



United **FRONT**

OSUCCC – James clinical and basic researchers unite to improve therapy for women with triple-negative breast cancer.



**NCI
CCC**

A Comprehensive Cancer Center Designated by the National Cancer Institute

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UPFRONT

The Director's Perspective

Reasons to Celebrate

Quality indicators continue to rise

Last year a panel of NCI experts arrived at the OSUCCC to look under the hood and see how we're doing. I'm pleased to report that they liked what they saw. Following that visit and a review of our core-grant renewal proposal, the NCI rated our program "exceptional," their highest descriptor, and recommended continued funding. The amount will be announced early this year.

The renewal enables Ohio State to retain its status as one of only 40 NCI-designated comprehensive cancer centers, a designation that the University has maintained since 1976. The NCI site review team also stated, "This Center should serve as the model for other matrix university-based Centers."

These remarkable outcomes were, of course, a team effort. They were achieved through the dedication,

cooperation and collaboration of our physicians, nurses, investigators and support staff.

Over the past five years, our NCI grant funding has grown by 96 percent, placing the OSUCCC 14th in annual NCI research funding nationally. Our goal is to break into the top 10. During the same period, our investigator-initiated therapeutic clinical trials jumped 258 percent, to 552, and we produced about 4,000 scientific publications. Half of those publications represent collaborations among disciplines—an amazing statistic that shows our investigators are working together to produce the best results.

Speaking of best results, the Leapfrog Group, a coalition of public and private purchasers of employee health coverage that works to improve healthcare quality, has



MICHAEL A. CALIGIURI, MD
DIRECTOR,
COMPREHENSIVE
CANCER CENTER
CHIEF EXECUTIVE
OFFICER, JAMES CANCER
HOSPITAL AND SOLOVE
RESEARCH INSTITUTE
THE OHIO STATE
UNIVERSITY, JOHN L.
MARAKAS NATIONWIDE
INSURANCE ENTERPRISE
FOUNDATION CHAIR IN
CANCER RESEARCH

again included the OSUCCC – James on its list of 65 "top hospitals." The selection comes from the organization's national survey of hospital performance in key areas of patient safety and quality.

Finally, the second annual Pelotonia, our grassroots bicycle tour held in August, generated \$7.8 million exclusively for cancer research at the OSUCCC – James. The event attracted 4,047 riders, nearly double the number who participated in the 2009 inaugural Pelotonia. Together, the two events raised more than \$12 million for cancer research here at home. We truly have reasons to celebrate.

THE OHIO STATE UNIVERSITY COMPREHENSIVE CANCER CENTER– ARTHUR G. JAMES CANCER HOSPITAL AND RICHARD J. SOLOVE RESEARCH INSTITUTE

Director, Comprehensive Cancer Center
Chief Executive Officer, James Cancer
Hospital and Solove Research Institute
The Ohio State University
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Executive Director for Administration
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Distinguished University Professor
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WINTER 2011



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JamesCare Comprehensive Breast Center opens its doors



ON THE COVER: TRIPLE-NEGATIVE BREAST CANCER TEAM FORMS A UNITED FRONT PHOTOGRAPH BY ROMAN SAPECKI

Quarantine?

Are Newly Treated Thyroid-Cancer Patients Hazardous to Our Health?



By **MATTHEW D. RINGEL, MD**, professor of Medicine in the Division of Endocrinology, Diabetes and Metabolism, with joint appointments in the Division of Medical Oncology and the Department of Molecular Virology, Immunology and Medical Genetics. In 2009, he was awarded the American Thyroid Association's Van Meter Award, which is presented to an investigator age 45 or under who has made outstanding contributions to thyroid disease research.

This autumn the question again arose in the media about whether thyroid-cancer patients just treated with a radioactive isotope of iodine pose a hazard to those around them. Should those individuals be quarantined before returning home following treatment? While this hazard may be theoretical, it is worth recalling that many members of the public have a limited understanding of radiation and an almost instinctive fear of radioactivity at any level.

Our post-911 world involves closer monitoring of low-level radioactivity at airports, landfills and tunnels, giving the question added life.

More than 44,000 new cases of thyroid cancer are expected to occur in Americans this year. Most of those individuals will be treated with I-131, a radioactive isotope of iodine that concentrates in the thyroid gland and destroys cancer cells. I-131 has been used for decades to treat thyroid cancer. Here at Ohio State, a major thyroid cancer referral center, we treat a large number of thyroid cancer patients, many of whom receive I-131 therapy during their management.

In 1997, the Nuclear Regulatory Commission (NRC) relaxed its guidelines governing the therapeutic use of I-131 to allow more patients to return home following therapy instead of quarantining them in the hospital. The change was made after studies concluded that most

patients treated with I-131 posed little risk to their families and others when patient doses and physical characteristics result in low exposure levels, and when simple and reasonable measures are followed after patients leave the clinic.

I-131 has a half-life of about eight days and is flushed from the body in the urine and the gastrointestinal tract. Thus, through both radioactive decay and excretion, the I-131 that is not taken up by the thyroid cells is quickly cleared from the body.

Patients treated for thyroid cancer at The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute are released in strict accordance with Ohio Department of Health regulations and in accordance with NRC guidelines. All patients receive detailed verbal and written instructions prior to treatment describing how to maximize the clearance of the



I-131 and how to minimize exposure to those in their homes. This education process begins in the treating clinician's office and continues with our thyroid cancer nurses after the visit, with our nuclear medicine physicians and with their radiation safety officers. Importantly, if the exposure exceeds 500 millirems, or if patients are unable to follow the recommendations for safe discharge home, they are admitted to the hospital until safe exposure levels are achieved.

These practices are the current standard of care and are endorsed by the American Thyroid Association,

The Endocrine Society, the Society of Nuclear Medicine, and the American Association of Clinical Endocrinologists. Per an October 20, 2010, joint statement from these organizations, "The American Thyroid Association has recently completed an examination of the current scientific evidence for any potential risks to the public from I-131 therapy of thyroid cancer. It is anticipated that the report will provide updated recommendations for best practices focusing on patient and public safety following I-131 treatment."

Our goal at Ohio State is to treat

all patients using evidence-based best practices. For patients with thyroid cancer, this often includes I-131 therapy. We are committed to using this treatment in a manner that is safe for patients, their families and the public. We believe this is best afforded by following current NRC and society-approved recommendations and guidelines, which include careful attention to administered dose, detailed patient education and instructions, and open communication between physicians and patients. 

BREAKTHROUGH

The Frontiers of Cancer Research

▶ MULTIPLE MYELOMA

PUT TO THE TEST

Second Ohio State cancer drug enters clinical trial



For the second time within 2½ years, an experimental drug created by cancer researchers at the OSUCCC – James is being tested in a clinical trial.

Last summer, adult patients began receiving oral doses of AR-42, which is in a new class of drugs called histone deacetylase (HDAC) inhibitors, or compounds designed to reactivate genes that normally guard against cancer but are turned off by the cancer process.

AR-42 is designed to treat relapsed or treatment-resistant multiple myeloma, chronic lymphocytic leukemia or lymphoma. **JOHN C. BYRD, MD**, initiated the drug's development with **CHING-SHIH CHEN, PhD**, a cancer researcher and professor of Medicinal Chemistry in the College of Pharmacy.

The phase I/IIa clinical trial is assessing the safety and initial evidence of anticancer activity of the drug. Byrd says Ohio State is the only site worldwide accepting patients to this trial.

“Early tests in cancer cell models showed that AR-42 is 10,000-fold more potent than the starting/parent agent,” says Chen.

In 2003, Byrd asked Chen to try to improve the potency of a short-chain fatty acid known to have a

weak inhibitory effect against cancer growth. Chen worked with cancer center and pharmacy colleagues to develop the drug, originally called OSU-HDAC42, a broad spectrum histone and non-histone deacetylation inhibitor (pan-DAC).

The agent has been licensed to the biopharmaceutical company Arno Therapeutics, Inc., for clinical development.

“It is exciting to see this very potent broad-class I/II HDAC inhibitor enter the clinic for treatment of blood cancers, and we look forward to meaningful results,” Byrd says, noting that **CRAIG HOFMEISTER, MD**, is principal investigator on the clinical trial.

In August 2009, the OSUCCC – James began enrolling patients in a clinical trial for AR-12, another anticancer agent designed by Chen's lab that also is being developed by Arno Therapeutics. AR-12 inhibits solid-tumor growth by triggering cancer cells to self-destruct.

Byrd is professor of Internal Medicine and the D. Warren Brown Designated Professorship in Leukemia Research. Chen holds the Lucius A. Wing Chair of Cancer Research & Therapy, and is professor of Medicinal Chemistry, of Internal Medicine, and of Urology.

▶▶ BRAIN CANCER

SLEEPERS AWAKE

Conscious sedation for brain surgery may shorten hospital stay

The recovery time and cost of brain-tumor surgery might be reduced if surgery is performed while patients are awake during part of the procedure, a study at the OSUCCC – James suggests.

The study examined the outcomes of 39 patients treated for glioma. Doctors wanted to learn whether patients who received conscious sedation had outcomes different from those who underwent general anesthesia.

“Our data suggest that patients who received conscious sedation had shorter hospital stays, and that this reduced the cost of treatment,” says study leader **E. ANTONIO CHIOCCA, MD, PhD**, professor and chair of Neurological Surgery. “This finding must be validated with a randomized prospective clinical trial, but if it holds true, it would mean that changing our way of delivering anesthesia may allow these patients to leave sooner and save resources.”

Neurosurgeons usually reserve conscious sedation for patients with tumors near the brain’s speech and sensorimotor centers, Chiocca says. The method was conceived in the 1950s to avoid or minimize damage to these centers. Since then, several studies have indicated that conscious sedation can result in more complications than general anesthesia, while other studies appear to show the opposite.

To investigate this question,



Chiocca and colleagues studied the outcomes of 20 cases that used conscious sedation during surgery for gliomas and compared them with 19 cases that used general anesthesia.

The researchers evaluated patients for the number of days they remained in the hospital and for the cost of four items related to the surgery: the operating room, anesthesia, neurosurgical intensive care and the hospital room. Each patient was also evaluated for neurological complications.

No significant differences were found in the percentage of complications. As for the costs, the expense associated with the operating room and anesthesia were the same in both groups, and both groups spent similar time in intensive care. However, patients receiving conscious

THE RESEARCHER

E. ANTONIO CHIOCCA, MD, PhD

professor and chair of Neurological Surgery, Dardinger Family Endowed Chair in Oncological Neurosurgery

sedation had shorter hospital stays after leaving intensive care than patients receiving general anesthesia—3.5 vs. 4.6 days.

And the shorter hospital stays led to an average 36-percent decrease in post-intensive-care direct cost for cases receiving conscious sedation.

