exposure to these pesticides. Other genes included O-6-methylguanine-DNA methyltransferase (MGMT) and telomerase reverse transcriptase isoform 3 (TERT) that exhibited increased methylation levels, and interleukin-1 receptor (IL1R1) that showed decreased methylation levels for all OPs. Aberrant promoter methylation of proliferation-related genes has also been detected, such as cyclin-dependent kinase 10 (CDK10) and protein tyrosine phosphatase, receptor type, K (PTPRK). In addition, we also observed genes/loci with unknown biological functions that exhibited significant fold changes in methylation level. DAZ interacting protein 1 (DZIP1), a mammalian protein with unknown biological function, showed large fold increases. A series of hypothetical protein showed large fold increases in DNA methylation level, such as hypothetical protein LOC123904 (UNQ2446) and LOC255101 (DKFZp434O0527) (Table 1).

**Discussion**

This is the first *in vitro* study of DNA methylation alteration in response to pesticide exposure using a genome-wide approach. Compared to controls and other pesticides, we observed distinct genome-wide DNA methylation patterns in relation to exposure to three commonly used pesticides that have been associated with cancers in cells, animals and epidemiology studies. We identified 1759, 1746, and 1580 CpG sites with significant methylation changes in response to exposure to fonofos, parathion and terbufos respectively. Out of these genes, 712 genes were seen for all three pesticides with similar levels of methylation changes. Gene ontology analysis demonstrated that some of these genes are directly implicated in carcinogenesis, some have functions related to cancer etiology, and some remain functionally unknown (Table 1).

Fonofos, parathion, and terbufos have been associated with various cancers (Table 2). When treated with fonofos, which has been associated with colon cancer, MSH2 exhibited about 3-fold increase in methylation level compared with control cells. Animal studies demonstrated that inactivation of MSH2, a mismatch repair gene, allows the proliferation of gastrointestinal (GI) tract cells damaged by methylating agents, and may increase the
<table>
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**Table 1. Selected genes based on GO analysis.** *OP-specific genes and common genes in 3 OPs were sorted by absolute fold change in fonofos-treated cells. CDCA1: cell division cycle associated 1; MSH2: mutS homolog 2; MYC1: 1-myc-1 proto-oncogene isoform 2; CDKN1B: cyclin-dependent kinase inhibitor 1B; CD1D: CD1D antigen; d polypeptide; RAP1: BRCA1 associated protein 1; MAGEC2: melanoma antigen family C; 2; INSIG1: insulin induced gene 1 isoform 1; RASL12: RAS-like; family 12 protein; CIAPIN: cytokine induced apoptosis inhibitor 1; TMEPAI: transmembrane prostate androgen-induced protein isoform a; GADD45G: growth arrest and DNA-damage-inducible, gamma; PITPK: protein tyrosine phosphatase, receptor type, K; MGMT: O-6-methylguanine-DNA methyltransferase; PDX1: pancreatic and duodenal homeobox 1; TER1: telomerase reverse transcriptase isoform 3; CDK10: cyclin-dependent kinase 10; ALX4: aristaless-like homeobox 4; PITRG: protein tyrosine phosphatase, receptor type, G; TP53I11: tumor protein p53 inducible protein 11; TNFRSF11B: tumor necrosis factor receptor superfamily, member 11b; TNFRSF25: tumor necrosis factor receptor superfamily, member 25; HIST4H4: histone H4; HDAC5: histone deacetylase 5; WINP5: WNT1 inducible signaling pathway protein 3; IL1R1: interleukin 1 receptor, type I; DZIPI: DAZ interacting protein 1; UNQ446: hypothetical protein LOC123904; DKFz434O0527: hypothetical protein LOC25510; C21orf77: hypothetical protein LOC55264; PAX7: paired box gene 7 isoform 1; EPHEA: EPH receptor A4; CHRNA3: cholinergic receptor, nicotinic, alpha 3; DRD1: dopamine receptor D1; EPHE7: EPH receptor A7.*
mutation of tumor suppressor genes. The methylation level of TEM1 doubled when cells were treated with fonofos and terbufos, which have been associated with prostate cancer (PC) . TEM1 is a potential tumor suppressor gene, and TMEPA1 expression is prostate abundant and restricted to prostatic epithelial cells. Among the three OPs, parathion is the only OP associated with breast cancer. Natural killer T cells could recognize CD1-presented antigen and initiate the cytolysis of the antigen-presenting cells, indicating loss of CD1D is associated with breast cancer metastasis. BAP1, a tumor suppressor gene, is a BRCA1 associated protein that functions in the BRCA1 growth control pathway. Methylation of the BRCA1 promoter has been shown to occur in approximately 20% of breast cancer patients. Both CD1D1 and BAP1 exhibited more than 2-fold increased methylation in parathion treated cells.

