Research Horizons

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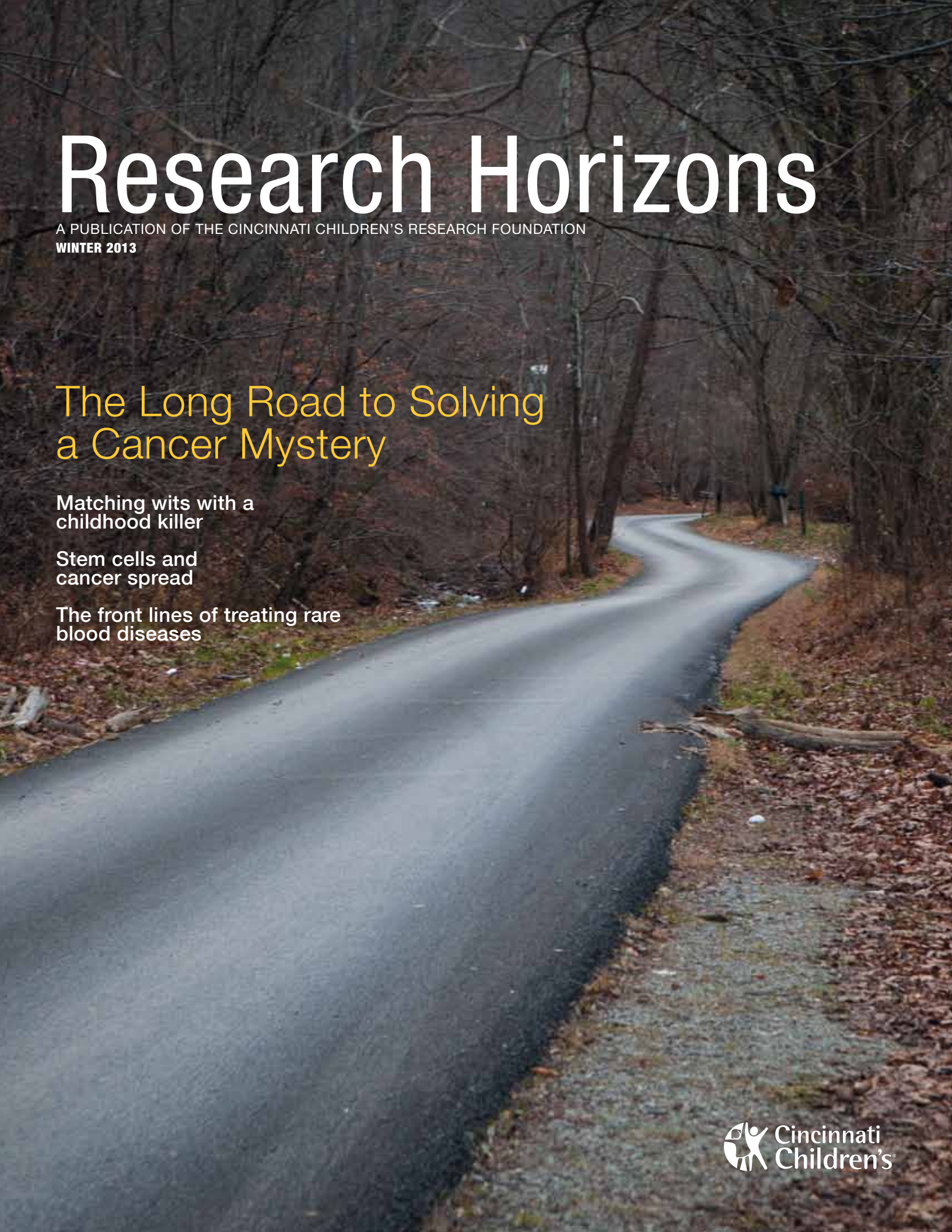
WINTER 2013



The Long Road to Solving a Cancer Mystery

Matching wits with a childhood killer

Stem cells and cancer spread

The front lines of treating rare blood diseases

Cincinnati Children's

Research Horizons

WINTER 2013

page 4
Robin Cotton, MD,
was awarded the William Cooper Procter Medallion, the highest honor bestowed by Cincinnati Children's

About the Cover: A rare disorder led a Cincinnati Children's researcher to the hills of rural Kentucky — and a genetic discovery.

Zubair Ahmed, PhD,

Ophthalmology, received a five-year, $1.8 million award from the National Institute on Deafness and Other Communication Disorders to study “Usher proteins in the inner ear structure and function.”

Chiou Fen Chuang, PhD,

Developmental Biology, received a five-year, $1.4 million award from the National Institute of General Medical Sciences to study “Specifications of stochastic left-right neuronal asymmetry in *C. elegans*.”

John P. Clancy, MD,

Pulmonary Medicine, was awarded a four-year, $2.2 million grant from the National Heart, Lung and Blood Institute to study “MR predictors of infection, inflammation and structural lung damage in CF.”

Charles Dumoulin, PhD,

Imaging Research Center, received a two-year, $1.2 million grant from GE Healthcare for the Ohio Third Frontier Imaging Program.

Prasad Devarajan, MD,

Nephrology, will use a five-year, $4 million grant from the National Institute of Diabetes and Digestive and Kidney Diseases to develop “Critical Translational studies in pediatric nephrology.”

Sudhansu K. Dey, PhD,

Reproductive Sciences, received a five-year, $1.4 million grant from the National Cancer Institute to work with the M.D. Anderson Cancer Center on “The role of bioactive lipids in inflammation and cancer.”

Hartmut Geiger, PhD,

Experimental Hematology, will join with the University of Kentucky, using a five-year, $1.3 million grant from the Edward Evans Foundation to study “Molecular mechanisms and therapies for radiation-induced myelodysplatic syndrome.”

Gurjit Khurana Hershey, MD, PhD,

Asthma Research, received a two-year grant of $1.1 million from the Ohio Department of Jobs and Family Services to work with Nationwide Children’s Hospital on the “Ohio Children’s Hospitals Task Force.”

Todd Jenkins, PhD,

Bariatric Surgery, will work with the University of Cincinnati on a $3.5 million, five-year grant to continue the “Teen longitudinal assessment of bariatric surgery.”

Theodosia Kalfa, MD, PhD,

Hematology, received $1.5 million over three years from the National Institute of Heart, Lung and Blood Diseases to study “Rho GTPases in terminal erythroid maturation.”

Research Horizons

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AWARDS & APPOINTMENTS

Long Lu, PhD,

Biomedical Informatics, will use a five-year, $2.6 million grant from the National Heart, Lung and Blood Institute to develop “A network-based approach to associate HDL subspeciation.”

Alexander Miethke, MD,

Gastroenterology, **Hepatology and Nutrition,** was awarded $1.6 million over five years by the National Institute of Diabetes and Digestive and Kidney Diseases, to examine “The role of regulatory T cells in biliary atresia.”

Stephanie Ware, MD, PhD,

Molecular Cardiovascular Biology, will use a grant of $5.9 million over four years from the National Heart, Lung and Blood Institute to work with the University of Miami on a study of “Genotype-phenotype associations in pediatric cardiomyopathy.”

Sing Sing Way, MD, PhD,

Infectious Diseases, received a three-year, $1 million grant from the National Institute of Allergy and Infectious Diseases to study how “Regulatory T cells dictate immunity during persistent salmonella infection.”

Susanne Wells, PhD,

Experimental Hematology, will use an award of $1.3 million over five years from the National Cancer Institute to study “The role and regulation of the human DEK proto-oncogene.”

Yi Zheng, PhD,

Experimental Hematology and Cancer Biology, will explore “Lineage determination and tissue homeostasis in the aged” with the help of a five-year, $1.8 million grant from the National Institute on Aging.

Basilia Zingarelli, MD, PhD,

Critical Care Medicine, was awarded $1.8 million over four years by the National Institute of General Medical Sciences to study “PPAR-γ and PPAR-γ agonists in septic shock.”

Aaron Zorn, PhD,

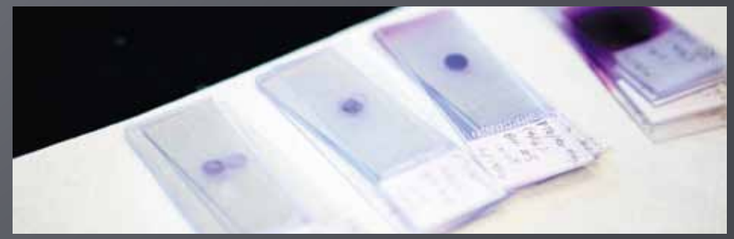
Developmental Biology, will use a five-year, $2.8 million grant from the National Heart, Lung and Blood Institute to study how “OSR transcription factors regulate embryonic lung development.”

Sam Kocoshis, MD,

Gastroenterology, Hepatology and Nutrition, received the Murray Davidson Award from the American Academy of Pediatrics in recognition of an outstanding clinician, educator and scientist who has made significant contributions to the field of pediatric gastroenterology and nutrition.

Sandra Degen, PhD,

Developmental Biology, was named a Charter Fellow of the National Academy of Inventors for outstanding contributions to innovation. Her research has focused on blood coagulation and cancer, work for which she holds three patents.



Robin Cotton, MD, is one of only nine physicians since 1960 to receive the William Cooper Procter Medallion, the highest honor bestowed by Cincinnati Children's.

The award was created in 1960 by A. Ashley Weech, MD, Chair of Pediatrics at Cincinnati Children's from 1941 to 1963. Albert Sabin, MD — inventor of the oral polio vaccine — was the first recipient. The most recent recipient was Jeff Whitsett, MD, honored in 1996 for his role in developing artificial surfactant, which has dramatically improved survival rates for preterm infants.

Cotton, who recently stepped down as Director of Pediatric Otolaryngology/Head and Neck Surgery, built the world's premier center for diagnosing and treating airway abnormalities at Cincinnati Children's. The center serves more than 33,000 outpatients and performs more than 11,000 procedures a year.

Tom Cody, Chairman of the Board at Cincinnati Children's, announced the honor on Nov. 26. "Dr. Cotton is a remarkable innovator, surgeon, educator and leader, whom families from around the world have traveled to see for tracheal reconstruction surgery."

Past Winners of the William Cooper Procter Medallion

Albert Sabin, MD
Ashley Weech, MD
Robert Lyon, MD
Edward Pratt, MD
Josef Warkany, MD
Waldo Nelson, MD
William Schubert, MD
Jeffrey Whitsett, MD

Breakthroughs, cures will come only from better funding of pediatric cancer research

We are pleased to highlight in this issue of *Research Horizons* some of the important research advances being made by scientists and doctors in the Cancer and Blood Diseases Institute at Cincinnati Children's.

We have one of the largest and most successful pediatric cancer and blood disease research programs in the country. Cincinnati Children's stands out as one of only a few centers nationally to have robust research that helps advance the understanding and treatment of childhood cancers and blood diseases.