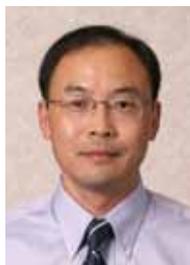


*Published in the journal
Cancer Prevention Research.*



SUPER SUBSTANCE

Dietary supplement may block cancer cells



XIANGHONG ZOU, PhD,
assistant professor of Pathology

Researchers at the OSUCCC – James have discovered how a substance produced when eating broccoli and brussels sprouts can block the proliferation of cancer cells.

Compelling evidence indicates that the substance, indole-3-carbinol (I3C), may have anticancer effects and other health benefits. The findings show how I3C affects cancer cells and normal cells.

The laboratory and animal study discovered a connection between I3C and a molecule called Cdc25A, which is essential for cell division and proliferation. Research showed that I3C causes the destruction of that molecule and thereby blocks the growth of breast cancer cells.

“Cdc25A is present at abnormally high levels in about half of breast cancer cases, and it is associated with a poor prognosis,” says study leader **Xianghong Zou, PhD**, assistant professor of Pathology, who notes that the molecule also occurs at abnormally high levels in cancers of the breast, prostate, liver, esophagus, endometrium and colon, as well as in non-Hodgkin’s lymphoma and other diseases such as Alzheimer’s.

“For this reason, a number of anti-Cdc25A agents have been identified, but they have not been successful for cancer prevention or treatment due to concerns about their safety or efficacy,” says Zou. “I3C can have striking effects on

cancer cells. A better understanding of this mechanism may lead to the use of this dietary supplement as an effective and safe treatment strategy for cancer and other diseases associated with overexpression of Cdc25A.”

For this study, Zou and colleagues exposed three breast cancer cell lines to I3C and observed that the substance caused the destruction of Cdc25A. They also pinpointed a location on that molecule that made it susceptible to I3C, showing that if that location is altered because of a gene mutation, I3C no longer causes the molecule’s destruction.



Published in the journal
Cancer Prevention Research.

DOSING BY THE NUMBERS

Model may simplify high-dose radiosurgery planning

There is no straightforward way to determine the optimal dose level and treatment schedules for high-dose radiation therapies such as stereotactic therapy, used to treat brain and lung cancer, or high-dose brachytherapy for treating prostate and other cancers.

However, radiation oncologists at the OSUCCC – James may have solved this problem by developing a mathematical model that encompasses all dose levels.

Typically, radiation therapy for cancer is given in daily low doses spread over many weeks. Oncologists often calculate the schedules for these fractionated, low-dose courses using a mathematical linear-quadratic (LQ) model that is also used to

evaluate radiation response, interpret clinical data and guide clinical trials.

“Unfortunately, the LQ Model doesn’t work well for high-dose radiation therapy,” says co-author **NINA MAYR, MD**, professor of Radiation Oncology. “Our study resolves this problem by modifying the current method to develop a Generalized LQ (gLQ) Model that covers all dose levels and schedules.”

First author, **JIAN WANG, PhD**, who passed away unexpectedly in June 2010, was largely responsible for developing the gLQ Model.

If verified clinically, Mayr says, the gLQ Model could guide the planning of dose levels and schedules needed for the newer radiosurgery and stereotactic radiation therapy and for

high-dose brachytherapy procedures that are increasingly used for cancer patients.

Mayr says the new model could allow oncologists to design radiation dose schedules more efficiently, help researchers conduct clinical trials for specific cancers more quickly, and make high-dose therapies available to cancer patients much sooner.

“Our Generalized LQ Model determines appropriate radiation levels across the entire wide spectrum of doses, from low to high, and from many to very few treatments, which is a new approach,” Mayr says.



Published in the journal
Science Translational Medicine

▶▶ BREAST CANCER

IN THE LOOP

Breast cancer cells regulate multiple genes in response to estrogen-like compounds

Cancer researchers at the OSUCCC – James have discovered a type of gene regulation and DNA behavior in breast cancer cells that may offer insight about environmental exposure to estrogen-like compounds.

The study provides the first evidence that cells can regulate many genes at once by looping their DNA, contributing to cancer when this process goes awry. This regulation, discovered in breast cancer cells as a response to estrogen, resulted in the silencing of 14 genes at once.

TIM H.-M. HUANG, PhD, professor of Molecular Virology, Immunology and Medical Genetics, and Pei-Yin Hsu, a visiting scholar in Huang's lab, located the DNA looping event in a breast cancer cell line gene cluster at chromosome region 16p11.2. They validated the finding using normal human breast epithelial cells and two animal models.

They also used the normal-cell model to determine if long-term exposure to nine estrogen-like chemicals can initiate gene silencing through this mechanism. These chemicals included diethylstilbestrol, two thalates and bisphenol A (BPA).

The suppressive effects varied in normal cells, but when investigators exposed rats to BPA for 21 days, they found concurrent suppression of 10 genes comparable to those located at 16p11.2. Huang says this suggests that continuous exposure to estrogen-like compounds might lead to permanent silencing of genes in this conserved cluster.

In healthy breast epithelial cells, 14



THE RESEARCHER

TIM H.-M. HUANG, PhD, (Left)

professor of Molecular Virology, Immunology and Medical Genetics at the OSUCCC – James

gene regulatory sites joined to form a temporary transcription site, Huang says. “But in breast cancer cells, there is no coordinated transcription site pairing, the DNA loops become tangled and the entire gene complex shuts down in a dead knot.”

In some cases, he adds, this multi-gene regulatory mechanism can increase gene expression and oncogenic activity, further contributing to cancer.

“We offer a new concept in this paper for the collective regulation of gene transcription,” says first author Hsu, who identified the loop structures and their significance. “We found that in normal breast cells, DNA looping is more flexible and

brings different promoters together temporarily. But in cancer, this complex just locks up and causes long-term suppression.”

For a demonstration of DNA looping, visit <http://www.youtube.com/watch?v=4y0e6oumqdo>



Published in the journal
Genome Research.

MOLECULAR MAYHEM

Targeted agent shows promise for chronic lymphoid leukemia



THE RESEARCHER

AMY JOHNSON, PhD,
*assistant professor of Hematology
 and Medicinal Chemistry*

Researchers at the OSUCCC – James have identified an experimental agent that targets chronic lymphocytic leukemia (CLL) and perhaps other proliferative disorders of lymphocytes.

A study of cells from patient tumors shows that the small-molecule inhibitor CAL-101 promotes apoptosis in CLL cells and disrupts several external survival pathways needed for CLL cell viability and proliferation. The agent blocks the PI3K-delta molecule, an isomer of the P13K (phosphatidylinositol-3 kinase) pathway, which is activated mainly in hematopoietic cells.

“Our findings provide a rationale for developing CAL-101 as the first in a new class of targeted therapies for CLL,” says principal investigator **AMY JOHNSON, PhD**, assistant professor of Hematology and Medicinal Chemistry. “A PI3K

inhibitor hasn’t been developed yet because this pathway is required for many essential cellular functions, but the identification of PI3K-delta, which is hematopoietic-selective, unlocks a potential therapy for B-cell malignancies.”

CLL is the most common adult leukemia in the United States. Patients with the asymptomatic phase can live for many years even without treatment. Once the disease reaches the symptomatic phase, treatment is required, usually chemotherapy that often induces remission. But current therapies are not curative; nearly all patients relapse.

The PI3K pathway is essential for survival of cells generally. This made it an unsuitable target for small molecule inhibitors until recently when research showed that PI3K-delta expression occurs mainly

in hematopoietic cell types. Preclinical studies suggest that blocking this molecule may kill B cells with little toxicity to other hematopoietic cells.

The OSUCCC – James study found that: CLL cells show high PI3K pathway activity and PI3K-delta expression; CAL-101 preferentially kills CLL cells compared with normal B-cells; CAL-101 inhibits PI3K-delta and promotes apoptosis in primary CLL cells while disrupting multiple external survival pathways; CAL-101 cell killing is caspase-dependent and not diminished by stromal cells; and CAL-101 does not kill normal T cells or natural killer (NK) cells or reduce antibody-dependent cellular cytotoxicity, but it does lower production of inflammatory and antiapoptotic cytokines by activated T cells.

A phase I clinical trial of CAL-101 is under way in select relapsed or refractory hematologic malignancies at Ohio State and other centers.



Published in the journal Blood

OF NOTE

*Recent Recognitions of
OSUCCC – James Physicians
and Researchers*

GRANTS



CARLO M. CROCE, MD, director of Human Cancer Genetics and the John W. Wolfe Chair in Human Cancer Genetics, **has**

received a \$1.9 million, five-year NCI grant to study microRNAs and ultraconserved noncoding RNAs as biomarkers for cancer risk, early tumor detection, tumor progression and response to treatment.



CHARLES SHAPIRO, MD, director of Breast Medical Oncology, and **KAY HUEBNER, PhD**, professor of Molecular

Virology, Immunology and Medical Genetics, **have received a \$2.5 million, five-year NCI grant** to study microRNA profiles of triple-negative breast cancer to define subgroups and therapeutic targets.



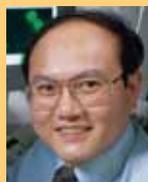
WOLFGANG SADEE, PhD, chair and professor of Pharmacology, of Pharmacy, of Internal Medicine, of Psychiatry and of Public

Health, **has received a \$9.1 million, five-year grant from the National Institute of General Medical Sciences** for a study titled "Expression Genetics in Drug Therapy." The grant also funds Ohio State as a member of a nationwide Pharmacogenomics Research Network.

AWARDS AND HONORS



MICHAEL A. CALIGIURI, MD, director of the OSUCCC and CEO of the James Cancer Hospital and Solove Research Institute, **is one of four scientists nationwide this year to receive a prestigious MERIT Award from the National Cancer Institute** for superior competence and outstanding productivity. The award provides up to 10 years of research support.



CHING-SHIH CHEN, PhD, Lucius A. Wing Chair of Cancer Research & Therapy, Professor of Medicinal Chemistry, of Internal Medicine, and of Urology, **has received the 2010**

Innovator of the Year award from The Ohio State University for his translational research and the success of his drug discovery program.



CARLO M. CROCE, MD, director of Human Cancer Genetics and the John W. Wolfe Chair in Human Cancer Genetics, **has been inducted into the American Academy of**

Arts & Sciences, an honorary society established in 1780 by John Adams and other founders of the nation to study complex emerging problems.