The similar methylation patterns observed for three individual OPs suggest that they may cause cancers via, at least partially, similar biological pathways. Aberrant tumor suppressor gene promoter methylation has emerged as one of the most important epigenetic mechanisms in the development of human cancer. In our study, several tumor suppressor genes were observed to have altered methylation levels in response to these three pesticides. Promoter hypermethylation of p53, a tumor suppressor gene, was found in individuals with skin, breast, and lung cancers. Methylation of ALX4 is a frequent event in colorectal cancers and other GI adenocarcinomas, indicating it as a potential marker for colon and other adenocarcinomas of the GI tract. We observed more than 12-fold increased methylation level of ALX in three OPs-treated cells, supporting that ALX4 gene methylation may serve as a specific marker for colon and other GI cancers. We also identify aberrant promoter methylation.

<table>
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Table 2. Profile of fonofos, parathion and terbufos. * Toxicity classification: Category I, most toxic; Category II, moderately toxic; Category III, slightly toxic; Category IV, practically nontoxic. ** EPA carcinogenicity classification: A, known to cause cancer in humans; B, probable human carcinogen; C, possible human carcinogen; D, not classifiable as to human carcinogenicity; and E, probably not carcinogenic.
of tumor suppressor genes, such as WISP3, a tumor suppressor gene in breast cancer\textsuperscript{33}, and PDX1, a tumor suppressor gene in gastric cancer\textsuperscript{34}. As shown in Table 2, the three OPs have been associated with various cancers, including colorectal, breast, lung and skin cancers.

MGMT is a DNA repair protein that specifically removes promutagenic alkyl groups from the O\textsuperscript{6} position of guanine in DNA\textsuperscript{35}. Aberrant hypermethylation in the promoter region of MGMT gene has been found frequently in different tumor types, including lung\textsuperscript{36} and colon cancers\textsuperscript{37}. We found more than 6-fold increase in methylation level of MGMT in three OPs-treated cells. Telomerase is a cellular ribonucleoprotein reverse transcriptase that stabilizes telomere length (TL) and is composed of telomerase RNA template and telomerase transcriptase protein (hTERT)\textsuperscript{38}. Altered methylation level in hTERT promoter may have influence on gene expression thus on telomerase activity\textsuperscript{39}. TERT promoter hypermethylation has been found in several cancer cell lines, including human breast cancer cell MCF-7, colon cancer cell SW480, and lung cancer cell SW2\textsuperscript{40-42}. Histone undergoes posttranslational modifications which alter their interaction with DNA and nuclear proteins\textsuperscript{43}. Histone modifications are also epigenetic changes that play important role in cancer development\textsuperscript{44}. Histone 4 is subject to covalent modification by methylation and acetylation, and thus influences chromatin structure and gene expression. Hypermethylation of TERT, HIST4H4 (Histone H4), and HDAC5 (histone deacetylase 5) observed in this study suggests that pesticides may exert their carcinogenic effects via, at least in part, histone modifications or TL shortening that are regulated by DNA methylation changes. These DNA methylation alterations may induce a wide range of potential abnormalities in gene expression patterns that can lead to dysregulation of processes related to cell transformation and tumorigenesis, including DNA repair, cell cycle control, genome stability and genome reprogramming\textsuperscript{45}.

In addition to tumor suppressor genes and genes with functions important in cancer development, several genes with unknown function also showed significantly altered methylation. For example, although the biological function of the paired box gene 7 (PAX7) is unknown; it plays critical roles during cancer growth and is speculated to involve tumor suppression\textsuperscript{46}. This is supported by our observation that all three OPs increased methylation level of PAX7. A series of hypothetical protein were differentially methylated, however, their existence has only been predicted without experimental evidence that it is expressed \textit{in vivo}.

Pesticides may affect DNA methylation through several cellular processes, including oxidative stress/reactive oxygen species generation\textsuperscript{4}, DNA methyltransferase activity alteration\textsuperscript{47}, and immunotoxicity\textsuperscript{48}. These cellular processes have been shown to be induced by exposure to pesticides and associated with altered DNA methylation patterns. In experimental and human studies, several OPs, including methyl parathion\textsuperscript{49,50}, have been shown to induce oxidative stress\textsuperscript{51,52}. DNA methylation reflects the cumulative oxidative stress, and ROS production has recently been shown to alter the expression of genes belonging to DNA methylation machinery\textsuperscript{53}. Some pesticides have immunotoxic effects\textsuperscript{54}, and are associated with elevated cytokine levels\textsuperscript{55}. Fonofos\textsuperscript{56} and terbufos\textsuperscript{20} are associated with NHL, and the leukemogenic effects may be related to immune function perturbation. It has been reported that elevated plasma cytokine levels were associated with hypermethylation of tumor-suppressor genes in peripheral lymphocytes\textsuperscript{48}. Despite the lack of clear evidence for carcinogenicity of OPs, these cellular processes have been postulated to induce abnormalities in gene expression patterns, which in turn could lead to tumorigenesis.