More than half our patients come from across the nation and around the world for specialized cancer therapies and bone marrow transplantation. Much of our research focuses on understanding the genetic causes of pediatric cancers and blood diseases. We use this understanding to develop more effective, targeted therapies that attack diseases at their source, while minimizing the impact on a child's healthy development.

The stories in this issue help illustrate why Cincinnati Children's is one of the nation's top two centers in overall pediatric research funding from the National Institutes of Health. Our faculty regularly win prestigious and competitive national research awards.

But research funding for childhood cancer and blood disease remains an overwhelming challenge. The National Cancer Institute (NCI) works hard to advance cures for children and adults, but its budget pales in comparison to the economic burden of cancer on our society because one of every two men, and one out of three women, will develop cancer.

Just 3 to 4 percent of the NCI's annual budget of $5 billion is dedicated to childhood cancers. The pharmaceutical industry spends about $70 billion on research and development each year. Seventeen to 18 percent of that is allotted to adult cancer and less than 1 percent goes to pediatric cancer.

Although this comparatively smaller investment in childhood disease does slow progress, the funds we receive are put to maximal use. Research has led to the cure of more than 80 percent of children with cancer, and as the articles featured here show, we are on the cusp of even greater breakthroughs every day. These new findings would advance much faster with better funding.

This is why we encourage fellow scientists, researchers and parents to speak out for enhanced funding of research into childhood cancer and blood diseases, as well as research on behalf of all childhood diseases. Our children's futures depend on it.

John Perentesis, MD
Executive Co-Director, Division of Oncology
Cancer and Blood Diseases Institute

Cancer and Blood Diseases Institute
Executive Co-Directors

Stella Davies, MBBS, PhD, MRCP, and
Lisa Filipovich, MD
Bone Marrow Transplantation and Immune Deficiency Research

Joseph Palumbo, MD
(Acting), Hematology

Yi Zheng, PhD
Experimental Hematology and Cancer Biology

Experimental therapy crosses blood-brain barrier to treat neurological disease

Researchers have overcome a major challenge to treating brain diseases by engineering an experimental molecular therapy that crosses the blood-brain barrier in mice.

Posted online in the *Proceedings of the National Academy of Sciences (PNAS)* early edition on February 4, the study was led by Dao Pan, PhD (below), a researcher in the Cancer and Blood Diseases Institute at Cincinnati Children's.

"This study provides a non-invasive procedure that targets the blood-brain barrier and delivers large-molecule therapeutic agents to treat neurological lysosomal storage disorders," Pan says. "Our findings will allow the development of drugs that can be tested for other brain diseases like Parkinson's and Alzheimer's."

Pan and colleagues assembled the new agent by merging part of a fatty protein called apolipoprotein E (apoE) with a therapeutic lysosomal enzyme called a-L-idurondase (IDUA). They then treated lab-cultured human cells and mouse models of the disease mucopolysaccharidosis type I (MPS I).

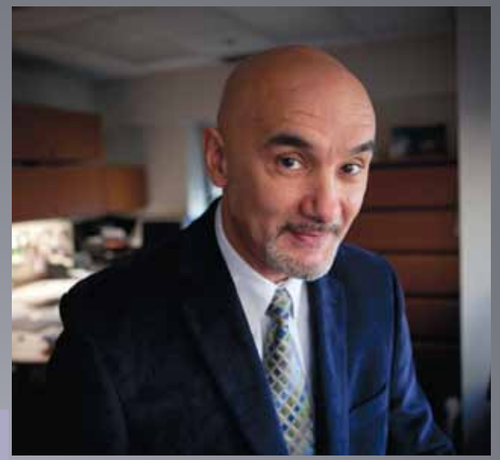
MPS I is one of the most common lysosomal storage diseases to affect the brain. The disease can lead to hydrocephalus, learning delays and other cognitive deficits.

In MPS I, cells lack the IDUA enzyme, allowing cellular debris to accumulate in the brain and other organs. The experimental therapy is exciting because the team found a way to carry supplemental IDUA across the blood-brain barrier. By tagging some apoE components to the IDUA enzyme, the modified protein could attach to endothelial cells and cross through the cells to reach brain tissues.

More research is needed to determine if the therapy can be applied to humans. The team also is exploring whether other brain disease can be treated using this new approach.

Gene test may become new weapon against septic shock

A researcher at Cincinnati Children's is evaluating a rapid gene test that could identify dangerous infections among hospitalized children before they suffer potentially deadly harm.



Hector Wong, MD (left), Director, Division of Critical Care Medicine, has been awarded a three-year, $1.5 million grant from the National Institute of General Medical Sciences to pursue new ways to battle septic shock, one of the leading causes of death among hospitalized children.

An estimated 20,000 to 42,000 children a year suffer severe sepsis in the United States and about 4,500 children a year die, according to a study recently published in *Critical Care Research and Practice* by Cincinnati Children's researchers Carley Riley, MD, and Derek Wheeler, MD.

Although survival rates have improved dramatically since the 1960s, better ways to detect the early signs of sepsis are needed to further reduce deaths.

"Through this new grant, our research program has evolved to a new phase," Wong says. "By leveraging genomic data, we can develop new diagnostic tools for septic shock that can enhance decision making in the ICU, support quality improvement work and help identify candidates for clinical trials."

Regulating protein could prevent scarring and inflammation. Findings have implications for heart failure, muscular dystrophy and pulmonary disorders

Researchers at Cincinnati Children's have discovered a previously unknown function for a protein that could lead to new drugs for battling inflammation and tissue fibrosis.

The study finds that heart attacks and other types of injury activate a tissue repair pathway controlled by the protein TRPC6. The protein prompts cells called fibroblasts to change into myofibroblasts, which in turn secrete extracellular matrix, an important substance needed for tissue remodeling. Over-activating this pathway can lead to excessive inflammation and scarring.

The study, led by Jeffery Molkentin, PhD, and Jennifer Davis, PhD, of the Heart Institute at Cincinnati Children's, was published September 27, 2012 in *Developmental Cell*.

"Our study suggests that a TRPC inhibitor could be a good anti-fibrotic or anti-inflammatory agent in heart failure, muscular dystrophy, pulmonary disorders and other diseases where tissue fibrosis becomes a problem," Molkentin says. "Meanwhile, activation of the TRPC pathway with an agonist compound could be used in select situations to enhance wound healing."

Prior to the new findings, TRPC6 had not been associated with fibrosis, although it has been linked to other cellular functions in skin cells, kidneys and the brain.

Some TRPC inhibitors already are in early-stage development, although their initial design has not targeted heart disease, inflammation or fibrosis. The new study may expand the development focus, Molkentin says.

Mangano named Chief of Pediatric Neurosurgery

Francesco Mangano, DO (right), has been named Chief of the Division of Pediatric Neurosurgery at Cincinnati Children's. He served as acting director prior to this appointment.

Mangano joined the Division in 2005 and has been instrumental in helping develop the medical center's nationally renowned Pediatric Epilepsy Program. His research projects include investigating advanced MRI imaging techniques in children with congenital hydrocephalus and diffusion tensor imaging of hydrocephalus, traumatic brain injury, and vascular and neoplastic brain lesions. He also is an expert in complex disorders of the spine and spinal cord.

Mangano earned his Doctor of Osteopathy degree from the Philadelphia College of Osteopathic Medicine; completed neurosurgery residency at Long Island Jewish Medical Center; and completed a neurosurgery fellowship at St. Louis Children's Hospital.

Nephrology named NIH Center of Excellence

Cincinnati Children's Division of Nephrology has been named an NIH Center of Excellence in pediatric nephrology. The honor went to only three pediatric nephrology centers nationwide.

Collaboration among disciplines is a major requirement of the $4 million, five-year award, as is serving as a resource to individuals around the world who are conducting research into pediatric kidney disease.

"Our goal is to tackle some of the most vexing and difficult problems afflicting children with kidney disease worldwide," says principal investigator and Division Director Prasad Devarajan, MD (at right).

The Division is known for research and clinical treatment of acute kidney injury, nephrotic syndrome and lupus nephritis. One breakthrough discovery is a biomarker test that identifies kidney injury early, before serious damage occurs. Center of Excellence funding will further the study of biomarkers to predict a child's vulnerability to kidney problems due to disease or treatment.

The award will fund staff in Cincinnati Children's core Genomics, Proteomics, and Biomarker laboratories who are focused on kidney disease. These staff will help develop new biomarkers and build basic science models; their findings will serve as a resource to doctors and researchers.

The goal is to use the Center of Excellence award to help change the course of a childhood killer.

"People used to think that children died *with* acute kidney injury, but we now know beyond the shadow of a doubt that children die *because of* acute kidney injury," Devarajan says. "It is a complication of the therapies we give our patients, and of advanced disease in other organ systems. It has generated an epidemic all over the world, and we need to put an end to it."

Anti-rejection drug everolimus shrinks kidney and brain tumors, shows promise for a growing number of disorders

An important Phase III clinical trial confirms that the anti-rejection drug everolimus can dramatically reduce brain tumor growth in patients with tuberous sclerosis complex (TSC).

The study -- published online November 14, 2012, in *The Lancet* -- was led by David Franz, MD (below), Director of the TSC Clinic at Cincinnati Children's.

"Every patient in this study experienced a decrease in size of their tumors, and no patient required surgery for their tumors after treatment with everolimus," Franz says. "Thirty-five percent of patients in this study on everolimus had at least a 50 percent reduction in tumor volume after an average of 42 weeks on medication."

Until recently, surgery was the standard therapy for treating subependymal giant astrocytomas (SEGAs), but everolimus offers a new alternative, Franz says.

Similar results from a smaller Phase II study of everolimus were published in 2010 in *The New England Journal of Medicine*. Based on that data, the U.S. Food and Drug Administration (FDA) granted accelerated approval of everolimus for patients with SEGAs. The FDA had already approved using the drug to treat TSC-related kidney tumors, based on studies by John Bissler, MD, a nephrologist at Cincinnati Children's.

About 1 million people worldwide live with TSC, including nearly 50,000 in the US. The TSC Clinic at Cincinnati Children's, which follows more than 800 children and adults, is believed to be the largest in the world.

Everolimus also may have benefits beyond treating TSC. The same mTOR signaling pathway associated with overactive cell growth in TSC also is implicated in Alzheimer's disease, type 2 diabetes, Parkinson's disease, Huntington's disease and autism. This makes everolimus, an mTOR inhibitor, a potential candidate to treat these disorders, Franz says.

Everolimus is marketed by Novartis, which provided drug and financial support for the study. In addition, several of the Phase III study co-authors are employees of Novartis.

Antommaria heads newly launched Ethics Center

As rapid advances in medical research and clinical care turn the impossible into the everyday, navigating the intricacies of healthcare decision-making is increasingly difficult.

So the appointment of Armand H. Matheny Antommaria, MD, PhD, FAAP, as Director of the new Ethics Center at Cincinnati Children's seems all the more timely.