FACULTY AND PROGRAMS

JOHN C. BYRD, MD, holder of the D. Warren Brown Family Designated Professorship in Leukemia Research at the OSUCCC – James, **has been named director of the new Division of Hematology in the Department of Internal Medicine.**



PHILIP PAYNE, PhD, a member of the Experimental Therapeutics Program, **has been named chair of the Department of Biomedical Informatics at Ohio State.**



RICHARD D. WHITE, MD, FACR, **has been appointed chair of the Department of Radiology and director of the Imaging Signature Program.**



NATIONAL LEADERSHIP



MICHAEL A. CALIGIURI, MD, director of the OSUCCC and CEO of the James Cancer Hospital and Solove Research Institute, **has been elected to serve as councilor on the American Society of Hematology's (ASH) Executive**

Committee in 2011. He will attend Executive Committee meetings and serve on a Clinical Research Training Institute Oversight Subcommittee, among other duties.



MICHAEL LAIRMORE, DVM, PhD, associate dean for research and graduate studies and professor in the College of Veterinary Medicine at The Ohio State

University, was named a member of the national Institute of Medicine, the health arm of the National Academy of Sciences.



WILLIAM E. CARSON III, MD, (left), associate director for Clinical Research, and **JEFF A. WALKER, MBA**, executive director of the OSUCCC – James,

have joined the board of directors for the National Comprehensive Cancer Network, an alliance of 21 leading cancer centers dedicated to improving the quality and effectiveness of cancer care.



THOMAS ROSOL, DVM, PhD, professor of Veterinary Biosciences and special assistant to the vice president for Research, **has been elected by the American Veterinary Medical Association to that organization's Council**

on Research. Rosol also serves on the NIH National Center for Research Resources Council and the federal USDA Advisory Board.

United FRONT

OSUCCC – James clinical and basic researchers come together to improve therapy for women with triple-negative breast cancer.



Left to right: KAY HUEBNER, PhD, professor of Molecular Virology, Immunology and Medical Genetics; BHUVANESWARI RAM ASWAMY, MD, assistant professor of Internal Medicine; CHARLES SHAPIRO, MD, director of Breast Medical Oncology and professor of Internal Medicine; EWA MROZEK, MD, assistant professor of Medicine; CHING-SHIH CHEN, PhD, professor of Medicinal Chemistry, of Internal Medicine, and of Urology.

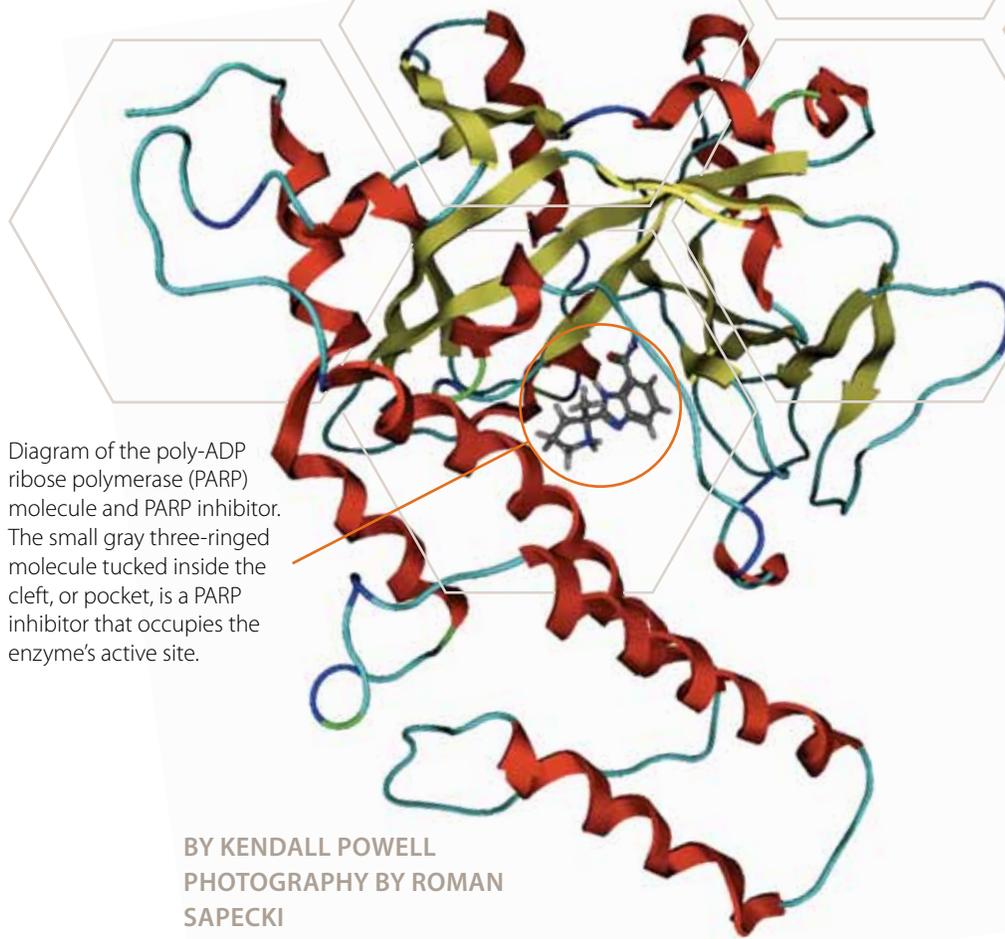


Diagram of the poly-ADP ribose polymerase (PARP) molecule and PARP inhibitor. The small gray three-ringed molecule tucked inside the cleft, or pocket, is a PARP inhibitor that occupies the enzyme's active site.

BY KENDALL POWELL
PHOTOGRAPHY BY ROMAN
SAPECKI

Twenty years ago, oncologists treated breast cancer as if it were one disease, with some patients responding better to therapy than others. “Today, we know that breast cancer has at least four molecular subtypes, enabling us to individualize therapy based on the molecular genetic characteristics of a patient’s tumor,” says Charles Shapiro, MD, director of Breast Medical Oncology and professor of Internal Medicine.

Patients with hormone-sensitive tumors—those that test positive for estrogen (ER) and/or progesterone receptors (PR)—will likely respond to antiestrogen drugs such as tamoxifen and aromatase inhibitors. Tumors that overexpress the HER2 receptor often respond well to trastuzumab (Herceptin), an antibody that blocks HER2.

However, in 10-15 percent of breast cancer patients, the tumor lacks the ER and PR receptors and does not *overexpress* HER2. These so called “triple negative breast cancers” (TNBC) disproportionately affect young women who are premenopausal, especially African-American women and those who inherit a *BRCA1* gene from either parent. These cancers are highly aggressive and characterized by early recurrences. Until recently the only therapeutic option was chemotherapy. When the cancer recurred it was often resistant to chemotherapy, resulting in poor patient outcomes.

“As an oncologist you see the devastation that this type of breast cancer brings to the woman and

her family,” Shapiro says. “It takes only one patient experience in which every chemotherapy regimen you try fails before you realize that we have to do better than chemotherapy alone. That’s what our work is all about. We are committed to improving therapy for this subtype of breast cancer.”

SPARK OF PROMISE

Hope for TNBC patients is emerging with a new class of agents called poly-ADP ribose polymerase (PARP) inhibitors, Shapiro says. He noted the findings of a phase II clinical trial that were reported at the 2009 American Society for Clinical Oncology meeting in Orlando. The trial investigated the use of the PARP inhibitor BSI-201 in combination with the standard chemotherapy regimen gemcitabine and carboplatin (GC) on 116 women with metastatic TNBC.

The study, led by Joyce O’Shaughnessy of US Oncology, showed that more than 60 percent of patients in the group receiving both BSI-201 and GC experienced clinical benefit, meaning their cancer either responded to the drugs or was stable for at least six months. By comparison, only 20 percent of those in the chemotherapy-alone group showed a benefit. Women receiving the PARP inhibitor had a median overall survival of

The New JamesCare Comprehensive Breast Center centralizes all aspects of breast-cancer care in one location— see page 31.

9.2 months compared to 5.7 months for women on chemotherapy alone. The addition of the PARP inhibitor also did not cause any worse side effects.

“To find a drug that improves survival is real progress, even if the improvement is only a matter of months,” says Shapiro. Although the results of the phase II trial need to be tested in a larger phase III trial, Shapiro speculates that PARP inhibitors will benefit some TNBC patients when combined with chemotherapy.

Shapiro’s optimism stems from the PARP inhibitor’s mechanism of action. There is evidence that triple negative breast cancers especially rely on PARP to repair breaks caused by DNA-damaging agents such as certain types of chemotherapy. PARP inhibitors prevent the polymerase from making that repair, causing the cells to self-destruct by apoptosis.

Shapiro is collaborating with two other OSUCCC – James researchers, Kay Huebner, PhD, and drug designer and medicinal chemist Ching-Shih Chen, PhD, to answer fundamental questions about TNBC and PARP inhibitors. For example, why is TNBC so sensitive to DNA damage, and what drugs other than chemotherapy drugs will augment the response of TNBC to PARP inhibitors?

Huebner, a specialist in the molecular genetics of cancer, notes that in the last decade, several research groups looked at the total

gene expression profiles of many breast cancers and found that the patterns clustered nicely into the subsets of breast cancer—ER/PR-positive, ER/PR/HER2-positive, ER/PR-negative and HER2-positive and TNBC. She and Shapiro are dissecting the molecular profile of TNBC itself.

“If we understood the DNA damage response in TNBC, it might be possible to identify subsets of TNBC patients who are most likely to respond to PARP inhibitors or other targeted therapies,” says Huebner, professor of Molecular Virology, Immunology and Medical Genetics.

The two have been using tissue microarrays—microscope slides with hundreds of roughly 1.0 mm cores from tumor biopsies for simultaneous study—to identify molecular markers that are unique to TNBC.

One of the team’s first tissue microarrays (TMA) had more than 400 breast cancer samples, all of which came from the Stefanie Spielman Tissue Bank, which houses tumor samples and associated clinical data from breast cancer patients treated at the OSUCCC – James. This tissue bank was made possible by the philanthropy of the late Stefanie Spielman and her husband, Chris, a former Ohio State and NFL football player.

The TMA, which included 40 TNBC samples, enabled the investigators to correlate molecular differences in the samples with each

patient’s age, ethnicity, response to therapy, course of disease, and ultimate outcome. The investigation confirmed that three molecular markers, a checkpoint protein, a mutated tumor-suppressor protein and a histone protein variant called gamma-H2AX were more commonly expressed in TNBC tumors. Expression of these proteins was an indication that the DNA damage response pathways were likely to provide future therapeutic targets for TNBC. To verify and extend the findings, Shapiro, Huebner and their colleagues have built another tissue microarray with nearly 200 TNBC cases that includes clinical and patient outcome data.

“This tissue array should enable us to refine the protein markers that are significant in TNBC,” Huebner says. As Shapiro explains, prognostic or predictive markers are helpful in the clinic, but they may also lead to a better understanding of TNBC biology and identification of key genes and proteins that can be targeted with new drugs.