This is the first study on DNA methylation alteration in pesticide-treated human cells using the array-based genome-wide site-specific Illumina HumanMethylation27 platform. The Infinium DNA methylation platform is highly suitable for novel DNA methylation marker discovery. Although the methylation site coverage of the Illumina HumanMethylation27 platform is moderate and some available platforms have higher methylation site coverage, such as NimbleGen’s (including 750K probes); however, the NimbleGen chip provides only a binary call of methylation for each gene (Yes/No, based on an unknown threshold of methylation). The Illumina methylation Chip has several advantages: 1) quantitative results (i.e., a precise measure reflecting percent methylation at any given
methylation site); 2) single site resolution, unequivocally measuring methylation of 27,578 individual methylation sites (NimbleGen is a tiling array in which the chip features are combined to produce just a call of methylation in large DNA sequences); 3) is based on bisulfite treatment, a highly standardized preprocessing that ensures high reproducibility. In addition, we applied stringent statistical criteria to screen the CpG sites with expected FDR small than 5%. Furthermore, we demonstrated that the CpG sites non-randomly distributed among functional categories by gene ontology analysis. Our results provided direct experimental evidence that pesticides may modify DNA methylation in gene promoter CpG sites, which may play a pathological role in cancer development. Further studies in other cell types and human samples are required.

Acknowledgement.
We thank Drs. Andrea Baccarelli, Harvard School of Public Health, Andrew D, Wallace, North Carolina State University, Department of Environmental and Molecular Toxicology, and Simon Lin and Pan Du, Northwestern University, Biomedical Informatics Center, and Nadereh Jafari, Northwestern University, Genomics Core Facility for their important contributions. This work was supported by NIH award 1RC1ES018461-01.

Reference
5 Wong PS, Matsumura F. Promotion of breast cancer by beta-hexachlorocyclohexane in MCF10AT1 cells and MMTV-neu mice. BMC Cancer. 2007;7:130.


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The Journal of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University, Vol. XIV


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 Lurie Cancer Center and support the Lurie Cancer Center’s mission to foster basic and translational research in the mechanisms and treatment of cancer.

Bioinformatics Core Facility
Director: Warren Kibbe, PhD
312.695.1334 or wakibbe@northwestern.edu

The Bioinformatics Core Facility provides analysis, support and design for microarrays, proteomics, clinical trial informatics as well as custom web-based database development for basic science and clinical projects.

Biostatistics Core Facility
Director: Alfred Rademaker, PhD
312.908.1970 or rademaker@northwestern.edu

The Biostatistics Core Facility provides biostatistical and data management support including such services as: data analysis, clinical trial design, database design and management, design and analysis of clustered data, diagnostic screening tests, protocol preparation, and sample size determination.

Cancer Therapeutics and Diagnostic Screening Core Facility
Director: Eric Weiss, PhD
847-491-5643 or elweiss@northwestern.edu

The Cancer Therapeutics and Diagnostic Screening Core Facility helps investigators design, validate, and conduct diverse high throughput assays. These can be virtually any assay with a photometric readout, such as absorbance, luminescence, and fluorescence polarization. The facility has recently added capability for high throughput microscopy, including sophisticated software for analysis of large image databases. Additionally, the facility provides access to advanced platforms for large scale liquid handling, plasmid preparation, generations and manipulation of arrayed microbial strains, and protein affinity purification.

Cell Imaging Core Facility
Director: Teng-Leong Chew, PhD
312.503.4445 or t-chew@northwestern.edu

The Cell Imaging Facility offer state-of-the art instrumentation and services for the study of biological processes at the tissue, cellular and subcellular levels. The facility’s services include light, fluorescence, confocal, and electron microscopy, microinjection, digitally controlled temperature stage for live cell observation, computerized image analysis, and digital image manipulation.
Clinical Research Office
Director: Timothy Kuzel, MD
Administrative Director: Renee Webb
312.908.4026 or t-kuzel@northwestern.edu
r-ripenburg@northwestern.edu

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.

Mary Beth Donnelley Clinical Pharmacology Core Facility
Director: Michael Avram, PhD
312.908.0638 or mja190@northwestern.edu

The Donnelley Clinical Pharmacology Core Facility was established to provide investigators with pharmacokinetic support for clinical studies, including Phase I and Phase II clinical trials, of cancer chemotherapeutic agents and analgesics. Support includes optimizing the design, conduct, analysis, and interpretation of the pharmacokinetic portion of the proposed clinical study. Chemotherapeutic and analgesic concentrations in body fluids are measured using a state-of-the-art Agilent high performance liquid chromatography system linked to an Applied Biosystems API 3000 triple quadrupole mass spectrometer. Drug concentration histories are fitted to various compartmental pharmacokinetic models using commercially available and specialized software. Standard statistical criteria are used for model selection.