The Ethics Center will support clinicians, researchers and administrators in weighing the ethical implications of healthcare and research options. Antommaria will lead programs in clinical, research and organizational ethics, provide education to staff and conduct research on behalf of the Center.

Antommaria, a general pediatrician, will spend about one-third of his time in clinical service as a hospitalist at Cincinnati Children's, something he sees as essential to his ethicist role.

"Ethics should be an adjunct to clinical activities," he says. "It should grow out of our clinical experience." His work as a pediatrician first sparked his interest in healthcare ethics.

"Particularly in an institution like Cincinnati Children's, where so many of the children have rare and complex conditions, there are ethical considerations in many of the decisions doctors and families must make," he says. He points to left ventricular assist devices and biorepositories as just two areas where rapid advances have raised important ethical questions.

He hopes to help with those decisions, drawn here by what he terms a real "openness" to discussing and addressing the difficult issues that accompany progress.

Antommaria earned his MD at Washington University School of Medicine in St. Louis, and his PhD in religious ethics at The University of Chicago Divinity School. He completed his pediatric residency at the University of Utah. Before coming to Cincinnati Children's, he served as associate professor of pediatrics and led ethics activities at Primary Children's Medical Center in Salt Lake City.

He has published a variety of articles that include organ donation after circulatory death, critical care triage and other ethical issues in journals including *JAMA* and the *Hastings Center Report*.

As Lee Ault Carter Chair in Pediatric Ethics and Director of the new Ethics Center, Dr. Armand Antommaria will support clinical and research staff in managing ethical questions posed by modern medicine.

Cincinnati Children's receives Autism Center of Excellence grant

A network of five leading medical centers, led by Cincinnati Children's Hospital Medical Center and Boston Children's Hospital, has received a five-year, $12.5 million grant from the National Institutes of Health (NIH) to learn more about how autism develops.

The study, headed by Darcy Krueger, MD, PhD, will enroll 150 children under the age of 3 who have tuberous sclerosis complex (TSC), a rare genetic disease that causes tumors to form throughout the body, including the brain.

About 50 percent of children with TSC develop autism or autism-like symptoms. These children offer a unique way to study autism because TSC can be diagnosed even before birth, making it possible to observe how the brain's circuitry develops before autism becomes apparent.

"If we can intervene before the onset of autism symptoms, we can perhaps offset future problems," Krueger says.

This project is one of several ways researchers at Cincinnati Children's are working to improve treatment for children with autism spectrum disorders. The projects range from refining the definitions of autism-like conditions to exploring the genetics of brain development to testing potential treatments.

Through the newly formed TSC Autism Center of Excellence Research Network (TACERN), and in close collaboration with the national Tuberous Sclerosis Alliance, researchers in the Autism Center of Excellence will track infants diagnosed with TSC using advanced brain imaging techniques.

Also participating in the study are the University of Alabama at Birmingham, Mattel Children's Hospital at UCLA and the University of Texas Medical School at Houston.

Dr. Darcy Krueger will lead a five-center study to explore why autism develops in nearly half the children with tuberous sclerosis complex.

Researchers discover gene linked to deafness

A team led by researchers at Cincinnati Children's has isolated a genetic mutation responsible for deafness associated with Usher syndrome type 1.

Usher syndrome causes deafness, night-blindness and a loss of peripheral vision through the progressive degeneration of the retina. The findings, published online September 30 in *Nature Genetics*, could lead to improved treatments.

Researchers conducted genetic analysis of 57 people from Pakistan and Turkey to pinpoint a gene mutation affecting the CIB2 protein that is associated with Usher syndrome type 1 and non-syndromic hearing loss.

The CIB2 mutation appears to interfere with normal calcium signaling that regulates the ear's ability to convert the mechanical energy of hair cells into electrical signals that the brain can recognize as sound.

"With this knowledge, we are one step closer to understanding the mechanism of mechano-electrical transduction and possibly finding a genetic target for future therapies," says Zubair Ahmed, PhD, the study's lead investigator.

Saima Riazuddin, PhD, (far right, with Ahmed) co-led the study. Others involved include Thomas Friedman, PhD, and Inna Belyantseva, MD, PhD, from the National Institute on Deafness and other Communication Disorders; and the teams of Suzanne Leal, PhD, of Baylor College of Medicine; and Gregory Frolenkov, PhD, of the University of Kentucky.

Cracking the Code of NF1

Dr. Ami Patel, a post-doctoral fellow, manipulates tumor cells in the laboratory. Known for her skill at performing gene knockdown tests, Patel did much of the legwork for finding new treatment targets for NF1. Researchers in the Division of Bioinformatics, Biostatistics and the Imaging Resource Center, and co-investigators at the National Cancer Institute, were also crucial to the research in a recently published study.

A new drug shows remarkable promise for shrinking tumors

Science may be on the verge of cracking the code of neurofibromatosis 1 (NF1) – long labeled a disease without treatments.

Finding clues to solve the NF1 puzzle – a genetic condition that causes non-cancerous tumors to grow in the nervous system – has been slow and tedious since first described in 1882 by German pathologist Friedrich von Recklinghausen.

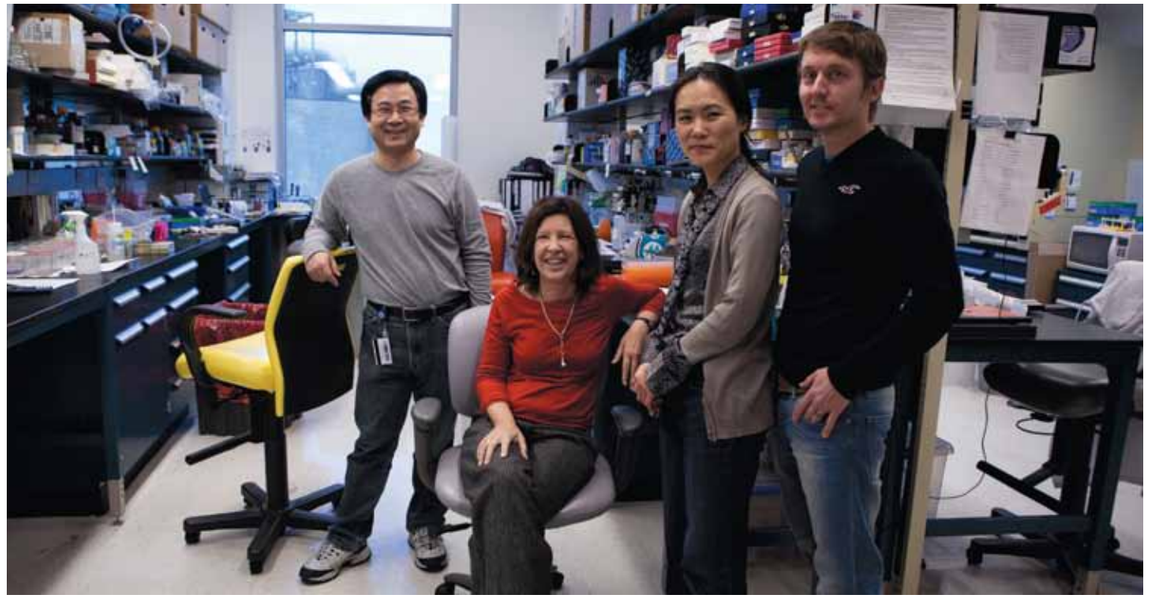
It is not from lack of trying. Researchers have devoted entire careers to understanding how NF1 works. They have tested chemotherapies, radiation and targeted molecular treatments in laboratories and, in some cases, on patients. Until recently, these efforts have been of little or no avail.

"We've never been able to shrink the tumors very well," explains Brian Weiss, MD, an oncologist in the Cancer and Blood Diseases Institute at Cincinnati Children's. "They have a lot of collagen and fibrous tissue in them. On average they grow very slowly. NF1 is just a very different animal than cancer."

Now, a multi-institutional study led by Cincinnati Children's researcher Nancy Ratner, PhD, indicates this "different animal" may almost be corralled. Published last December in the *Journal of Clinical Investigation*, the study demonstrates that using an experimental cancer drug to block a single, critical protein in the NF1 molecular process, MEK, is effective in mouse models of plexiform neurofibromas.

Working with the Neurofibromatosis Preclinical Consortium, the research team showed this targeted molecular therapy generated unprecedented results. In tests on mice, MEK inhibition significantly shrunk more than 80 percent of plexiform neurofibromas – the nerve tumors caused by NF1. The data were so compelling that researchers reported the study provided "strong rationale" for testing the drug in a clinical trial.

Working with a national group called the Neurofibromatosis Clinical Consortium – of which Cincinnati Children's is a charter mem-



ber – Weiss is finalizing plans for a multi-institutional clinical trial to determine whether the MEK inhibitor is effective in NF1 patients.

As to why MEK appears to shrink neurofibromas when other treatments have failed, Weiss says it may be MEK's proximity in the cellular growth pathway to the source of the problem – the mutated NF1 protein. This makes it harder for the disease process to perform a molecular "end run," which can happen when molecular targets are far downstream from the inciting molecular lesion.

It also may help explain why other tested drugs have had less success. For example, sirolimus blocks a molecule called mTOR, which is frequently implicated in other tumors and cancers. Weiss says mTOR is five steps away from the NF1 protein. Although sirolimus has worked with some tumors and cancers, it has not been effective at shrinking NF1 neurofibromas.

LIKE A CAR WITH NO BRAKES

NF1 is inherited. Only one defective copy of the NF1 gene is needed to trigger the disease. If one parent has the disease, then children have a 50 percent chance of getting NF1. The prevalence of NF1 is about one of every 3,000 children born. More than 100,000 people in the United States have the disease.

The NF1 gene normally acts to prevent uncontrolled cell growth in nerves and to block tumor formation. When the NF1 gene mutates and loses function, "it's like hitting the gas pedal on a car with no brakes," according to Weiss. Cells in the nerves grow and accumulate abnormally.

Neurofibromas can grow to be quite large – unsightly and disfiguring as well as sometimes painful and dangerous. In some cases, large plexiform neurofibromas can turn into deadly malignant peripheral nerve sheath tumors (MPNSTs). Exactly what causes this malignant transformation remains unknown. Other than being able to perform the occasional surgery in certain cases, there still is no way to treat plexiforms or MPNSTs.

Although the recent MEK inhibitor study showed nominal ability to slow growth of MPNSTs in mice, the tumors refuse so far to yield to available treatments.

Still, many people with NF1 can live long lives. Weiss notes that NF1 tumors tend to grow slowly, so years can pass before they become problematic, if at all. Although an advantage for patients, slow tumor growth can be a challenge when trying to design a clinical trial.

Plexiform neurofibromas can remain stable for almost a year, making it difficult and time consuming to evaluate medicines that may only slow or prevent the growth of tumors rather than shrink them. NF1 patients would also need to be on medicines for longer periods of time compared to people with faster-growing tumors.

"It comes down to managing acceptable levels of risk for the patient," Weiss says. "In cancer, where the result of not treating is death, you may be willing to take certain risks. In NF1, it can be a different set of considerations."