Huebner and Shapiro are joint recipients of a \$3 million grant from the National Cancer Institute (NCI) that is dedicated to further refining subtypes of TNBC by microRNA profiling and to identifying new treatment targets.

“Understanding what drives these tumors is the first step toward understanding what potential drug targets are available,” Shapiro says. Ideally, the studies with Huebner will lead to new targets that can

be handed off to Chen, professor of Medicinal Chemistry and holder of the Lucius A. Wing Chair of Cancer Research and Therapy, who can then design drugs to attack those targets.

DESIGNER DRUGS FOR TNBC
Shapiro and Chen are working to

learn more about PARP inhibitors. Each drug in this class has been developed by different pharmaceutical companies, and the drugs don't all behave the same way. Chen wants to determine their mechanisms of action and whether their differences can be exploited to improve efficacy or modified to

produce new therapies.

In addition, Shapiro and Chen are investigating whether combining PARP inhibitors with a second drug will make them more effective against cancer cells. This second drug, currently used to treat a neurological disorder, activates a protein called PKC-delta. This pro-

TRIALS FOR TRIPLE NEGATIVE BREAST CANCER

Two phase I clinical trials are under way at the OSUCCC – James for women with triple negative breast cancer (TNBC). The trials test two targeted experimental agents in combination with standard chemotherapy. Both trials were designed by OSUCCC – James investigators and funded by the National Cancer Institute's Cancer Therapy Evaluation Program (CTEP).

In addition to determining the maximum tolerated dose of the new agents, the trials will gather data that should give researchers new insight into the biology of these aggressive tumors.

OSU-10080 offers carboplatin and the PARP inhibitor ABT888 to patients with metastatic TNBC. Recent studies suggest that the benefit of platinum-based drugs such as carboplatin for TNBC patients may be improved when combined with a PARP inhibitor.

Breast oncologist Bhuvanewari Ramaswamy, MD, principal investigator (PI) for the trial, notes that previous trials involving PARP inhibitors have used a three-drug cocktail, but the toxicity of the combination limited the dose of the PARP inhibitor that could be used.

Ramaswamy wants to learn if using two drugs instead of three will permit a higher PARP-inhibitor dose and greater effectiveness.

The study uses PET scans and blood tests to determine the degree of DNA damage the drug combination is producing as a surrogate marker of effectiveness. "We want to learn if we can identify tumors that respond to this regimen," she says.

OSU-10011 is aimed at women with newly diagnosed, locally advanced TNBC (clinical stages II-III). This trial combines carboplatin and paclitaxel with the experimental notch pathway inhibitor, RO4929097. The notch pathway is overactivated in about 40 percent of all breast cancers, including a high percentage of TNBC cases.

The trial provides three drugs as neoadjuvant therapy with the goal of achieving complete pathological response, i.e., the absence of any residual tumor cells in resected breast or axillary lymph node tissue at the time of definite breast surgery. "Patients with complete pathological responses have a three-year survival rate of 95 percent," says Ewa Mrozek, MD, PI for the trial and a breast cancer specialist in the Division of Oncology.

"Women with TNBC have an increased rate of disease recurrence and death within five years of diagnosis. We want to protect those patients in every way we can from recurrence," Mrozek says. The best way to do that, she says, is to boost the numbers of TNBC patients who respond completely to neoadjuvant therapy. The trial opened in November: Mrozek will enroll 15 to 18 patients.

Tumor samples taken before and after therapy will be used to learn more about the regimen's effects on tumor cells in patients. "Both of these trials include important correlative studies. They are at the cutting edge of research and will provide further insight into the biology of these cancers," says Charles Shapiro, MD, director of Breast Medical Oncology.

tein is part of a signaling pathway that is activated after DNA damage and directs the cell toward apoptosis.

“We hypothesize that once standard chemotherapy drugs and PARP inhibitors cause DNA damage, adding this second drug will enhance the killing of TNBC, producing a synergistic combination,” says Chen, professor of Medicinal Chemistry, Internal Medicine, and Urology.

“The beauty of this is that both the PARP inhibitors and the neurological drug are already being tested in humans,” Shapiro says. The team could therefore test this synergy hypothesis in a phase I study relatively quickly.

Finally, one of the oldest known characteristics of tumor cells may lead to an entirely new therapy for TNBC, Chen says. In the 1950s, Nobel laureate and biochemist Otto Warburg discovered that tumor cells take up glucose from the blood at a far greater rate than healthy cells. This “Warburg effect” boosts the cells’ energy production and drives their proliferation, and uncontrolled proliferation is one of the hallmarks of cancer. Chen’s laboratory has developed several potent candidate agents that block this increased glucose uptake and choke the growth of cancer cells in laboratory studies.

Eventually, he hopes to test a more fully developed drug in TNBC patients.

“Doing drug discovery in academia, we have the advantage of strong interactions with our colleagues in

both basic science and clinical science,” says Chen. “More importantly, we see how hard our clinicians work to save patients’ lives. We understand the urgency, and we can do things more efficiently.”

TRANSLATIONAL MEDICINE AT ITS BEST

That efficiency emerges from collaborative research and the cycle of translational medicine: Information flows from the clinic to the laboratory bench and back out again to be tested in clinical trials. Currently, the OSUCCC – James is conducting two clinical trials to test new drug regimens for TNBC (see sidebar, opposite page).

Both are phase I trials under an NCI U01 contract awarded to Michael Grever, MD, professor and chair of Internal Medicine, and co-director of the OSUCCC – James Experimental Therapeutics Program.

The two trials are specific for triple negative breast cancer patients: one is led by breast cancer medical oncologist Bhuvana Ramaswamy, MD, who is evaluating the combination of carboplatin and the PARP inhibitor ABT-888 in women with metastatic TNBC who have received two or fewer prior chemotherapy regimens for treatment of metastatic disease.

The other trial is led by Ewa Mrozek, MD, also a breast cancer medical oncologist, who is evaluating two chemotherapy drugs, paclitaxel and carboplatin, in combination

with a drug that inhibits the notch pathway in TNBC.

The notch pathway seems to be especially important in TNBC. Unlike the first trial, this is designed for women with localized, non-metastatic TNBC who are receiving neoadjuvant treatment.

Both trials represent the cutting edge of research and clinical care, which Ohio State’s Medical Center and the OSUCCC – James are dedicated to providing, Shapiro says. These trials, in turn, will provide tumor samples that may influence the next steps taken in laboratory experiments.

Shapiro and his colleagues in the Breast Program bring their clinical knowledge of how TNBC plays out in patients, and that provides the motivation to find better, targeted treatments through Huebner’s and Chen’s basic and translational science efforts.

“The pressing need right now is TNBC—that’s what is motivating all of us,” says Shapiro. “This is truly translational research that goes both ways. Clinical observations in these first trials could have an important impact on the next generation of experiments in the lab.” 

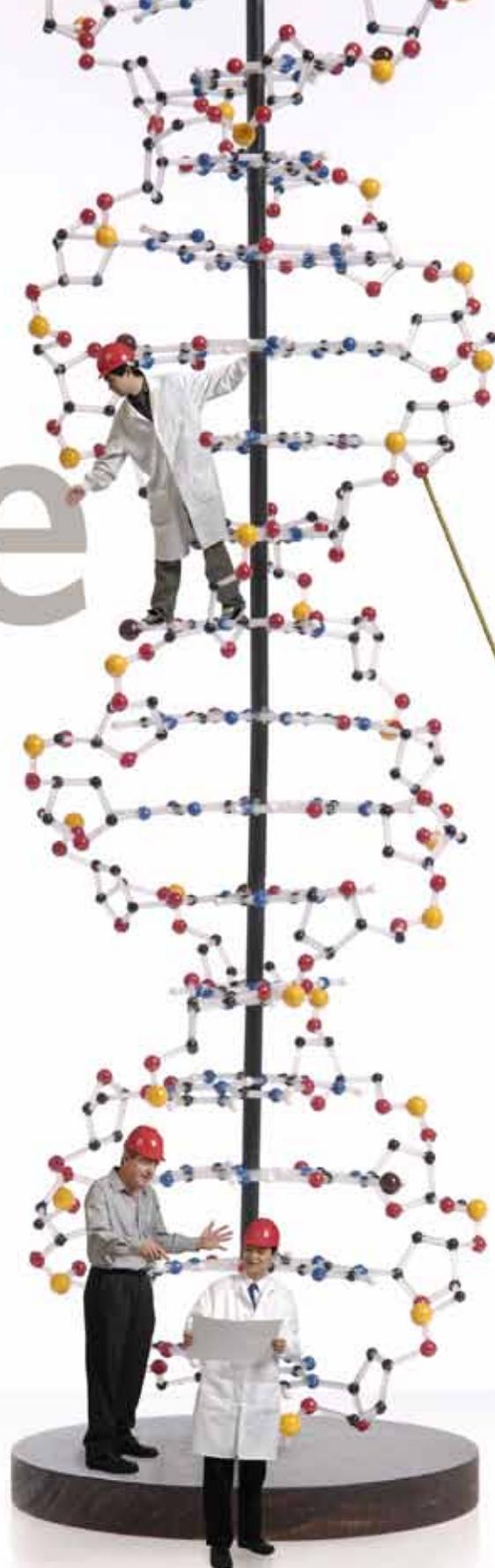
SMALL

Science

Nanotechnology may provide new biocompatible materials that could improve how we treat, detect and study cancer

CHENQUANG ZHOU
PhD candidate, Pharmaceuticals

JAMES LEE, PhD,
professor and director of the National Science Foundation Center for Affordable Nanoengineering of Polymeric Biomedical Devices at Ohio State



JOHN LANNUTTI, PhD,
professor of Engineering

ROBERT LEE, PhD,
professor of Pharmacy

BY BOB HECKER

PHOTOGRAPHY BY ROMAN SAPECKI

Researchers in several colleges at The Ohio State University are teaming up to apply nanotechnology to improve the detection and treatment of cancer.

“Nanotechnology offers a new tool for more effective cancer detection techniques and treatment,” says James Lee, PhD, the Helen C. Kurtz Professor of Chemical and Biomolecular Engineering and

director of the National Science Foundation (NSF) Center for Affordable Nanoengineering of Polymeric Biomedical Devices at Ohio State.

“Through nanotechnology, we can make multifunctional materials and devices for detecting, diagnosing and perhaps treating cancer by delivering therapeutic genetic material into cells with minimal toxicity,”

says Lee, who also is a member of Ohio State’s Comprehensive Cancer Center – James Cancer Hospital and Solove Research Institute (OSUCCC – James).