Flow Cytometry Facility
Director: Charles Goolsby, PhD
312.908.1294 or c-goolsby@northwestern.edu

The Flow Cytometry Core Facility provides cell sorting services and access to routine flow cytometry assays such as immunophenotyping and DNA analysis as well as guidance, technical assistance and equipment for the investigators to utilize more complex multi-parametric, multi-laser measurement and cell sorting in their research. The recent acquisition of the MoFlo high-speed sorter has increased the capabilities. The facility serves as a focus for studies of cellular heterogeneity in disease. Services range from consultation on experimental design, sample preparation and data analysis to instrument operation and set-up for cell sorting and multi-laser operation.

Genomics Core Facility
Director: Nadereh Jafari, PhD
312.503.3702 or n-jafari@northwestern.edu

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 throughput DNA extraction by Autopure LS from Gentra.

Keck Biophysics Facility
Director: Amy C. Rosenzweig, PhD
847.491.7610 or amyr@northwestern.edu

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.

Mouse Histology and Phenotyping Laboratory
Director: Warren G. Tourtellotte, MD
Facility Manager: Donna Emge
312.503.2679 or d-emge@northwestern.edu

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 312.503.9804 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: Piotr Kulezsa, MD, PhD Administrative Director: Eric Odulio Facility Manager: Adekunle Raji 312.908.9595 or p-kulezsa@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: Rajeshwar Awatramani, PhD Director of Core Operations: Lynn T. Doglio, PhD 312.503.0088 or r-awatramani@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.

Transcription factor KLF11 integrates progesterone receptor signaling and proliferation in uterine leiomyoma cells.


Abstract
Uterine leiomyoma is the most common tumor of the female genital tract and the leading cause of hysterectomy. Although progesterone stimulates the proliferation of uterine leiomyoma cells, the mechanism of progesterone action is not well understood. We used chromatin immunoprecipitation (ChIP)-cloning approach to identify progesterone receptor (PR) target genes in primary uterine leiomyoma smooth muscle cells. We identified 18 novel PR-binding sites, one of which was located 20.5 kb upstream of the transcriptional start site of the Kruppel-like transcription factor 11 (KLF11) gene. KLF11 mRNA levels were minimally downregulated by progesterone but robustly upregulated by the progesterone antagonist RU486. Luciferase reporter assays showed significant baseline and RU486-inducible promoter activity in the KLF11 basal promoter or distal PR-binding region, both of which contained multiple Sp1-binding sequences but lacked classic progesterone response elements. RU486 stimulated recruitment of Sp1, RNA polymerase II, PR, and the coactivators SRC-1 and SRC-2 to the distal region and basal promoter. siRNA knockdown of PR increased KLF11 expression, whereas knockdown of KLF11 increased leiomyoma cell proliferation and abolished the antiproliferative effect of RU486. In vivo, KLF11 expression was significantly lower in leiomyoma tissues compared with adjacent myometrial tissues. Taken together, using a ChIP-cloning approach, we uncovered KLF11 as an integrator of PR signaling and proliferation in uterine leiomyoma cells.


Cortical neural precursors inhibit their own differentiation via N-cadherin maintenance of beta-catenin signaling.


Abstract
Little is known about the architecture of cellular microenvironments that support stem and precursor cells during tissue development. Although adult stem cell niches are organized by specialized supporting cells, in the developing cerebral cortex, neural stem/precursor cells reside in a neurogenic niche lacking distinct supporting cells. Here, we find that neural precursors themselves comprise the niche and regulate their own development. Precursor-precursor contact regulates beta-catenin signaling and cell fate. In vivo knockdown of N-cadherin reduces beta-catenin signaling, migration from the niche, and
neuronal differentiation in vivo. N-cadherin engagement activates beta-catenin signaling via Akt, suggesting a mechanism through which cells in tissues can regulate their development. These results suggest that neural precursor cell interactions can generate a self-supportive niche to regulate their own number.

Galbaugh, T.; Feeney, Y. B.; Clevenger, C. V.

Prolactin receptor-integrin cross-talk mediated by SIRPalpha in breast cancer cells.