CHALLENGES REMAIN

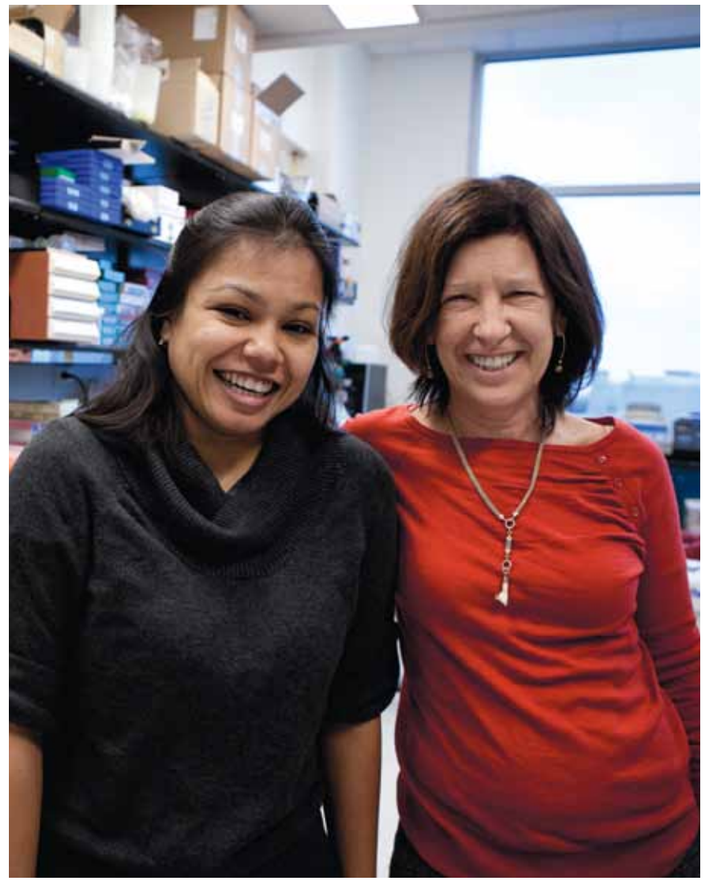
Ratner has studied NF1 her entire career and produced milestone findings on how the disease process works. Although she is pleased to see clinical trials open for a drug that may be able to stop or shrink NF1 plexiform neurofibromas, it is only one part of the NF1 puzzle.

"Being able to manage the disease would be a huge step forward," she says. "But our goal is curative therapy. We want to prevent tumors from forming or completely ablate them."

Despite these remaining challenges, Ratner is hopeful about finding other pieces of the puzzle. She was there when good answers were far and few between, and when research more or less centered on dissecting patient tumor samples and analyzing them under a microscope. But technology such as bioinformatics and advanced MRI capabilities -- along with talent and hard work -- are producing new possibilities.

"The success we have seen gives me real hope that soon we will be able to identify drugs that really do melt these neurofibromas so they won't come back," Ratner says.

Her team also has a list of candidate molecular targets that might kill off MPNST cells. The results, however, are still early. "We need more verification before we can discuss it." ■



This Page: Dr. Nancy Ratner, with members of her laboratory team at Cincinnati Children's, says real success in NF1 research will come from preventing or curing the disease. **Following Page, Top:** Dr. Brian Weiss is developing a multicenter clinical trial of a drug to shrink plexiform neurofibromas in patients with NF1. **Bottom:** Ratner with post-doctoral fellow Ami Patel.

HLH Meets its Match

Persistence and smart medicine just might outwit this deadly disorder

The child had a host of life-threatening complications: an enlarged liver and spleen, low blood count, inflammation in the brain. No one knew what it was, or what to do about it.

But Lisa Filipovich, MD, was certain of one thing. Figuring it out would be her life's work.

It was some 30 years ago, Filipovich's first day as a faculty member at the University of Minnesota Hospital. But she remembers it as if it were yesterday.

"It was one of the most fascinating and challenging areas of medicine I'd experienced. But it wasn't anything we had learned in training. No one knew what it was. It hadn't even been described in the medical literature."

She knows now that "it" was hemophagocytic lymphohistiocytosis (HLH), a life-threatening immunodeficiency. For people with HLH, the body's normal reaction to infection goes into overdrive, resulting in swelling, pain, and eventually, life-threatening damage to organs and systems throughout the body.

"One of the problems is, no one stumbles to the diagnosis soon enough. And it's the 'soon enough' that is the problem."

THE FIRST MAJOR STEP

Filipovich, now Director of the Immune Deficiency and Hystiocytosis Program at Cincinnati Children's, and one of the world's leading authorities on HLH, says a big breakthrough in understanding the disease came in the late '90s, when the first genetic cause was identified. People who shared the disorder's unique set of abnormalities had a common genetic mutation that kept them from producing perforin, a protein crucial to the function of the immune system.

"It began to explain why the natural killer cells and other cytolytic cells in this disease don't work," says Filipovich, "but it still didn't tell us why patients got the disease.

The process of better understanding and managing HLH is one that has occupied Filipovich and her team for the last 20 years. And although it has not yet yielded a cure, it has certainly led to more successful treatment.

STEPS TOWARD PROGRESS

"There are two steps in treating this disease," says Filipovich. "First is to stop the destructive inflammatory process. But many patients then need their immune system changed over with a bone marrow transplant."

HLH has both inherited and acquired forms. It can occur at almost any age, although it most often strikes infants and young children, even babies *in utero*. It typically starts with an infection. In highly susceptible individuals, it can be triggered by a childhood immunization.

"At first, the immune system turns on as it should, to contend with whatever the infectious agent is," explains Filipovich. "The problem is, it gets going and can't turn off. That's what leads to the toxicity, to the destruction of the blood cells."

From there begins a downward spiral that can include liver failure, respiratory distress, inflammation in the brain and the inability to fight infection.

EARLY DETECTION IS KEY

Because HLH is considered a "rare" disease (statistics say it occurs in about one in a million children), many healthcare providers are unfamiliar with its symptoms, and it often goes undiagnosed for too long.

"One of the problems is, no one stumbles to the diagnosis soon enough," Filipovich says. "And it's the 'soon enough' that is the problem."

To halt the rampant inflammation of HLH, doctors currently use high-dose steroids, but with a number of side effects. Filipovich and other members of her team (see story that follows) are working on studies to evaluate therapies with fewer toxic effects.

"It would be a great advance if the disease could be controlled with far less collateral damage," she says.

OVERHAULING THE IMMUNE SYSTEM

Once the inflammation is under control, most patients must have their immune systems replaced by means of a bone marrow transplant. The team at Cincinnati Children's does more bone marrow transplants for HLH than any other center in the United States or Europe, with impressive results.

"We have made pivotal advances in this area," Filipovich says. One of the advances is the use of "reduced intensity conditioning," which uses a milder biologic agent to prepare a child for bone marrow transplant. The approach has far less systemic toxicity than the usual chemotherapy. It cannot be used for every child, says Filipovich, but for those who are eligible, it has improved long term survival to 90 percent.

What "long term" survival means for children with HLH is still to be determined, Filipovich says. She continues to follow patients that she treated more than a decade ago, but acknowledges that there is a long way to go.

"We're getting there," Filipovich says. "But there are people who, for reasons we don't understand, are far more fragile than others. Sometimes there are clues in the way they've responded to drugs or other things in the past. But we're not yet smart enough to use that information to personalize their treatment."

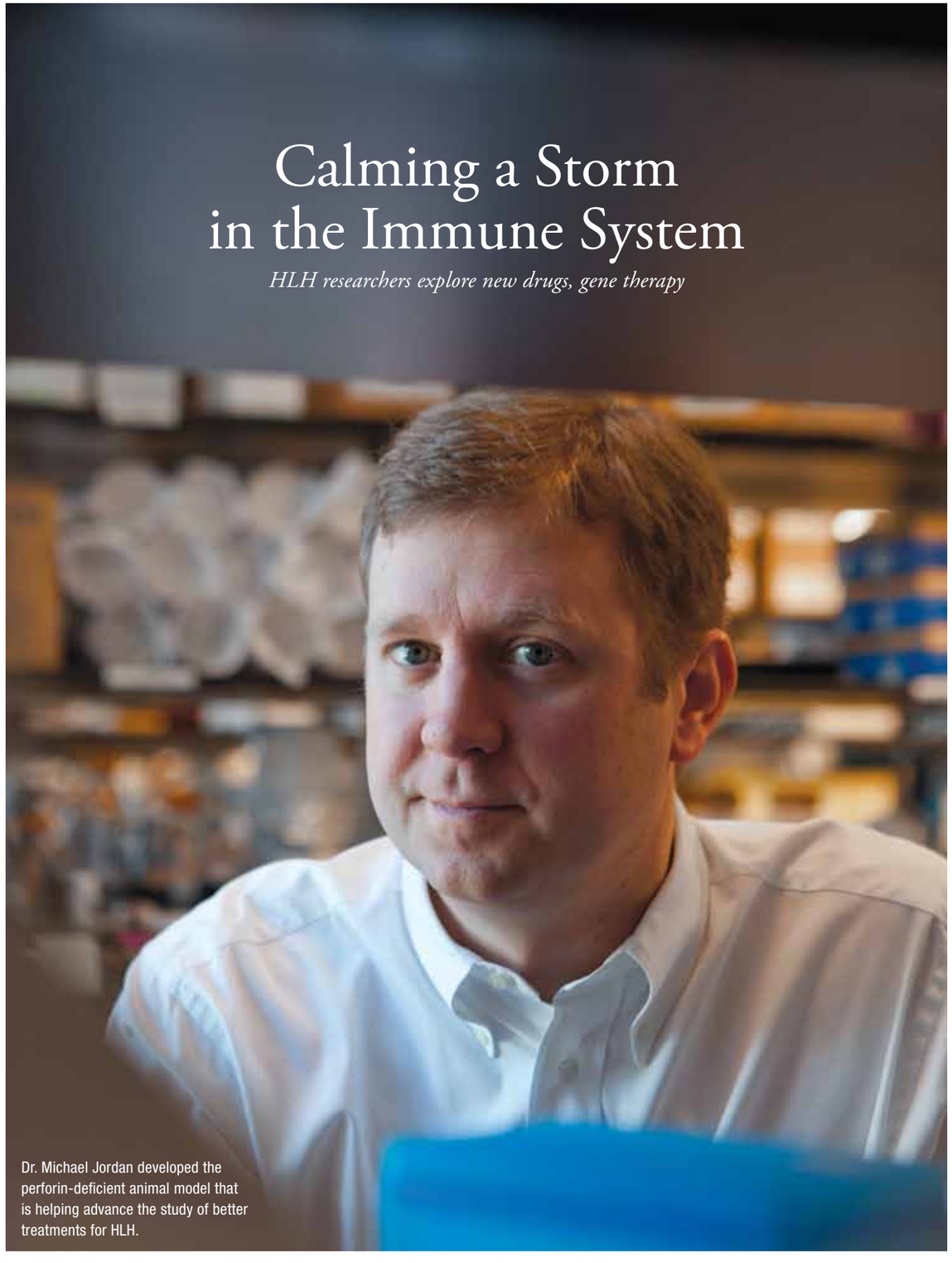
Even so, Filipovich and her colleagues have made an enormous difference for many children with HLH.