Nanotechnology enables scientists to create products with new structural attributes by engineering their molecular features, a process that involves working at or around

JEFFREY CHALMERS, PhD,
*professor of Chemical and
Biomolecular Engineering*



KRIS JATANA, MD,
*assistant professor of
Otolaryngology – Head
and Neck Surgery*



MARIANO VIAPIANO, PhD,
*assistant professor of
Neurological Surgery*

the scale of a nanometer, or one billionth of a meter. “The products themselves don’t have to be on the nano scale, but they have an altered nanostructure that gives them special characteristics,” Lee explains.

Ohio State’s Nanoengineering Center is one of 19 Nanoscale Science and Engineering Centers funded by the NSF. It is the only one that solely develops polymer-based bionanotechnology, or creating soft materials primarily for medical use. Lee says softness is important for biocompatibility in working with tissue and proteins.

“Ohio has always been strong in the polymer industry—automotive and aerospace are examples—and we have a strong University workforce at Ohio State, where we’re training the next generation of researchers in the polymer field,” he adds. “We took advantage of this when we applied for our NSF grant by proposing to apply polymer-based nanotechnology to the medical field.”

A five-year, \$12.9 million NSF grant established the Center in 2004. The grant was renewed in 2009 for another five years at \$12.5 million. The Center comprises approximately 25 Ohio State faculty, mainly from the colleges of Engineering, Arts and Sciences, and Pharmacy, who are collaborating with scientists and physicians in the College of Medicine and the OSUCCC – James on some 20 joint projects. The Center also has a medical advisory and evaluation board consisting of physicians and

researchers from the College.

In addition, the Center works with seven partner institutions: Duke University, University of Illinois at Urbana-Champaign, University of Michigan, Massachusetts Institute of Technology, Oakwood University, University of California at San Francisco, and State University of New York at Albany.

The Center’s research vision is aggressive: to revolutionize medical diagnosis and treatment by developing affordable, environmentally and biologically benign nanoengineering techniques that use polymers, biomolecules and nanoparticles to design and produce biomedical and therapeutic devices.

In its first five years, the Center yielded more than a dozen patents and nine commercial spin-off companies. Among its developments were polymer scaffolds that support the growth of blood vessels for transplant, techniques for shaping DNA into structures that might form sensors for biological agents, and a compact disk that contains tiny channels that transport fluids for medical testing.

Now the Center is integrating its nanomaterials and technologies into sophisticated drug- and gene-delivery methods, and into cell-sorting and analysis techniques to promote personalized health care, Lee says.

The ultimate goal, he adds, is to build a nanofactory that integrates nanofluidic circuits and synthetic chemistry into continuous produc-

tion of nanostructures and devices for treating cancers, chronic infections and central nervous system diseases, as well as expediting vaccine delivery.

“Our proposed nanofactory will combine several technologies to do things more powerfully and efficiently, possibly leading to breakthroughs,” Lee says.

Here’s a look at three promising cancer studies at Ohio State that involve collaborative work in nanotechnology.

MIMICKING MALIGNANT MIGRATION

Cancer researchers investigating how malignant brain tumor cells migrate into surrounding tissue have worked with engineers at the Center to develop biocompatible nanofibers that mimic the neural topography used by migratory cells during metastasis.

Mariano Viapiano, PhD, a researcher at the OSUCCC – James, says growing tumor cells on nanofibers instead of in petri dishes produces behavior that more closely mimics the behavior of cells in actual tumors.

Viapiano and John Lannutti, PhD, of the College of Engineering, have been leading collaborators on this project for more than two years. Lannutti heads the design of nanofibers—which can be observed only through a scanning electron microscope—and Viapiano studies the behavior of cells cultured in these fibrous molecular scaffolds and their response to novel drugs.

Nanofiber Solutions, a company formed by Lannutti and his student, Jed Johnson, is marketing these nanofibers commercially and has received NIH and NSF small-business funding to aid in the transition to market.

“Dr. Lannutti has investigated an impressive number of chemical compound combinations and physical processes to make fiber scaffolds that increasingly resemble the texture of the brain,” Viapiano says. “My lab designs methods to analyze cell motility, performs biochemical and genetic analyses of the cells, and tests experimental compounds that could be anti-invasive *in vivo*.”

He explains that cells cultured on petri dishes must adapt to a homogenous, rigid surface that alters their migratory mechanisms.

“But by providing the cells with an elastic 3D scaffold with complex topography, we challenge them with physical conditions that more closely resemble the natural tissue environment,” Viapiano says.

He notes that some drugs known to inhibit cell migration in brain tissue are ineffective when the same cells are cultured on petri dishes, but the drugs are effective again when the cells are cultured on nanofibers.

Viapiano says researchers have learned from this new model of cell culture that they can reproduce, at least in part, complex mechanisms of migration using a controllable *in vitro* model that allows them to perform analyses not possible with tissue samples.

“Our next steps are to increase the throughput of this model to perform comparative studies in cultured cells and eventually in fresh biopsy samples,” he says. “The ultimate benefit will be having a reproducible culture environment that can be used for basic research and as a potential bioassay for clinical applications.”

DETECTING CTCs

Bioengineers and cancer specialists have produced a blood test that uses nanotechnology to reveal circulating tumor cells (CTCs) and thus determine the aggressiveness of squamous cell carcinoma of the head and neck (SCCHN).

Jeffrey Chalmers, PhD, a professor of Chemical and Biomolecular Engineering and a researcher at the OSUCCC – James, where he directs the Analytical Cytometry Shared Resource, says the technology, which was developed at Ohio State and the Cleveland Clinic Foundation, is a negative depletion process that uses magnetic nanoparticles to isolate and quantify CTCs from the blood of patients with SCCHN.

Reporting in the *Journal of the American Medical Association's* subspecialty journal, *Archives of Otolaryngology – Head and Neck Surgery*, the researchers identify three general CTC detection methods: immunocytochemistry, which implies visual observations; flow cytometry or image cytometry; and reverse transcriptase polymerase chain reaction. Using a negative depletion technique before employ-

ing one of these methods can greatly increase the sensitivity and specificity of detection, the authors say.

“Our negative depletion process enriches for CTCs from human blood by removing normal blood cells using immunomagnetic separation,” Chalmers explains, noting that the technology is being commercialized by a local company.

In a study of 48 patients with SCCHN who were undergoing surgery, the investigators determined that, when they used this technique, their patients who were found to have no CTCs had a statistically significant improved disease-free survival. A blood test with such a prognostic capability could have important clinical implications, the scientists say.

“With prospective clinical follow-up now as far out as three years after surgery, we have seen correlations suggesting that the presence of these cells in the blood may be related to a worse outcome,” says Kris Jatana, MD, an assistant professor in the Department of Otolaryngology – Head and Neck Surgery who has been involved in this work with SCCHN since its conception at the OSUCCC – James. Jatana was co-first author on the published manuscript. “This may help identify patients with more aggressive cancers and enable us to customize treatments accordingly. Our goal is to improve patient outcomes.”

Jatana says there is still no standardized prognostic blood test for SCCHN, or even for cancer surveil-

lance, although many experimental techniques have been described.

“We believe our technique is superior to others as it removes normal cells from the blood, allowing for the detection of any abnormal cells—the CTCs,” he says. “Many techniques done throughout the United States and internationally identify only cells with a specific surface marker, which creates an intrinsic bias and the potential to miss abnormal cells.”

Jatana finds their work extremely exciting. “With continued investigation we hope to further characterize these cells and determine if this technology can also be used in surveillance for the earliest detection of microscopic cancer recurrence.”

DYNAMIC DELIVERY

One problem with using viral vectors for gene therapy is that some viruses generate immune responses that complicate or hinder the treatment. Scientists are thus pursuing nonviral vector techniques as well.

At Ohio State, researchers have designed nanoparticles that appear to deliver genetic material into cells with minimal toxicity. In laboratory studies, the researchers found that this vector can deliver DNA deeply enough into a cell to allow activation of its passenger gene.

Made of calcium phosphate in a lipid shell, the biocompatible nanoparticle protects DNA on its journey into the target cell and then dissolves via complex chemical reactions.

This research, published in the *International Journal of Pharmaceutics*, involves scientists from the colleges

of Engineering, Pharmacy and Medicine, several of whom are affiliated with the Nanotechnology Center, including its director James Lee and colleague Robert Lee, PhD, a professor in the College of Pharmacy who is a member of the OSUCCC – James and an expert in targeted nanoparticle and liposomal drug-delivery systems.

First author Chenguang Zhou, a PhD candidate in Pharmaceutics, notes that other attempts to use liposomes as nonviral vectors for gene therapy have protected the passenger DNA but have not adequately released the material into the cell.

“While calcium phosphate has been used to deliver plasmid DNA for decades, the method is typically characterized by low and irreproducible transfection efficiency,” Zhou says. “But our novel lipid-coated nano-calcium phosphate vector provides consistently efficient and satisfactory delivery. This superior stability makes our vector a promising candidate for clinically useful gene delivery.”

The team next plans to test the nanoparticle’s ability to travel through the bloodstream and enter target cells in animals.

“We need to study how the vector maintains its integrity and protects the plasma DNA in the bloodstream. We also must learn how to avoid the unwanted internalization of the vector by immune cells,” Zhou says. “Then we may want to add targeting moieties that increase the binding and internalization of the vector to the target cells.”

When the experimental vector

does reach the clinic, Zhou speculates that it may be first applied in patients with leukemias or liver cancer.