**Abstract**
The hormone prolactin (PRL) contributes to the pathogenesis of breast cancer in part through its activation of Janus-activated kinase 2 (Jak2)/signal transducer and activator of transcription 5 (Stat5), a PRL receptor (PRLr)-associated pathway dependent on cross-talk signaling from integrins. It remains unclear, however, how this cross-talk is mediated. Following PRL stimulation, we show that a complex between the transmembrane glycoprotein signal regulatory protein-alpha (SIRPalpha) and the PRLr, beta(1) integrin, and Jak2 in estrogen receptor-positive (ER(+)) and ER(-) breast cancer cells is formed. Overexpression of SIRPalpha in the absence of collagen 1 significantly decreased PRL-induced gene expression, phosphorylation of PRLr-associated signaling proteins, and PRL-stimulated proliferation and soft agar colony formation. In contrast, overexpression of SIRPalpha in the presence of collagen 1 increased PRL-induced gene expression; phosphorylation of Jak2, Stat5, and Erk; and PRL-stimulated cell growth. Interestingly, overexpression of a tyrosine-deficient SIRPalpha (SIRPalpha-4YF) prevented the signaling and phenotypic effects mediated by wild-type SIRPalpha. Furthermore, overexpression of a phosophatase-defective mutant of Shp-2 or pharmacologic inhibition of Shp-2 produced effects comparable with that of SIRPalpha-4YF. However, the tyrosine phosphorylation of SIRPalpha was unaffected in the presence or absence of collagen 1. These data suggest that SIRPalpha modulates PRLr-associated signaling as a function of integrin occupancy predominantly through the alteration of Shp-2 activity. This PRLr-SIRPalpha-integrin complex may therefore provide a basis for integrin-PRLr cross-talk and contribute to the biology of breast cancer.


Interferon consensus sequence binding protein (ICSBP) decreases beta-catenin activity in myeloid cells by repressing GAS2 transcription.


**Abstract**
The interferon consensus sequence binding protein (ICSBP) is an interferon regulatory transcription factor, also referred to as IRF8. ICSBP acts as a suppressor of myeloid leukemia, although few target genes explaining this effect have been identified. In the current studies, we identified the gene encoding growth arrest specific 2 (GAS2) as an ICSBP target gene relevant to leukemia suppression. We find that ICSBP, Tel, and histone deacetylase 3 (HDAC3) bind to a cis element in the GAS2 promoter and repress transcription in myeloid progenitor cells. Gas2 inhibits calpain protease activity, and beta-catenin is a calpain substrate in these cells. Consistent with this, ICSBP decreases beta-catenin protein and activity in a Gas2- and calpain-dependent manner. Conversely, decreased ICSBP expression increases beta-catenin protein and activity by the same mechanism. This is of interest, because decreased ICSBP expression and increased beta-catenin activity are associated with poor prognosis and blast crisis in chronic myeloid leukemia (CML). We find that the expression of Bcr/abl (the CML oncoprotein) increases Gas2 expression in an ICSBP-dependent manner. This results in decreased calpain activity and a consequent increase in beta-catenin activity in Bcr/abl-positive (Bcr/abl(+)) cells. Therefore, these studies have identified a Gas2/calpain-dependent mechanism by which ICSBP influences beta-catenin activity in myeloid leukemia.

Moulder, S.; Li, H.; Wang, M.; Gradishar, W. J.; Perez, E. A.; Sparano, J. A.; Pins, M.; Yang, X.; Sledge, G. W.

A phase II trial of trastuzumab plus weekly ixabepilone and carboplatin in patients with
HER2-positive metastatic breast cancer: an Eastern Cooperative Oncology Group Trial.


Abstract

The epothilone B analogue, ixabepilone, binds to β-tubulin, is effective for taxane-refractory metastatic breast cancer (MBC), and may be given every 3 weeks or weekly. We evaluated the efficacy of weekly ixabepilone (I) plus trastuzumab (T) and carboplatin (C) as first line therapy in HER2+ MBC. Patients with HER2+ (3+ by IHC or FISH amplified) MBC received I (15 mg/m² IV) and C (area under the curve, AUC = 2 IV) on days 1, 8, and 15 of a 28-day cycle for a maximum of 6 cycles, plus weekly T (4 mg/kg loading dose then 2 mg/kg IV) during chemotherapy then every 3 weeks (6 mg/kg IV) until disease progression. The primary objective was to determine whether the combination was associated with a response rate (RR) of at least 75%. Fifty-nine patients were treated, and 39 had HER2 overexpression confirmed in a central lab (cHER2+). For all treated patients, objective response occurred in 26 patients (44%; 95% CI 31-58%), median time to progression was 8.2 months (95% CI 6.3-9.9), and median overall survival was 34.7 months (95% CI 25.7 to [not reached]). Results were comparable for cHER2- cancers. Grade 3-4 adverse events included neutropenia (49%), thrombocytopenia (14%), fatigue (12%), nausea (7%), diarrhea (7%), and neuropathy (7%). One patient died from treatment complications during cycle 1. Weekly ixabepilone and carboplatin plus trastuzumab have an acceptable toxicity profile, but are not likely to be associated with an RR of 75% in HER2+ MBC. Efficacy appears comparable to paclitaxel, carboplatin, and trastuzumab.

Hardy, K. M.; Kirschmann, D. A.; Seftor, E. A.; Margaryan, N. V.; Postovit, L. M.; Strizzi, L.; Hendrix, M. J.

Regulation of the embryonic morphogen Nodal by notch4 facilitates manifestation of the aggressive melanoma phenotype.