"We have built an extremely talented team with a strategy of providing treatment in safer, smarter ways," she says. "In the old days, if a patient was in desperate situation, we would go right to transplant. But we've learned to move beyond desperation medicine. We have to do smart and calculated medicine. In the end, you have to move quickly but in a thoughtful way." ■

Dr. Lisa Filipovich has spent her career unraveling the mysteries of the childhood immune disorder hemophagocytic lymphohistiocytosis (HLH). Her focus now is to improve treatment and ultimately, prevent the disease.

Calming a Storm in the Immune System

HLH researchers explore new drugs, gene therapy



Dr. Michael Jordan developed the perforin-deficient animal model that is helping advance the study of better treatments for HLH.

Although science has come a long way in understanding HLH, its treatment presents significant challenges. Each step in treatment – getting the raging immune response under control, then replacing the immune system with bone marrow transplantation – wages its own set of assaults on young bodies. Cincinnati Children's physician-researchers are working to find more effective, less toxic alternatives.

COMBINATION THERAPY

One approach is a multicenter clinical trial led by Michael Jordan, MD, of the Division of Bone Marrow Transplantation and Immune Deficiency. The trial combines two currently used approaches into a unique treatment, "hybrid immunotherapy." It is designed to improve the first step of treating HLH – quieting the inflammatory response. The Hybrid Immunotherapy for HLH (HIT-HLH) trial is the first-ever US-based trial for the condition and the only one funded by the NIH. It involves 10 centers throughout the U.S. and Canada.

"This study focuses on controlling the disease process, not fixing the underlying problem," says Jordan. "But we believe it may give more rapid and complete control of inflammation. We hope it will be a real improvement."

Treatment involves a combination of three drugs, anti-thymocyte globulin (ATG), etoposide and dexamethasone. The HIT-HLH trial has enrolled 12 children so far and plans to enroll up to 40 more. So far, researchers are pleased with the early results, Jordan says.

THE INTERFERON CONNECTION

Jordan and Lisa Filipovich, MD, also are collaborating with colleagues overseas and a Swiss pharmaceutical company on a clinical trial recently launched in Europe to test the effects of an antibody, NI-0501, on the protein gamma interferon (IFNγ). This protein, crucial in the immune process, is believed to be excessively elevated in patients with HLH. NI-0501 targets and neutralizes the protein. If the theory that elevated IFNγ causes HLH proves true – and data so far support it – then targeting the protein should shut down the disease process, Jordan says.

Filipovich, Jordan and others hope to approach the FDA this year for permission to test the drug in the U.S., with Cincinnati Children's a likely trial site.

GIVING PERFORIN A BOOST

Although most children with mutations in perforin – a protein crucial to immune response – develop HLH in early infancy, some develop the disease later. Children with "later onset" HLH are able to create some perforin, though not enough to avoid eventually succumbing to the disease.

For these children, Kimberly Risma, MD, PhD, Division of Allergy and Immunology, is looking at how targeted drug therapy might boost perforin production, which could help them avoid the ravages of HLH and the rigors of bone marrow transplantation.

"The type of perforin mutations impact the age of onset," Risma says. "So late onset - after 18 months - means a child is producing some small amount of functional protein. If onset occurs after age 5, the child is producing even more."

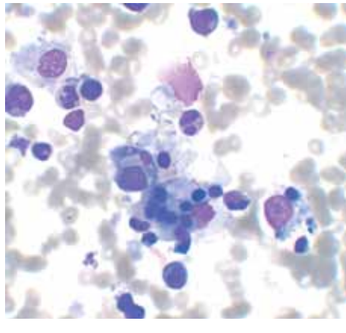
FINDING THE 'ENHANCERS'

Risma has developed a unique assay to measure the killing function of cytotoxic lymphocytes. Using a high-throughput screen, she hopes to evaluate thousands of small-molecule compounds to see which might best serve to boost the lymphocytes' killing capacity.

She will first screen FDA-approved compounds, using the resources of the University of Cincinnati's Drug Discovery Center. She will then look for similar compounds in the Center's library, which contains 300,000 pharmaceutical-quality compounds used by researchers nation-

"Many children with HLH do not survive bone marrow transplant. That could change if this works."

In hemophagocytic lymphocytosis (HLH), certain immune cells engulf other cell types, a condition called "hemophagocytosis" or "blood-eating," which damages the bone marrow and other organs. This image is from the bone marrow of a patient affected by HLH.



Dr. Kimberly Risma will search thousands of small-molecule compounds for one that boosts perforin production in children with 'later onset' HLH. Such a compound could prevent the need for bone marrow transplants in these children.

wide to hunt for potential treatments.

"My hope is that we will find compounds that are orally bioavailable and will have an impact on cytotoxic cells," Risma says.

COLLABORATION WITH GREAT ORMOND STREET

Jordan, Risma and Punam Malik, MD, at Cincinnati Children's, have also teamed with colleagues at London's Great Ormond Street Hospital for a study of perforin gene replacement therapy to treat HLH.

The study will use perforin-deficient mice – a model developed by Jordan during his training – and inject them with healthy stem cells carrying normal perforin genes. The mice will then be infected with a virus that triggers HLH. The goal is to see if the mice begin producing perforin and are protected from getting the disease.

"If the experiment works and the mice are protected, it will be an important step forward in the development of gene therapy for perforin-deficient HLH patients," Jordan says. "For patients who have no perforin function, there is no way to fix it apart from a bone marrow transplant, which has significant risks. If this is successful, we could fix the perforin deficiency with gene therapy, replacing it in their own cells."

GETTING THE VECTOR RIGHT

Researchers at Great Ormond Street turned to Cincinnati Children's because of our experience producing lentiviral vectors for gene therapy and Jordan's development of the pre-clinical mouse model for HLH. But getting it right is a painstaking process as scientists move their work from *in vitro* studies, conducted at Great Ormond Street, to an animal model here.

Refining the vector that will carry the perforin gene is especially complicated, says Jordan, because the vector is not a passive transport mechanism – it actually controls how much of the gene product is expressed, and when.

"Getting it controlled in the right cells at a sufficient level that can be modulated by inflammation is the ultimate goal," Jordan says.

He is optimistic that they will get it right and the animal studies will proceed. And he is cautiously optimistic that the research could lead to a game changer for HLH. But clinical trials remain several years and a great deal of work away.

"We already know that many children with HLH do not survive bone marrow transplant," he says. "That could change if this works." ■

Breakthrough for 'Bubble Boy' disease?

For children with X-SCID, a new gene therapy could be the answer

It started as a small bump on his hip. "They thought it was an infected hair follicle, so they gave him antibiotics," says Jennifer Golliday. "It didn't go away."

Jennifer is the mother of Jameson, a lively, impish little boy who turned one year old last October. Looking at him, you would never suspect the ordeal he went through in his first year of life.

When the bump grew larger and more infected, doctors in the Gollidays' hometown in Illinois took a biopsy. They told the Gollidays that Jameson had a rare form of cancer. He was five months old.

But one oncologist at their local hospital was not convinced. She thought it unusual for a baby to have such a rare cancer. Jameson was breathing rapidly and his overall condition was worsening. She kept looking for another explanation and found it: Jameson's symptoms mimicked those of a rare immune disorder, severe combined immunodeficiency. A blood test confirmed that Jameson had a form of the disorder known as X-SCID, which affects only boys.

The disorder is caused by a defect in one of the genes crucial to the body's production of immune cells. Like all children with SCID, Jameson had virtually no functioning immune system and was unable to fight any sort of infection. He was immediately transferred to Cincinnati Children's by medical jet.

"He was very sick," says Lisa Filipovich, MD, Director of the Immune Deficiency and Histiocytosis Program at Cincinnati Children's. She became Jameson's physician. "But he was lucky because what he had was treatable."

Jameson had developed a virulent form of pneumonia that required several rounds of antibiotics to get under control. The bump on his hip was not a malignancy but a cluster of uncontrolled B-cells, the result of his dysfunctional immune system.

Most children with SCID require a bone marrow transplant to rebuild their immune systems. But Jameson qualified for a clinical trial of a gene therapy being carried out at Cincinnati Children's.

Doctors at Cincinnati Children's removed some of Jameson's bone marrow and treated it in the laboratory with the corrected gene. They then returned the treated stem cells to Jameson via a simple IV infusion. The goal is that the corrected cells will proliferate and restore his immune system.

"Nine weeks after his infusion, he started making his own T cells," Jennifer says. "He had a runny nose and cough from his pneumonia, and it cleared up the week after he received the T cells. It was pretty exciting to see those symptoms disappear."

"He was treated at 8 months of age and so far, it's working," says Filipovich. "His immune system is developing exactly as we would expect."