COLLABORATIONS CONTINUE

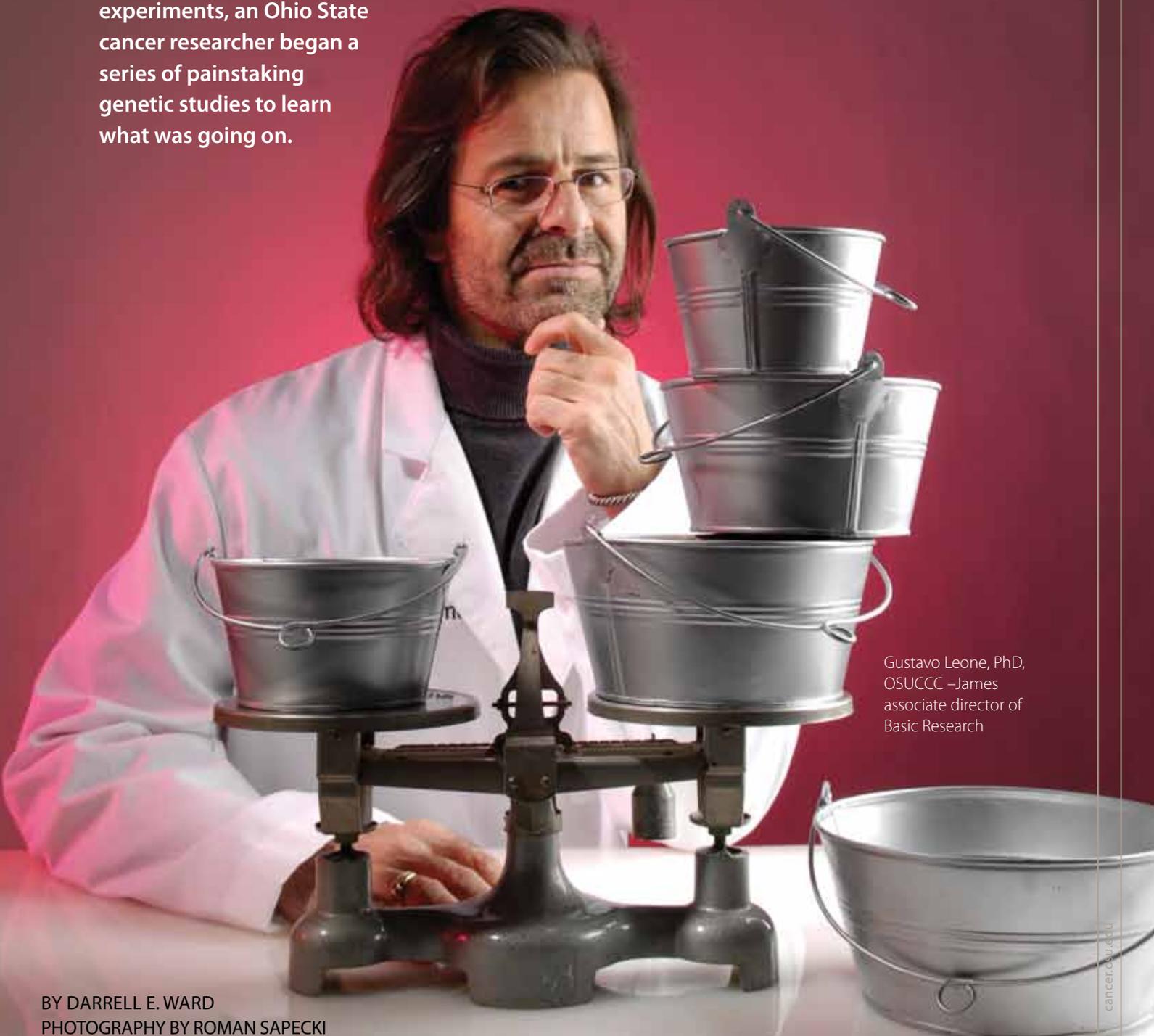
“Our Center so far has developed a number of gene/drug delivery vectors,” Zhou says. “We keep close collaboration with oncologists at the OSUCCC – James to understand the clinical demand for gene therapy and to test our vectors in clinically relevant animal models. We also work with pharmaceutical companies for evaluating our formulations in clinical settings.”

“Nanotechnology allows us to achieve a degree of refinement and control in analyzing biological processes that would’ve been unthinkable 10 years ago,” adds Mario Viapiano, referring not only to his team’s brain cancer studies but to malignant mechanisms in general. “Nanoparticle applications provide tools for specific targeting of tumors or for bioassays with diagnostic potential, enhancing personalized medicine. This should make the technology very exciting for physicians and their patients.”

“But none of this can be accomplished by one discipline alone,” says Nanotechnology Center Director James Lee. “Our collective goal is to make a difference, and collaboration is the key.” ■

Dissecting the Bucket Brigade

When a family of transcription factors failed to perform as expected in animal experiments, an Ohio State cancer researcher began a series of painstaking genetic studies to learn what was going on.



Gustavo Leone, PhD,
OSUCCC –James
associate director of
Basic Research

BY DARRELL E. WARD
PHOTOGRAPHY BY ROMAN SAPECKI

HUMAN GENES ARE SPELLED IN UPPERCASE ITALICS. ANIMAL GENES ARE IN MIXED CASE. GENE PRODUCTS ARE IN NORMAL TYPE.

When researchers at the Massachusetts Institute of Technology isolated and cloned the gene responsible for retinoblastoma in 1986, it was a stunning breakthrough. That gene, *RB1*, was the first example of a tumor-suppressor gene, “that priceless category of genes that, among other tasks, protect us from developing cancers,” as Nicholas Dyson described them in a 2003 commentary in the journal *Nature*.

But how did this tumor suppressor gene work and how did its loss contribute to cancer? Many thought that the answers to those questions were well in hand following 10 years of mainly cell culture *in vitro* experiments. Then Ohio State cancer researcher Gustavo Leone—who had made significant contributions to those cell-culture studies—began verifying the findings in animal models.

“That’s when things got interesting,” says Leone, PhD, associate director of Basic Research at the cancer center.

Getting it right was important. “Understanding RB and its fellow molecules is critical for understanding cancer,” says Michael Ostrowski, PhD, chair of Molecular and Cellular Biochemistry, co-leader of the Molecular Biology and Cancer Genetics research program, and one of Leone’s collaborators. “Mutations in both copies of *RB1* lead to retinoblastoma, a malignancy in children, and families with *RB1* mutations are predisposed to cancer. The RB pathway is also inactivated in most

human cancers, so knowledge of the molecular interactions involved is central to cancer biology.”

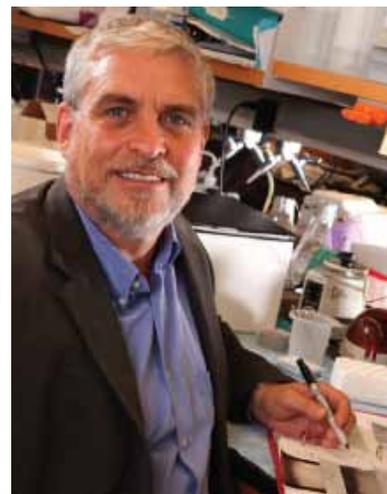
Basic research often begins with little knowledge of the thickets that lie ahead. The cloning of RB was followed a few years later by the discovery of a protein that binds both the RB1 protein and the early 2 (E2) protein encoded by adenoviruses. The new molecule was called E2 Factor 1, or E2F1. It was a transcription factor, a protein that binds with specific DNA sequences to activate or repress genes. A Duke University group found the protein; a Harvard group cloned the gene.

ALL IN THE FAMILY

The discovery of *E2F1* soon led to the discovery of a family of genes: *E2F2* through *E2F6*. Cell culture studies revealed that the E2F1, 2 and 3 proteins were gene activators and the only ones that bind with RB1 to regulate the cell cycle.

In 1990, Leone, then a graduate student at the University of Calgary, was attending an international virology conference in Berlin where he heard talks by the groups from Duke and Harvard about their discoveries.

“The investigators showed that RB bound to the E2F protein, and that the two of them together somehow executed some function in cancer,” Leone says. “They also had found that three cancer-causing viruses—adenovirus, papillomavirus and polyomavirus—encoded proteins that could bind RB1 and release an



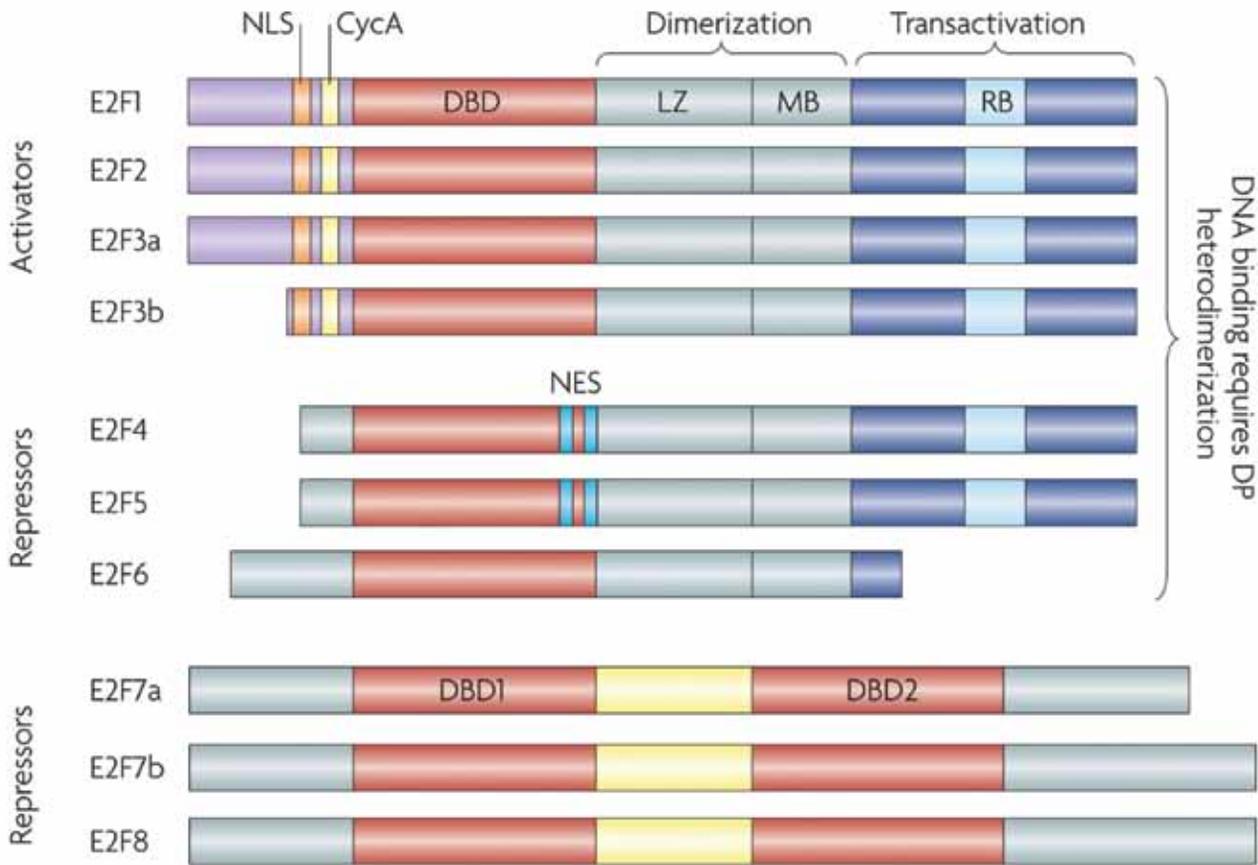
MICHAEL OSTROWSKI, PhD, chair of Molecular and Cellular Biochemistry and co-leader of the Molecular Biology and Cancer Genetics Program

activity that people didn’t understand.”

“They had linked RB1, tumor-causing viruses and E2F, and they speculated that this was important for cell-proliferation control and was likely involved in cancer.”

Leone was fascinated. “I thought, ‘Wow, I’d like to work on that.’” He joined the Duke group as a post-doctoral candidate in 1994 and has studied the *E2F* family ever since.