Abstract

Reactivation of the embryonic morphogen Nodal in metastatic melanoma has previously been shown to regulate the aggressive behavior of these tumor cells. During the establishment of left-right asymmetry in early vertebrate development, Nodal expression is specifically regulated by a Notch signaling pathway. We hypothesize that a similar relationship between Notch and Nodal may be reestablished in melanoma. In this study, we investigate whether cross talk between the Notch and Nodal pathways can explain the reactivation of Nodal in aggressive metastatic melanoma cells. We show a molecular link between Notch and Nodal signaling in the aggressive melanoma cell line MV3 via the activity of an RBPJ-dependent Nodal enhancer element. We show a precise correlation between Notch4 and Nodal expression in multiple aggressive cell lines but not poorly aggressive cell lines. Surprisingly, Notch4 is specifically required for expression of Nodal in aggressive cells and plays a vital role both in the balance of cell growth and in the regulation of the aggressive phenotype. In addition, Notch4 function in vasculogenic mimicry and anchorage-independent growth in vitro is due in part to Notch4 regulation of Nodal. This study identifies an important role for cross talk between Notch4 and Nodal in metastatic melanoma, placing Notch4 upstream of Nodal, and offers a potential molecular target for melanoma therapy. Cancer Res; 70(24); 10340-50. (c)2010 AACR.

Nichole R. Blatner, Andreas Bonertz, Philipp Beckhove, Eric C. Cheon, Seth B. Krantz, Matthew Strouch, Juergen Weitz, Moritz Koch, Amy L. Halverson, David Bentrem, Khashayarsha Khazaie

In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction.


Abstract

T-regulatory cells (Treg) and mast cells (MC) are abundant in colorectal cancer (CRC) tumors. Interaction between the two is known to promote immune suppression or loss of Treg functions and autoimmunity. Here, we demonstrate that in both human CRC and murine polyposis the outcome of this interaction is the generation of potently immune suppressive but proinflammatory Treg
(ΔTreg). These Treg shut down IL10, gain potential to express IL17, and switch from suppressing to promoting MC expansion and degranulation. This change is also brought about by direct coculture of MC and Treg, or culture of Treg in medium containing IL6 and IL2. IL6 deficiency in the bone marrow of mice susceptible to polyposis eliminated IL17 production by the polyp infiltrating Treg, but did not significantly affect the growth of polyps or the generation of proinflammatory Treg. IL6-deficient MC could generate proinflammatory Treg. Thus, MC induce Treg to switch function and escalate inflammation in CRC without losing T-cell–suppressive properties. IL6 and IL17 are not needed in this process.


Irreversible electroporation therapy in the liver: longitudinal efficacy studies in a rat model of hepatocellular carcinoma.


Abstract
Irreversible electroporation (IRE) is an innovative local-regional therapy that involves delivery of intense electrical pulses to tissue to induce nanoscale cell membrane defects for tissue ablation. The purpose of this study was to investigate the feasibility of using IRE as a liver-directed ablation technique for the treatment of hepatocellular carcinoma (HCC). In the N1-S1 rodent model, hepatomas were grown in 30 Sprague-Dawley rats that were divided into treatment and control groups. For treatment groups, IRE electrodes were inserted and eight 100-mus 2,500-V pulses were applied to ablate the targeted tumor tissues. For both groups, magnetic resonance imaging scans were performed at baseline and 15-day follow-up intervals to determine tumor sizes (one-dimensional maximum diameter, D(max); estimated two-dimensional cross-sectional area, C(max)) as a tactic to assess longitudinal outcomes. Additional groups of treated animals were sacrificed at 1-, 5-, and 7-day intervals posttherapy for pathology assessment of treatment response. Magnetic resonance images showed significant tumor size reductions within 15 days posttherapy (32 +/- 31% D(max) and 52 +/- 39% C(max) decreases compared with 110 +/- 35% D(max) and 286 +/- 125% C(max) increases for untreated tumors). Pathology correlation studies documented progression from poorly differentiated viable HCC tissues before treatment to extensive tumor necrosis and full regression in 9 of 10 treated rats 7 to 15 days after treatment. Our findings suggest that IRE can be an effective strategy for targeted ablation of liver tumors, prompting its further evaluation for HCC therapy.


The MMSET histone methyltransferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells.


Abstract
The MMSET (Multiple Myeloma SET domain) protein is overexpressed in multiple myeloma patients with the translocation t(4;14). Although studies have shown the involvement of MMSET/WHSC1 in development, its mode of action in the pathogenesis of multiple myeloma (MM) is largely unknown. We found that MMSET is a major regulator of chromatin structure and transcription in t(4;14) MM cells. High levels of MMSET correlate with an increase in lysine 36 methylation of histone H3 and a decrease in lysine 27 methylation across the genome, leading to a more open structural state of the chromatin. Loss of MMSET expression alters adhesion properties, suppresses growth and induces apoptosis in MM cells. Consequently, genes affected by high levels of MMSET are implicated in the p53 pathway, cell cycle regulation and integrin signaling. Regulation of many of these genes required functional histone methyl-transferase (HMT) activity of MMSET. These results implicate MMSET as a major epigenetic regulator in t(4;14)+ MM.