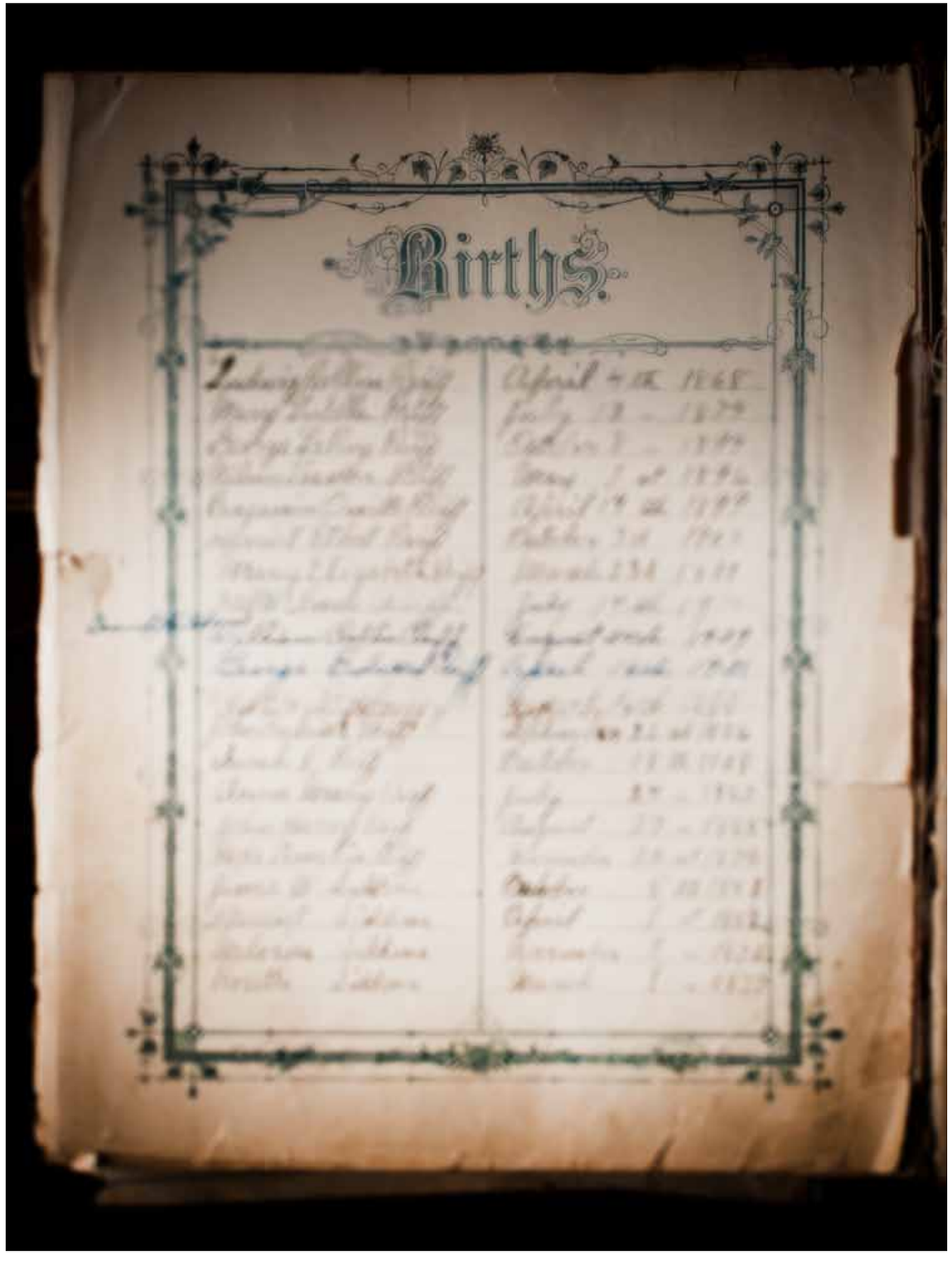
Jameson and his mom were able to resume life at home just before this past Thanksgiving. The only medication he will require is gamma globulin replacement for up to two years, until doctors are certain that his B-cell function is working as it should.

An initial attempt at gene therapy for X-SCID was halted worldwide some years ago when several children developed leukemia from the treatment. It was believed to be a result of the vector used in the therapy. Researchers now use a completely different vector, and doctors and families are cautiously hopeful that gene therapy will be the future go-to treatment – and possibly the cure – for X-SCID.

"I consider any family who volunteers for this a critical partner in this process. We are experimenting together," says Filipovich. "With the internet and Facebook, families know the good, the bad and the ugly about gene therapy. They have seen it all, in a way we could never describe to them. They come to us with well thought-out decisions and choices. Now that we've lived through an era when bad things happened with gene therapy, having families who want to go forward is very empowering."

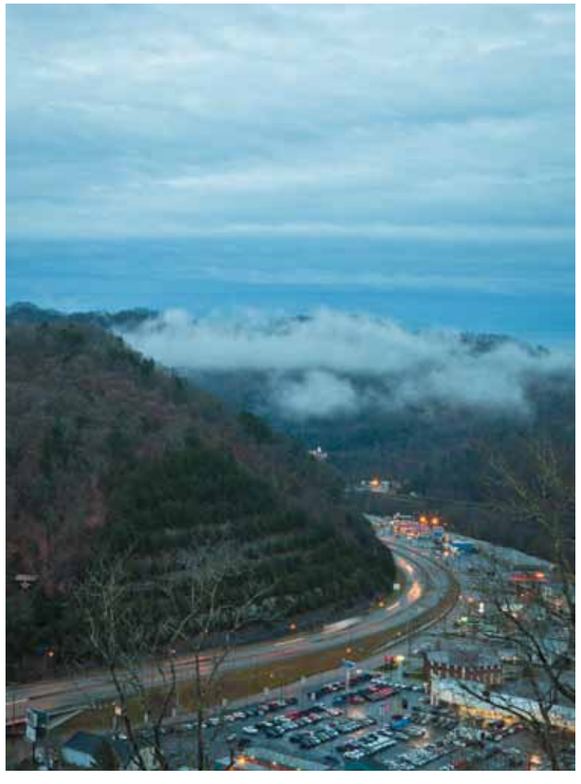
Jameson Golliday's was one of those families. And so far, they are happy with what they are seeing.

"His T-cell numbers are looking good," Jennifer says. "He is full of energy – he runs everywhere." ■



Medical Mystery Reveals Lethal Legacy

Rare gene mutation gives researchers a puzzling opportunity



After a chilly overnight rain, sunrise in the mountains of Southeast Kentucky reveals halos of mist that drape the coal-country ridges around Pikeville.

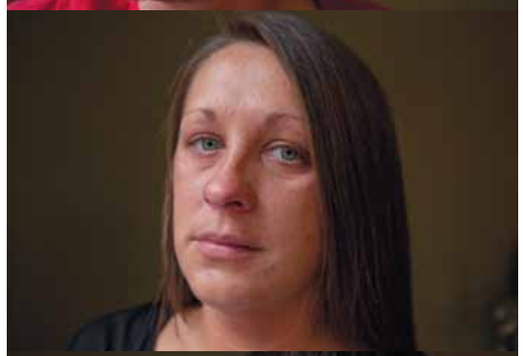
Down the road and up a long hollow lives a family of proud, hardworking people. They have forged their lives from these mountains – mining coal, rearing children and embracing their faith as they care for each other more than anything else.

Now those lives are shrouded by a baffling and potentially deadly medical mystery.

It started with Cincinnati Children's physicians trying to unravel the complicated case of three-year-old Dalson Cable. They discovered his family is prone to a rare mutation involving a gene called RUNX1. It puts family members at high risk for a rare blood disorder and an aggressive form of acute myeloid leukemia (AML), according to Ashish Kumar, MD, PhD, a hematologist in the Cancer and Blood Diseases Institute at Cincinnati Children's.

"The type of mutation is so unique it has previously been described in only one other family in the world," Kumar explains. "In fact, the mutation is not picked up by gene sequencing tests and at first we missed it."

Dalson was first referred to Kumar for what appeared to be a mild case of idiopathic thrombocytopenic purpura (ITP) – a bleeding disorder in which the immune system destroys platelets necessary for normal blood clotting. Dalson had low blood platelets as a baby and suffered from skin rashes and infections.



DIAGNOSTIC DEAD ENDS

While examining Dalson, Kumar learned other family members – including the boy's mother, Toni Cable – also have low blood platelets and skin rashes. What puzzled Kumar is that ITP is not known to be hereditary, so he had other family members screened for gene mutations associated with familial thrombocytopenia. The tests were negative - no mutations.

"In mild cases of ITP, health is not seriously affected," Kumar says. "At first I was not that concerned. Everyone appeared to be pretty healthy, but things started to not add up."

When Dalson had to be examined for an apparent eye infection, his ophthalmologist and pediatrician were concerned about certain aspects of the boy's appearance. They referred him to rheumatologists at Cincinnati Children's, who recommended additional genetic tests. Geneticists from the medical center probed more deeply into the family's medical history, revealing that at least two of Dalson's great maternal uncles and a distant cousin had died from leukemia.

"The geneticists here then made a connection between thrombocytopenia and familial leukemia," Kumar says.

They also saw potential for a rare condition called familial platelet disorder with propensity to AML (FPD-AML), where the RUNX1 gene is mutated. They ran RUNX1 sequence tests on family members, and those also came back normal.

For Kumar and his colleagues, it appeared to be another diagnostic dead end.

BIRTH OF A CLUE

A major break in the case came by happenstance, when a premature baby boy was born to a family cousin. The child had low blood platelets. Doctors in Southeastern Kentucky decided to screen the infant for possible genetic disorders, but they ordered a different kind of genetic test.

Rather than analyze gene sequence, the test looked at the number of copies of genes at the chromosomal level. It detected an abnormality in chromosome 21 - part of the chromosome was duplicated. The affected segment of chromosome 21 included RUNX1. High-resolution testing revealed part of the RUNX1 gene was duplicated, likely making it non-functional.

The baby's test was reviewed by the same Cincinnati Children's geneticist who had looked at Dalson's genetic screen. Doctors at Cincinnati Children's then ran the same test on Dalson and his mother, Toni. Those results were positive, confirming a rare genetic diagnosis and hereditary FPD-AML.

The diagnosis came full circle when Toni's father, a disabled coalminer with black lung disease, had an accident requiring medical treatment. Tests revealed Homer Tackett has advanced AML. His brothers were the great uncles who had died from leukemia.

Chemotherapy followed by bone marrow transplant is the only treatment that might help Tackett. With mounting family financial problems and his black lung, Tackett says he is an unsuitable candidate for further treatment. He prefers to focus his remaining time on enjoying his family.

"Maybe they can do some research to help," Tackett says. "My hope is for the younger ones."

FAMILY PUSHES TESTING, RESEARCH

As Toni helps Kumar locate family members willing to be tested, the details of everyday life weigh on her, like painful blisters that break out on her hands and keep her from bathing Dalson. "It's just been one thing after another," she says.

Kumar has come to know the family well, describing them as "very good and humble people." He is moved, although not surprised, that many members of the extended Tackett family are agreeing to be tested and participate in research.

"This is not a simple thing for them to do, but they have agreed to do it," he says.

Kumar now maintains an ever-expanding chart that resembles a handwritten family tree. It denotes which family members have been tested and results. Of the just less than half testing positive for the mutation so far – including Dalson and Toni Cable – current knowledge suggests about 35 percent will develop AML.

Cincinnati Children's scientists are eager to begin studying blood samples to look for answers. The research opportunity is both challenging and somewhat unprecedented, Kumar says. Right now he wants to make sure willing family members get tested and channeled into appropriate medical care, if needed.

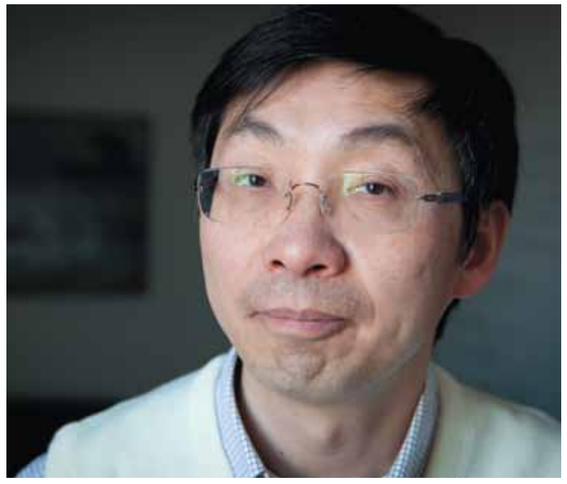
"The case involving this family gives us an excellent opportunity to study the biology of a potentially large repository of human cells, some in people who have the mutation but have not developed leukemia," Kumar says. ■

MORE ONLINE:
Watch the family tell their story at www.cincinnatichildrens.org/mystery

Above: Dr. Ashish Kumar says this rare family case allows scientists to gather and study important genetic information about familial platelet disorder and the risks of developing leukemia. **Below:** The baffling case of 3-year-old Dalson Cable helped scientists at Cincinnati Children's discover a rare gene mutation among Dalson's family (shown on preceding page) that puts them at risk of developing leukemia.