At Duke, Leone studied the similarities and differences among the family members, work that helped establish their importance in cell-cycle regulation. “The evidence suggested that E2Fs played a straightforward role in regulating the cell cycle,” he says. Gene activators E2Fs 1, 2 and 3 advanced the cycle, and E2Fs 4, 5 and 6 suppressed it. (At Ohio State in 2003 and 2005,



The mammalian E2F family of transcription factors. The members occupy eight chromosomal locations that encode nine distinct proteins. Traditionally, the family has been divided into gene activators (E2F1 to E2F3) and repressors (E2F4 to E2F8). A few key facts about the family:

- The first six E2Fs encode proteins that bind to DNA only after coupling with a second protein, called a dimerization partner protein (DP).
- Only the proteins encoded by E2F1 to E2F3 bind with the retinoblastoma protein, RB.
- The repressors E2F7 and E2F8 are structurally unique.
- The E2F7a and E2F7b proteins are produced by alternate splicing of the E2F7 primary messenger RNA. The two forms of the E2F3 protein (E2F3a and E2F3b), on the other hand, are transcribed from two distinct promoters within the gene.

his lab discovered *E2F7* and *E2F8* respectively, the last of the family. They, too, encode repressor proteins.)

The link between E2Fs and cancer is more tenuous, Leone says. “We need to know how these molecules function normally. If we don’t know that, it’s hard to imagine what they might be doing in cancer.”

CONTRADICTIONS OF INTEREST

When Leone joined The Ohio State University Comprehensive Cancer Center – Arthur G. James Cancer Hospital and Richard J. Solove Research Institute (OSUCCC – James) in 1999, he and his laboratory set out to verify what was known about the

family *in vivo*, in animal models.

“We generated mouse strains that had mutations in each of these genes, and we began looking at their role in normal development and in cancer,” Leone says. Based on the earlier *in vitro* studies, they had an idea of what these experiments should show. “But we observed

nothing like what we expected,” Leone says. “For example, loss of the genes sometimes had little or no effect on cell growth during development, and in other cases it had a dramatic effect but not the type that we had predicted.

“What we had supposed was true based on cell-culture experiments was not the full story,” Leone says. “In fact, some of it was wrong, so we began revisiting the initial questions much more rigorously.”

They designed experiments that were as thorough as possible. Focusing only on the gene activators—*E2F1*, *E2F2* and *E2F3*—Leone and his collaborators generated double- and triple-knockout mice that lacked different combinations of the three genes. In some strains they used conditional gene targeting to knock out E2Fs at will.

Their findings, published in *Nature* (2001), showed that mice underwent normal embryonic development in the absence of *E2f1* and 2, but that *E2f3* was required.

Furthermore, cells from the embryos that lacked *E2f1* and 2 proliferated robustly in culture, but those without *E2f3* did not proliferate. Overall, the study provided the first genetic evidence that these three genes play an essential role in cell-cycle progression, proliferation and development. The work succeeded because the conditional gene knockout strategy enabled the researchers to overcome the lethal effects of inactivation of multiple E2Fs in earlier animal models.

WHY SO MANY?

Nematodes and insects have one E2F activator and one repressor. More complex animals have families of transcription factors. Why do mammals have a large family of E2F genes to accomplish what just one activator and one repressor could do?

The same question applies to other transcription factors, Leone says. “Why are there so many members in the *p53* family, the *MYC* family and the *ETS* family? The assumption was that each *E2F* family member regulates specific genes.”

The function of *E2F* family members grew more complicated following a study published in *Molecular and Cellular Biochemistry* (2000) for which Leone was first author. It showed that *E2F3* actually had two distinct promoters and expressed two related proteins, E2F3a and E2F3b.

“We wanted to learn how the three activator genes functioned in concert during development and to dissect out the ‘a’ and ‘b’ dichotomy,” he explains.

The three genes were clearly working to the same end, but were they doing the same thing? “Each family member can carry a bucket, and together they will fill the tub,” he says. “Or each could do a specific job—one holds the bucket, one fills the bucket, one carries the bucket, and one empties the bucket—and work in concert to fill the tub.”

The ongoing thinking was that each of the genes did something

different. “We used combinatorial knockout models to investigate that question at the genetic level.” Their findings, published in *Nature* (2008), showed that *E2f3a* is the most important gene. Its presence alone was enough for development through adulthood, Leone says.

But what made *3a* so special? “This was the exciting part of the study,” he says.

The investigators swapped one family member for another. They took out the *E2f3a* and replaced it first with *E2f3b* and then with *E2f1*. “If *3a* was doing something really special, neither *3b* nor *E2F1* should be able to replace it; the embryos shouldn’t develop. Well, in both cases, the animals developed just fine.”

It turned out the *3a* protein wasn’t special at all. “What was critical was the regulation of the *3a* gene locus—the timing of its activation and the levels of expression,” Leone says.

Still, the findings beg the question: Why do mammals have so many *E2Fs*? Leone and his collaborators offer a hypothesis. The range of *E2Fs* may not be essential for development, but they might be required for long-term survival. “We think that mammals have so many of these genes because they are needed for living long term,” he says. They are investigating that question now.

ROLE REVERSAL

Tantalizing findings published in

Nature (2009) by Leone and his colleagues could have important implications for the role of E2Fs in cancer.

That study shows that *E2F 1-3* are, in fact, activators in stem cells, but that their role changes as cells differentiate. In differentiating cells, the E2F activators switch roles and become repressors. “Their function as gene repressors in differentiated cells is opposite what we thought for nearly 20 years,” Leone says.

The study also shows how the switch is made. Finally, the researchers show that when *RB1* is lost in mature cells—as happens in most cancers—these same *E2fs* once again become activators.

“In normal cells, these *E2fs* have a repressor role, but in cancer cells they are activators.” There, they could provide a new therapeutic target, he says. “If we can inactivate these *E2fs* in cancer cells, we may prevent cancer cell proliferation with few major effects on normal differentiated cells.”

The findings provide a better understanding of cell proliferation and death. In a developing animal, the change of *E2fs* from activators to repressors allows stem cells to make the transition to differentiated cells.

“This is important for differentiation,” he says. “These *E2fs* regulate the proliferation that parallels differentiation.”

They observed the switch in the differentiating cells of the intestinal crypt, Leone says. “If it doesn’t happen as these cells differentiate, they

accumulate DNA damage and start dying.”

Leone likened the parallel nature of the two events to turning off the lights and closing the door when you leave the room. “To leave the room correctly, you need both actions, which are independent of one another.”

Similarly, differentiating cells need to exit the cell cycle, and these *E2fs* are important for that. “Before this, these molecules were thought to be important only in proliferating cells like stem cells,” Leone says. “Because differentiated cells don’t proliferate, these activators were thought to be irrelevant. But we show that that’s not the case. It’s just the opposite.”

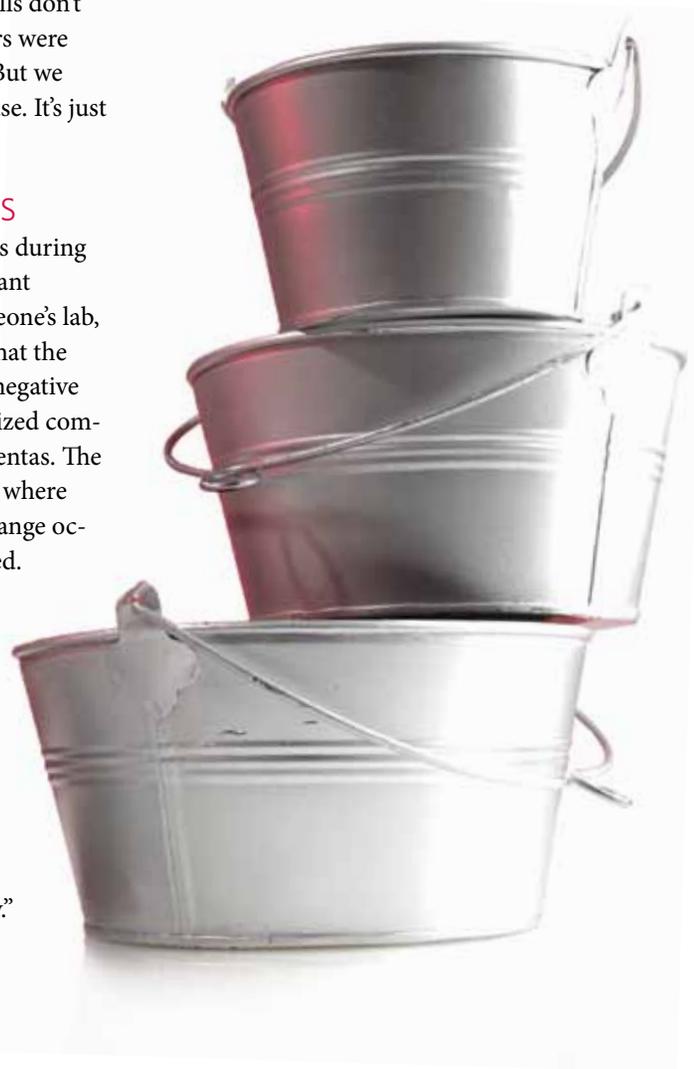
PLACENTAL EFFECTS

While examining embryos during the *E2f* studies, an observant postdoctoral student in Leone’s lab, Alain de Bruin, realized that the placental tissue from *Rb*-negative mice was highly disorganized compared with wild type placentas. The layer of the placental wall where oxygen and nutrient exchange occurs was severely disrupted.

“The question was,” Leone says, “were the problems of the fetuses in the *Rb*-deficient animals a side effect of placental disruption, or were they due to *Rb* deficiency? Numerous previous studies attributed the problems to *Rb* deficiency.”

Leone and his collaborators reported their findings in *Nature* (2003), noting that the layer’s trophoblast cells were poorly differentiated, neoplastic and dying by apoptosis. Placentas that were *Rb*-negative also showed poor transport of essential fatty acids and decreased surface area for oxygen and nutrient exchange.

Next, the researchers used a conditional gene-knockout model to restore *Rb* function in the placenta and this avoided many of the embryonic abnormalities



seen in the *Rb*-deficient fetuses. The findings showed that the lethal effects on *Rb*-deficient embryos are due to placental abnormalities and not to the *Rb* deficiency.

“We showed for the first time that *Rb* is important in the placenta,” Leone says.

In a follow-up study, Leone and his collaborators used a mouse strain that permitted conditional deletion of both *Rb* and *E2f3*. That study, published in *Genes and Development* (2006), showed that during mouse placenta development, *Rb* has a crucial function in placental stem cells, i.e., trophoblast stem cells.

Furthermore, deleting both the *Rb* and *E2f3* genes enabled the embryos to live three additional days and reduced trophoblast cell proliferation. “There’s obviously still something wrong, but it extended gestation,” Leone says. “This strongly suggests that E2f3 protein is an important partner in these responses.”