A Novel Nanoparticulate Formulation of Arsenic Trioxide with Enhanced Therapeutic Efficacy in a Murine Model of Breast Cancer


Abstract
PURPOSE: The clinical success of arsenic trioxide (As$_2$O$_3$) in hematologic malignancies has not been replicated in solid tumors due to poor pharmacokinetics and dose-limiting toxicity. We have developed a novel nanoparticulate formulation of As$_2$O$_3$ encapsulated in liposomal vesicles or “nanobins” [(NB(Ni,As)] to overcome these hurdles. We postulated that nanobin encapsulation of As$_2$O$_3$ would improve its therapeutic index against clinically aggressive solid tumors, such as triple-negative breast carcinomas. EXPERIMENTAL DESIGN: The cytotoxicity of NB(Ni,As), the empty nanobin, and free As$_2$O$_3$ was evaluated against a panel of human breast cancer cell lines. The plasma pharmacokinetics of NB(Ni,As) and free As$_2$O$_3$ were compared in rats to measure drug exposure. In addition, the antitumor activity of these agents was evaluated in an orthotopic model of human triple-negative breast cancer. RESULTS: The NB(Ni,As) agent was much less cytotoxic in vitro than free As$_2$O$_3$ against a panel of human breast cancer cell lines. In contrast, NB(Ni,As) dramatically potentiated the therapeutic efficacy of As$_2$O$_3$ in vivo in an orthotopic model of triple-negative breast cancer. Reduced plasma clearance, enhanced tumor uptake, and induction of tumor cell apoptosis were observed for NB(Ni,As). CONCLUSIONS: Nanobin encapsulation of As$_2$O$_3$ improves the pharmacokinetics and antitumor efficacy of this cytotoxic agent in vivo. Our findings demonstrate the therapeutic potential of this nanoscale agent and provide a foundation for future clinical studies in breast cancer and other solid tumors.

Hillhouse, J.; Turrisi, R.; Stapleton, J.; Robinson, J.

Effect of seasonal affective disorder and pathological tanning motives on efficacy of an appearance-focused intervention to prevent skin cancer in individuals reporting seasonal affective disorder (SAD) symptoms and pathological tanning motives. DESIGN: Randomized, controlled clinical trial. SETTING: College campus. PARTICIPANTS: Four hundred thirty adult female indoor tanners (200 in the intervention group and 230 control participants). INTERVENTION: A booklet discussing the history of tanning, current tanning norms, UV radiation’s effects on skin, recommendations for indoor tanning use focusing on abstinence and harm reduction recommendations, and information on healthier, appearance-enhancing alternatives to tanning. MAIN OUTCOME MEASURES: Self-reported attitudes, intentions, and tanning behaviors; pathological tanning motives assessed by a questionnaire developed for this study; and SAD symptoms assessed by the Seasonal Pattern Assessment Questionnaire. RESULTS: Two of the 4 pathological tanning scales, opiatelike reactions to tanning and dissatisfaction with natural skin tone, were significant moderators demonstrating stronger treatment effects for individuals scoring higher on these scales. Treatment effects were equivalently positive (ie, no significant moderator effects) for all levels of SAD symptoms and all levels of the other 2 pathological tanning motive scales (ie, perceiving tanning as a problem and tolerance to the effects of tanning). CONCLUSIONS: The appearance-focused skin cancer prevention intervention is robust enough to reduce indoor tanning among tanners who exhibit SAD symptoms or pathological tanning motives. Tailored interventions may address individuals’ motivations for tanning and their relation to maladaptive behavior, such as dissatisfaction with appearance or the need for relaxation because of anxiety.


Imaging response in the primary index lesion and clinical outcomes following transarterial locoregional therapy for hepatocellular carcinoma.

Abstract

CONTEXT: Response Evaluation Criteria in Solid Tumors (RECIST) (unidimensional), World Health Organization (WHO) (bidimensional), and European Association for Study of the Liver (EASL) (necrosis) guidelines are commonly used to assess response following therapy for hepatocellular carcinoma (HCC). No universally accepted standard exists.

OBJECTIVES: To evaluate intermethod agreement between these 3 imaging guidelines and to introduce the concept of the "primary index lesion" as a biomarker for response.

DESIGN, SETTING, AND PARTICIPANTS: Single-center comprehensive imaging analysis including 245 consecutive patients with HCC who were treated with chemoembolization or radioembolization between January 2000 and December 2008. Computed tomography and magnetic resonance imaging scans (N = 1065) were reviewed to assess response in the "primary index lesion," defined as the largest tumor targeted during first treatment.