Preventing Cancer Recurrence

Disrupting stem cells could halt cancer spread



As terrible as a cancer diagnosis can be, even more devastating are the words, "Your cancer has relapsed."

Oncologists know all too well that many cancers surge back despite initial success from surgeries, radiation treatments and chemotherapy. Relapses occur even among patients who have lived for several years in complete remission.

The question facing the research community has been, "Why?"

Recent research into the inner workings of cancer cells might hold the answer. Some scientists contend that cancer stem cells find ways to avoid or survive initial treatment, then begin churning out new cancer cells to replace those destroyed by treatment.

Researchers at Cincinnati Children's are working on ways to target and kill cancer stem cells -- or at least disrupt their function. If successful, this work may lead to longer remissions for more children and adults with cancer. For some, the new approach might even cure their cancer.

WHAT IS A CANCER STEM CELL?

The cancer stem cell theory asserts that the maintenance of cancer is driven by a small subset of cancer cells that possess stem cell-like properties. There are various kinds of cancer stem cells, but they are the only cells that can initiate tumor growth.

Exactly how these cells cause cancer relapse is not fully understood. The stem cells may go dormant, avoiding drugs that attack fast-dividing "daughter" cells. They may lack key receptors that make them virtually invisible to chemotherapy agents. Or they may hide in microenvironments that treatments simply cannot reach.

One way or another, once treatment begins destroying the most common cancer cells, the cancer stem cells survive treatment to re-activate and churn out more cancer cells. In many cases, the new generations are highly resistant to follow-up treatment.

NEW WEAPON AGAINST ALL

Much of the early work on cancer stem cells has occurred in leukemia.

"Most chemotherapy agents kill cancer cells while they are dividing. Dormant stem cells are not well-targeted," says Jose Cancelas, MD, PhD. "But even dormant cells have needs. So to kill them, we need to find and block those needs."

Cancelas directs research at the Hoxworth Blood Center, which is affiliated with the University of Cincinnati Medical Center, and he leads the cancer stem cell program at Cincinnati Children's. An expert in blood cancers, Cancelas studies the microenvironments where cancer stem cells live. He focuses on signaling proteins called Rac-GTPases, which play a role in regulating how cancer cells migrate.

Blocking the function of Rac-GTPases could prevent cancers from metastasizing. However, previous versions of blocking compounds have proven too toxic to serve as effective treatments.

Now Cancelas and colleagues may have uncovered a new approach. In a paper published online in June 2012 in the journal *Blood*, Cancelas' team, in collaboration with researchers in the laboratory of Yi Zheng, PhD, reported on a new molecular pathway. The Vav3 pathway plays an important role in cell proliferation in acute lymphoblastic leukemia (ALL), but has a minimal role in non-cancerous cells.

In addition to identifying this important pathway, the research team identified a small-molecule agent that can block it. They are testing the agent in "humanized" mice, which are bred to exhibit human forms of cancer by a colleague at Cincinnati Children's, James Mulloy, PhD. (For more about these mice, see page 29)

"So far, the therapy has been effective in mouse models. But there is still a long way to go before it can be ready for human clinical trials," Cancelas says.

If a drug can be developed to control the Vav3 pathway, Cancelas predicts it would be used in combination with existing chemotherapies, rather than as a first-line treatment. And preventing relapse in people with ALL may be just the beginning.

"Cancer stem cell target therapies, if proven to be successful against one type of cancer, could revolutionize how we do therapy for many kinds of cancer," "Cancer stem cell target therapies, if proven to be successful against one type of cancer, it could revolutionize how we do therapy for many kinds of cancer". Cancelas says.

EXPLORING RNA THERAPEUTICS

In a related effort to prevent cancer recurrence, Lee Grimes, PhD, and colleagues at Cincinnati Children's are studying how to use microRNA therapy as a weapon against cancer – especially

Opposite Page, Clockwise:
Dr. Lee Grimes is working on a potential RNA therapy that has been shown to cure cancer in mice with infant-onset AML.
Dr. Yi Zheng and colleagues have identified several small-molecule inhibitors that can disrupt the function of cancer stem cells.
Dr. James Mulloy has achieved breakthroughs in developing "humanized" mice to serve as improved animal models for studying cancers and potential cures.
Dr. Jose Cancelas and colleagues have identified a molecular pathway that could lead to better treatments for ALL.

"Cancer stem cell target therapies, if proven to be successful against one type of cancer, could revolutionize how we do therapy for many kinds of cancer."

against rare and powerful forms of acute myeloid leukemia (AML) that strike during infancy.

Grimes directs the Cancer Pathology Program at Cincinnati Children's and co-leads the Program in Hematologic Malignancies with James Mulloy, PhD. Much of Grimes' translational work is funded by the National Institutes of Health and the Leukemia and Lymphoma Society of America.

In the past 15 years, microRNAs (miRNAs) have emerged as important regulators of gene expression. Abnormal levels of miRNA have been linked to several forms of cancer, as well as other diseases. Such findings have triggered a global race to develop compounds that can control miRNA expression.

Grimes is working on a potential RNA therapy that could help infants born with mutations involving the 11q23 chromosome. Unlike adult-onset leukemia, AML strikes children with these gene mutations during infancy. Their cancers are so strong that the patients are much less likely to survive, even with stem cell transplants and other advanced therapy.

Grimes and colleagues identified a signaling pathway that regulates oncoproteins related to infant-onset AML. They also learned that the pathway can be blocked by a specific gene, Gfi1, which encodes a protein that represses the expression of other genes. Years of work have since confirmed the pathway and the repressor function in fruit flies, mice and humans.

In humans, the Gfi1 repressor itself cannot yet be affected by drug therapy. However, the researchers have identified microRNA inhibitors that can mimic Gfi1 repressor function. "We have found that antagonists of these microRNA, as a surrogate for the transcriptional repressor, can actually cure the leukemia in the mouse model," Grimes says.

The next step is to test the RNA therapy in "humanized" mice to determine its potential for clinical use. The team also is studying whether this microRNA inhibitor also can be used against adult leukemias.

PROMISING CANCER TREATMENTS TAKE TIME, MONEY

Yi Zheng, PhD, Director, Division of Experimental Hematology and Cancer Biology, leads an institution-wide hunt for small-molecule inhibitors that can disrupt the function of cancer stem cells. The team has already found several potential candidates.

In 2004, the Zheng lab discovered a first-generation small inhibitor against Rac-GTPases that was later shown to work against leukemia stem cells in mouse models. In 2008, Zheng presented early findings at the American Society of Hematology that a lead drug candidate dubbed "CASIN" – a Cdc42 small-molecule inhibitor – plays a role in mobilizing normal blood stem cells from bone marrow into the bloodstream. Since then, Zheng and colleagues have reported that CASIN also helps push leukemia-initiating cells into the bloodstream, where they become much more vulnerable to chemotherapy. Cincinnati Children's is working to further develop this potential treatment, which could make stem cell transplants much more effective.

Meanwhile, in June 2012, Zheng and colleagues reported in *Chemistry & Biology* that in laboratory tests, a lead drug candidate dubbed "Rhosin" stopped breast cancer cells from spreading. The inhibitor targets RhoA, one component of a family of cell signaling proteins known as Rho-GTPases. These proteins help regulate cell movement and growth throughout the body. Rhosin is one of several projects using innovation funds from Cincinnati Children's to move promising therapies closer to human clinical trials.

Now, Zheng and colleagues are studying another lead compound, dubbed Y16, that appears to work in conjunction with Rhosin to further disrupt cancer cell signaling. New findings related to this research were published in the journal *Proceedings of the National Academy of Sciences (PNAS)* in February.

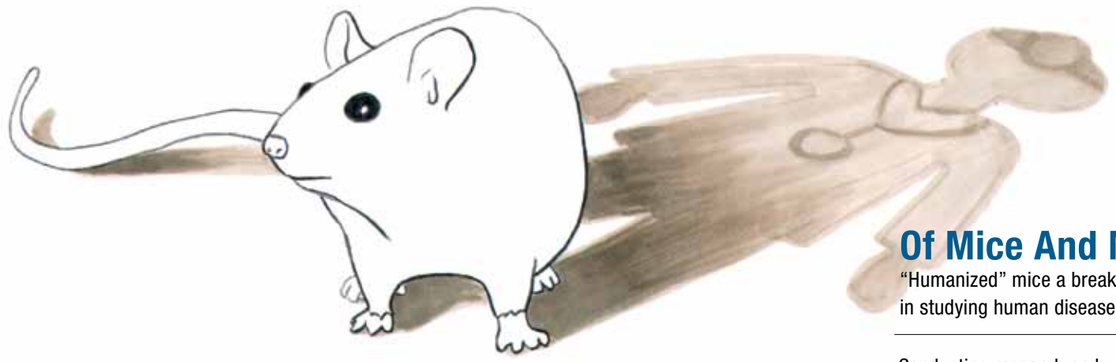
Despite the promise these treatments show, the challenge remains to find the funding to move these discoveries beyond the lab.

"Institutional support has become very important to move lead candidates toward commercialization. We are fortunate that Cincinnati Children's has the vision to provide such support," Zheng says. "Going from the lab to clinical trials requires enormous resources."

Zheng cautions that it will take several years for any of these drugs to reach market. He also predicts that none will be the single magic bullet that can wipe out cancer.

"This is a very dynamic and complicated process. Not all cancer stems cells are the same. Some are more potent than others, some are more sensitive to drugs than others, and by the time you find a promising treatment, the cancer mutates," Zheng says. "Now our goal is combination therapy. Instead of one magic bullet, we will need lots of different magic bullets." ■

How the humanized mouse has transformed research into human cancers



Of Mice And Men

"Humanized" mice a breakthrough in studying human disease

Conducting research on human diseases in animal models has long been a challenge for scientists. After all, there are differences between the two species that go way beyond ears and tails. So creating a mouse that more closely simulates the human condition has been a major breakthrough for scientists.

Humanized mice are bred to be immune deficient, which makes it possible for them to carry functioning human genes, tissues, even cancer tumors. These mouse models have become a major platform for analyzing the safety and effectiveness of potential new drugs.

James Mulloy, PhD, directs the Humanized Mouse Resource Core at Cincinnati Children's, which worked for more than two years to generate a colony of immune-deficient mice that remain strong enough to grow and develop while carrying human forms of leukemia.