The findings have important implications for cancer, which sometimes arises from a select few cells that have characteristics of stem cells, he says. “Here we show in a developmental system that the function of a major tumor suppressor is important in a stem cell compartment, and that the function of the gene is different in stem cells compared with their derivative cells. This adds to the evidence that tumor suppressors are important in stem cells.”

THE MICROENVIRONMENT

The finding in the placenta was completely unexpected. It told Leone and his collaborators that genes can have distant effects. “It told us to think broadly, and that led us to the microenvironment and the idea that genes inside tumor cells can influence surrounding cells in ways that favor tumor growth.”

In one of those studies, Ostrowski and Leone showed in *Nature* (2009) for the first time that gene changes in normal tumor fibroblasts foster tumor growth and progression. The work, which involved loss of a gene called *Pten* from normal mammary fibroblasts in mouse tumors, also provided the first animal model that accurately represents the microenvironment within human breast tumors.

“We found that normal stromal fibroblasts play an important role in suppressing cancer development and may explain why some human breast cancer patients respond to a standard therapy while others with apparently identical disease do not,” says Ostrowski, the co-principal investigator on the study with Leone.

The study also identifies new biomarkers specific to the fibroblasts that may help guide breast-cancer therapy and new molecular targets for developing therapies aimed at gene changes in stromal cells. The findings might also improve the understanding of other pathological conditions influenced by the tissue microenvironment, such as autoimmune disease, lung fibrosis and neurodegenerative diseases.

“Our findings reveal a new role for this gene in the tumor environment, which could lead to entirely new treatments for breast cancer and perhaps other solid tumors using agents that target stromal cells,” Leone says.

THE ROAD FROM HERE

Leone’s lab is now exploring the many functions and interactions of the E2F activators and repressors using a systems biology approach.

“We are using genomics, protein binding, genome and chip sequencing and gene expression profiles,” Leone says. “We’ll first use genomics and expression profiling to narrow down which combinations are probably working together in orchestration and then focus on those.”

Their goal is to learn how the entire E2F program is coordinated to regulate cell proliferation *in vivo*. “We want to identify and understand all the genes in this network, how they function and how they influence the tumor microenvironment,” Leone says.

How does the RB tumor-suppressor gene work and how does its loss contribute to cancer? The answers are still not “well in hand,” though they are “better in hand.” On a broader scale, the effort to understand RB illustrates that cancer yields its secrets only to thorough, sound science. 

BENCH TO BEDSIDE

From the Laboratory to the Pharmacy

OSU-10079 – A Phase 1 Study of Lenalidomide Maintenance Following Allogeneic Hematopoietic Cell Transplantation in Patients with Select High-Risk Hematological Malignancies

HYPOTHESIS: The use of lenalidomide post-transplant in patients with high-risk acute myeloid leukemia (AML), non-Hodgkin's lymphoma (NHL), or chronic lymphocytic leukemia (CLL) will be safe and will not significantly increase the risks of graft-vs-host disease (GVHD), graft rejection, or infection. If efficacious, this therapy could promote durable remission in patients who are treated with reduced-intensity conditioning regimens and are otherwise destined to relapse.

RATIONALE: Lenalidomide belongs to class of compounds called immunomodulatory drugs. It was derived from thalidomide and selected for clinical development after it was found to be more stable and 50,000-fold more potent at inhibiting tumor-necrosis factor alpha than thalidomide.

Lenalidomide has multiple potential antitumor mechanisms of action, but which mechanisms are responsible for clinical activity in patients who respond to therapy is unclear, and they may differ according to tumor type. These mechanisms include the modulation of cytokines and of T cells and natural-killer (NK) cells, the inhibition of blood-vessel growth, and direct effects on tumor cells.

Studies show that allogeneic blood or marrow transplantation (alloBMT) benefits many patients with advanced hematological

malignancies. For patients in remission, it often offers the best opportunity for long-term disease-free survival. For some patients who have relapsed or become refractory to conventional therapy, it is the only therapeutic option associated with durable remission.

More recently, reduced-intensity conditioning regimens have been used prior to transplantation to reduce transplant-related complications, toxicity and mortality. They allow donor-marrow engraftment to occur without the widespread tissue damage associated with standard myeloablative conditioning.

Unfortunately, recent data suggest that reduced-intensity conditioning is associated with higher rates of disease relapse and chronic GVHD.

Thus, while a reduced-intensity conditioning regimen allows most patients to survive the early post-transplant period and achieve initial engraftment of donor cells, they are often left with residual disease that eventually proliferates before they can develop an adequate immune response from donor-derived T and NK cells.

In this dose-escalation trial, we evaluate whether the addition of the immunomodulatory agent lenalidomide will mitigate relapse risk following reduced-intensity conditioning alloBMT. Detailed correlative studies will assess lenalidomide's effects on immunologic response and specific

anticancer activity. If this clinical approach proves feasible, we will proceed to a follow-up phase II study examining this treatment in each of the disease groups studied.

AT A GLANCE

Clinical trial OSU 09120

PI: **LESLIE A. ANDRITSOS, MD**, assistant professor of Medicine, Division of Hematology, Blood and Marrow Transplantation

Phone: 614-293-8074

Email: leslie.andritsos@osumc.edu

Eligibility: Patients with high-risk AML, NHL or CLL will be divided into three strata based on disease subtype. Each stratum has separate inclusion criteria, but all patients must meet the following criteria: be four weeks or more from prior chemotherapy (excluding steroids), radiation, or radioimmunconjugate therapy; age 18 to 75; ECOG performance status 0-2; DLCO greater than 40 percent with no symptomatic pulmonary disease; LVEF by Echo or MUGA 30 percent; HIV negative; creatinine clearance of 60 cc/min according to the Cockcroft-Gault equation; total bilirubin less than 2 mg/dL; AST/ALT less than or equal to 2.5 x ULN; no uncontrolled infection or diabetes mellitus; eligible donor identified.



NEED TO KNOW

Resources for Professional Development

SHARED RESOURCES

CAPTURE THE GLOW

A multispectral fluorescence imaging system is now available to OSUCCC – James investigators through the Wright Center of Innovation’s Molecular Imaging Agents Laboratory.



The Maestro EX, manufactured by Cambridge Research & Instrumentation, has a number of advantages over CT and other instruments that rely on radiation, says Michael Tweedle, PhD, director of the Molecular Imaging Agents Laboratory. It handles up to three animals at a time, captures imaging data in seconds, and is safer for both animals and investigators.

“The instrument works best with target concentrations typical for fluorescence experiments, not bioluminescence,” says operator Michelle Carlton. It is sometimes possible for investigators to test the suitability of a given model on a trial basis, and some introductory time on the machine may be available at no cost. “We want to offer investigators a low-risk opportunity to evaluate novel approaches to answering questions relevant to their research,” Tweedle says.

THE JAMES IS A LEAPFROG GROUP ‘TOP HOSPITAL’



The Leapfrog Group, a national coalition of public and private purchasers of employee health coverage who collectively work to improve healthcare quality, has included the OSUCCC – James on its 2010 list of 65 “top hospitals.”

The Dec. 1 announcement marks the second consecutive year that the OSUCCC – James has been listed

among the group’s “top hospitals,” which were selected from a field of nearly 1,200 university and other teaching hospitals, children’s hospitals and community hospitals in rural, suburban and urban settings. The selection is based on results from a Leapfrog Group national survey that measures hospital performance in key areas

of patient safety and quality. The results, which Leapfrog calls “the most complete picture available of a hospital’s quality and safety,” are posted at www.leapfroggroup.org.

FULL-SERVICE BREAST CARE UNDER ONE ROOF

New OSUCCC – James Comprehensive Breast Center Opens Its Doors

The new JamesCare Comprehensive Breast Center, located near The Ohio State University Medical Center, began welcoming patients in mid-January. The four-story, multidisciplinary center combines all facets of breast care in one location, including annual mammograms, complete diagnostic services, comprehensive breast-cancer treatment, access to clinical trials, reconstructive breast surgery, medical nutrition services, survivorship support, risk counseling, financial counseling, a library and resource center, a meditation room and private and semi-private waiting areas.

The center also houses Hope's Boutique, a shop that offers a full range of products and services designed to help women look and feel their best during and after cancer treatment. The front of the new boutique is a public shopping area, while the back offers privacy and assistance with bra and prosthesis fittings.



Conference Calendar

WHAT'S NEW IN ROBOTIC SURGERY ACROSS SPECIALTIES?

February 25, 2011

FOCUS: The conference will identify the application of robotic surgery across surgical specialties, review developments and emerging issues related to new robotic techniques, define patient populations that can benefit from robotic surgery and describe the potential benefits of robotic surgery over standard open surgery.

For more information, contact Katie Jones at 614-366-5183.



To register and view the agenda, visit <http://cancer.osu.edu/go/Robotics>.

2011 MELANOMA SYMPOSIUM: THE CHANGING LANDSCAPE

February 12, 2011

FOCUS: The day-long melanoma conference includes updates on new dermatologic approaches, recognizing molecular markers, new surgical considerations and the role of novel therapeutics. Also included are new modalities and therapies for ocular melanoma. For oncologists, surgeons, dermatologists, radiation oncologists, primary-care physicians, nurses and other healthcare professionals who diagnose and treat patients with melanoma.

For more information, contact Nancy Jones 614-293-3688.



To register and view the agenda, visit <http://cancer.osu.edu/go/Melanoma>.

▶▶ FUNDRAISING

A REWARDING RIDE

Pelotonia 10 boosts cancer research at the OSUCCC – James by \$7.8 million

On Nov. 27, The Ohio State University cancer program received a check for \$7.8 million in funds raised by the second annual Pelotonia, a grassroots bicycle tour held in August to generate support for cancer research at the OSUCCC – James. The 2010 event attracted 4,047 riders, nearly double the number who participated in the 2009 inaugural Pelotonia, which raised \$4.5 million.

Some of the money is funding cancer-related research by 64 talented Ohio State undergraduates, and 10 “idea grants” that support the preliminary work of OSUCCC investigators whose promising ideas fall outside of the mainstream.



IN THE NEXT ISSUE OF **frontiers**...

ONE-STOP DRUG-DISCOVERY WORKSHOP

It is now possible for investigators to design anticancer agents and move them through preclinical testing and into phase I and II clinical trials, all at the OSUCCC - James. It is drug discovery at its most efficient, and it is leading to a complete drug-development pipeline at The Ohio State University.

TARGETING GASTROINTESTINAL CANCERS

Continuous growth in patient volume over the past few years has prompted the OSUCCC – James to expand and strengthen its Gastrointestinal Oncology Program under the medical direction of Tanios Bekaii-Saab, MD. This expansion has led to promising new studies in GI malignancies, including pancreatic cancer.