MAIN OUTCOME MEASURES: Intermethod agreement (kappa statistics) between RECIST, WHO, and EASL guidelines response; correlation of WHO and EASL response in the primary index lesion with time to progression and survival. RESULTS: Kappa coefficients were 0.86 (95% confidence interval [CI], 0.80-0.92) between the WHO and RECIST guidelines, 0.24 (95% CI, 0.16-0.33) between RECIST and EASL, and 0.28 (95% CI, 0.19-0.36) between WHO and EASL. Disease progressed in 96 patients; 113 died. The hazard ratio for time to progression in responders compared with nonresponders was 0.36 (95% CI, 0.23-0.57) for WHO, 0.38 (95% CI, 0.24-0.58) for RECIST, and 0.38 (95% CI, 0.22-0.64) for EASL. Hazard ratios for survival in responders compared with nonresponders in univariate and multivariate analyses were 0.46 (95% CI, 0.32-0.67) and 0.55 (95% CI, 0.35-0.84) for WHO and 0.36 (95% CI, 0.22-0.57) and 0.54 (95% CI, 0.34-0.85) for EASL. Hazard ratios for survival in responders vs nonresponders in patients with solitary and multifocal HCC were 0.39 (95% CI, 0.19-0.77) and 0.51 (95% CI, 0.32-0.82) for WHO and 0.26 (95% CI, 0.10-0.67) and 0.47 (95% CI, 0.28-0.79) for EASL.

CONCLUSIONS: Among a group of patients with HCC, agreement for classification of therapeutic response was high between the RECIST and WHO guidelines but low between each of these and EASL. Application of these methods to measure response in a primary index lesion resulted in statistically significant correlations with disease progression and survival.


An integrated network of androgen receptor, polycomb, and TMPRSS2-ERG gene fusions in prostate cancer progression.


Abstract

Chromosomal rearrangements fusing the androgen-regulated gene TMPRSS2 to the oncogenic ETS transcription factor ERG occur in approximately 50% of prostate cancers, but how the fusion products regulate prostate cancer remains unclear. Using chromatin immunoprecipitation coupled with massively parallel sequencing, we found that ERG disrupts androgen receptor (AR) signaling by inhibiting AR expression, binding to and inhibiting AR activity at gene-specific loci, and inducing repressive epigenetic programs via direct activation of the H3K27 methyltransferase EZH2, a Polycomb group protein. These findings provide a working model in which TMPRSS2-ERG plays a critical role in cancer progression by disrupting lineage-specific differentiation of the prostate and potentiating the EZH2-mediated dedifferentiation program.
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PROFESSIONAL EDUCATION PROGRAMS
Throughout the year, the Lurie Cancer Center offers professional education programs on various cancer specialties.

Listed below are some of the programs planned for 2012. Visit cancer.northwestern.edu to view an up-to-date list of educational opportunities with details and online registration, or call 312.695.1304.

January
Brain Tumor Symposium
Drug Delivery and Nanomaterials Symposium

March
8th Annual Thoracic Surgical Oncology Nursing Conference

April
Malkin-Kraft Lectureship
Speaker: Scott W. Lowe, PhD
9th International Chicago Lymphoma Symposium
(Presented by Northwestern University in conjunction with The University of Chicago and The University of Massachusetts)
Translational Cancer Research Workshop

May
6th Annual Connie Moskow Memorial Lectureship
Speaker: Paul Goss, FRCP, MB, BCh, PhD
H Foundation Basic Science Symposium

June
2012 Oncology Review
23rd Annual Scientific Poster Session

September
7th Annual Northwestern Radiosurgery Symposium
Nathaniel Berlin Lectureship
Speaker: Tyler Jacks, MD

October
14th Annual Lynn Sage Breast Cancer Symposium

December
15th Annual Oncology Nursing Conference
PATIENT AND PUBLIC 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.

Listed below are some of the programs planned for 2012. Visit cancer.northwestern.edu to view an up-to-date list of programs with details and online registration, or call 312.695.1304.

Brain Tumor Patient and Caregiver Forum
19th Annual Cancer Survivors’ Celebration & Walk
  Leukemia & Lymphoma Town Hall
Lynn Sage Breast Cancer Town Hall Meeting
  Prostate Cancer Forum
  Thyroid Cancer Education Program
Young Adult Cancer Survivorship Program

ONGOING PROGRAMS

Cancer Connections
An opportunity for patients and families to learn about local support groups, educational programs, wellness activities and community resources.

Gilda’s Club Chicago at the Lurie Cancer Center
Patients and families at the Lurie Cancer Center have on-site access to a wide range of programs and activities offered by Gilda’s Club Chicago. Designed to be fun, informative and reduce stress, all of the activities are offered free of charge.
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 40 cancer centers in the nation to hold this NCI distinction.

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