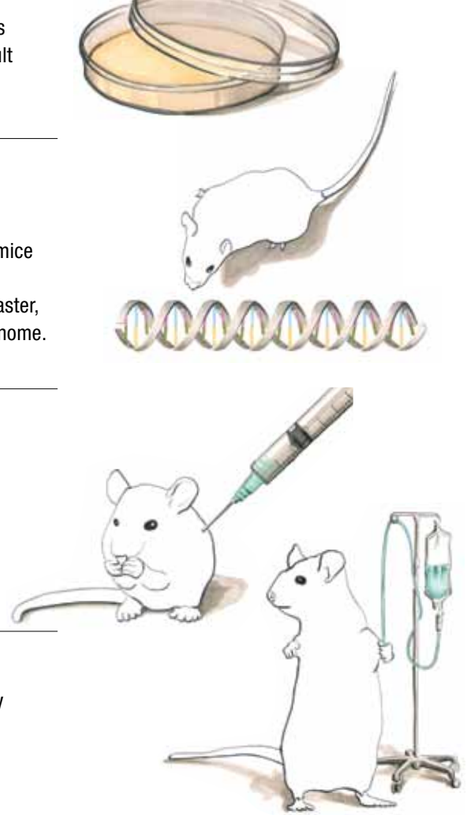
Most of the mice are used within Cincinnati Children's and the University of Cincinnati. Some are sold to researchers around the world. "Not a lot of centers can do what we are doing," Mulloy says.

In many types of cancer, human cancer cells do not grow well in culture, making it difficult to study what kills them.

Humanized mice are bred to lack immune responses that would reject human cells as foreign invaders. Some lines of humanized mice date back to the 1960s, but their uses have exploded with new technology that allows faster, more precise manipulation of the mouse genome.

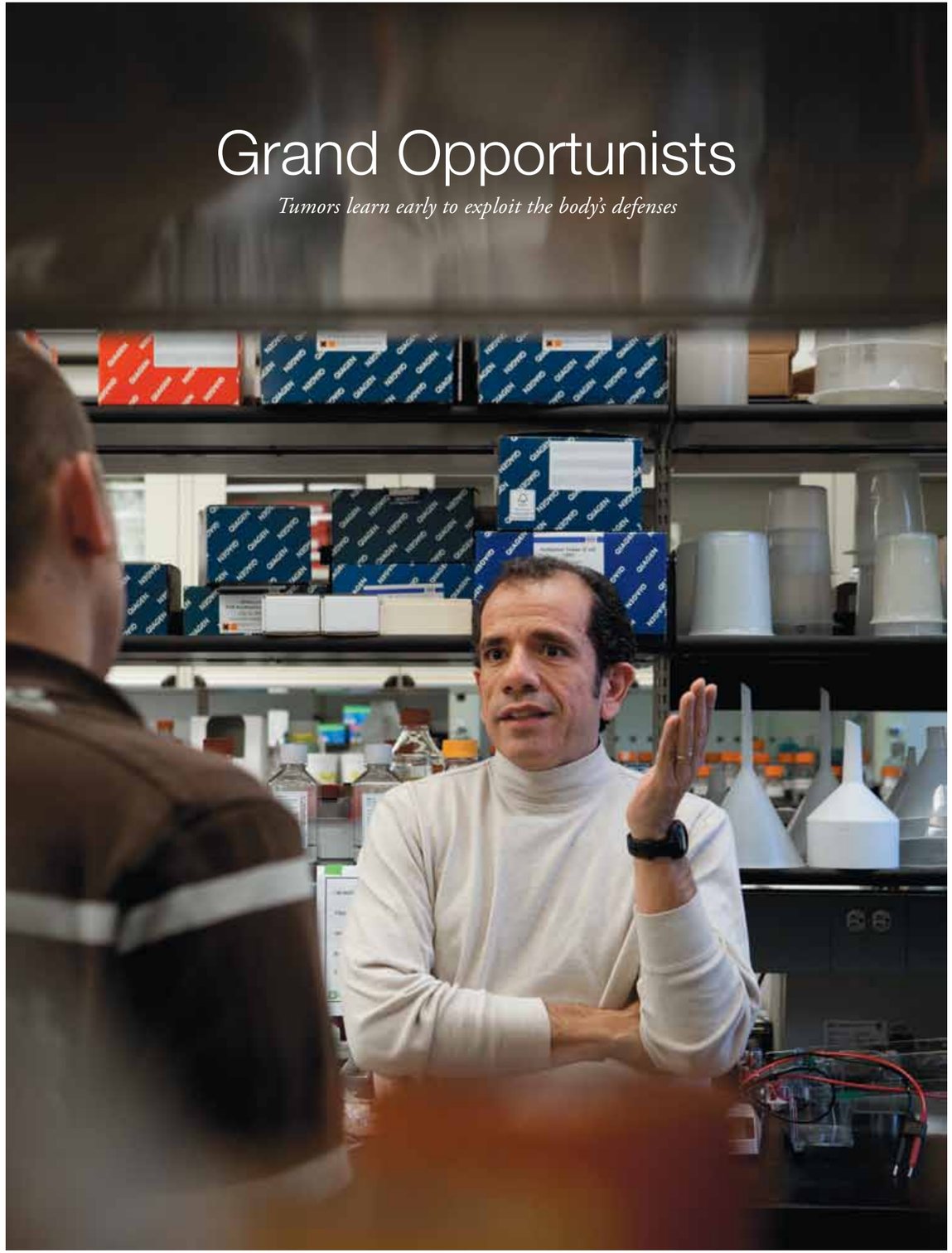
The mice receive cancer cells from a biobank of human tumor samples. As many as 70 percent of the cancer cell samples successfully engraft and expand.

Once the mice develop human cancers, they can receive experimental treatments.



Grand Opportunists

Tumors learn early to exploit the body's defenses



Joseph Palumbo, MD, has taken his research a few significant steps backward.

After years of studying what fuels the aggressive spread of tumors, says the cancer researcher, "We've wound the pathogenesis clock backwards."

So far backwards, in fact, that his latest findings seem to offer a way to prevent certain tumors from starting at all.

Palumbo, currently Acting Director of the Division of Hematology, has spent most of his career studying the role of the hemostatic system in cancer metastasis. These days, he is also looking at how the clotting system causes tumors to start in the first place.

Much of his work has been based on the pioneering studies of Harold Dvorak, who in the 1980s proposed that the tumor's environment is like a wound that does not heal.

"We used to think of tumors as just a collection of tumor cells," says Palumbo, "but they represent a pathologic organ. And like any organ, they have a blood supply and multiple supportive stromal cells. Dvorak noticed that the stromal elements in the tumor resembled stromal elements in a healing wound, and hypothesized that tumor cells could hijack this normal reparative system and use it to promote their own growth and survival."

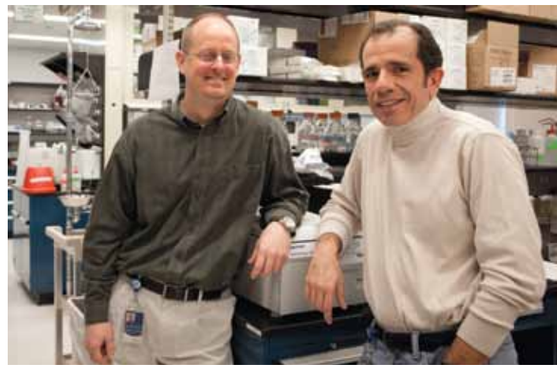
Although Palumbo saw the wisdom in Dvorak's hypothesis, he did not find evidence of a connection to the hemostatic system, a key system in wound repair, when he studied tumor growth using highly transformed transplantable tumor models. While these experimental systems were very informative for dissecting the mechanisms coupling hemostatic factorts to metastasis, an end stage in cancer progression, they were far less informative regarding early tumor development.

"By focusing on the later stages of tumor progression, we were missing key earlier steps in cancer pathogenesis – the evolution from a normal cell to a highly malignant cell," he says. "I started thinking, could it be that the hemostatic system has a role to play in tumor growth, but it's happening earlier in the process than we're studying? We're missing it because at the stage where the tumor is capable of metastatic spread, the tumor cells have already evolved to a point where they can generate everything needed to maintain a supportive stroma, including the ability to manipulate inflammatory cells in the tumor microenvironment."

FROM HEALTHY – TO HAYWIRE

So he returned to early tumor development, in search of the fine line where the body's healing mechanism goes haywire and becomes chronic inflammation.

Below, top: Palumbo with Dr. Matthew Flick. Drs. Flick and Jay Degen developed a mouse model that is protected from inflammation-driven cancer.
Bottom: Palumbo and his laboratory team.



“In the case of chronic inflammation, you have a pathologic setting where this normal reparative process is not being turned off and healing never occurs. You have a chronic problem that developing tumor cells can use to nurture their own growth and development.”

The Repair System

The body's normal repair system is an intricately designed process that starts the moment you, for example, slice into a finger while chopping an onion. The inflammatory response signals the hemostatic system to kick in. In order for your blood to produce a clot that will stem the bleeding and begin the healing process, it turns the blood-clotting protein prothrombin into thrombin, an enzyme that then converts fibrinogen to fibrin, which is crucial to the clotting process.

Normally, this cross-talk between the inflammatory and the hemostatic systems quiets down once healing is underway. But with tumors, it does not. Within the tumor, the processes continue unabated, providing malignant cells with a rich environment for growth.

“The first things that happen after a wound has occurred are the activation of the hemostatic and the inflammatory systems, both of which require tight regulation,” Palumbo says. “But in the case of chronic inflammation, you have a pathologic setting where this normal reparative process is not being turned off and healing never occurs. You have a chronic problem that developing tumor cells can use to nurture their own growth and development.”

This view is consistent with studies showing that chronic inflammation drives the development of multiple cancers, including cancers of the lung, pancreas and colon. Recent evidence has also shown that hemostatic factors are fundamental regulators of the inflammatory response in conditions that include bacterial infection and arthritis. Based on the dual importance of the clotting system in both cancer biology and regulation of inflammation, Palumbo reasoned that inflammation-driven cancers are likely to represent an important context where hemostatic factors play a role in early stages of tumor development and growth.

LOOKING FOR THE SOURCE

So he and his team began to study colitis-associated colon cancer in mouse models. They found what they believe is the culprit in one of the clotting and healing cascade’s starting lineup, the clotting protein prothrombin.

“If we genetically dialed down prothrombin by just 50 percent, we got a major decrease in the number of inflammation-driven adenomas that formed in the colon,” Palumbo says. “In fact, a significant portion of mice with low levels of prothrombin developed no tumors whatsoever. “

Less prothrombin, less cancer. But why?

To understand this, Palumbo’s team focused on one of prothrombin’s “downstream targets,” fibrinogen. They found that fibrinogen also played a role in inflammation driven colon cancer progression, but surprisingly this role had nothing do with clot formation.

STOPPING INFLAMMATION – AND CANCER

The discovery came about as a team effort. Fellow researchers Matthew Flick, PhD, and Jay Degen, PhD, had developed a mouse with a genetically modified fibrinogen. The mutant fibrinogen was designed so that it would clot normally, but would not bind with the leukocyte integrin receptor mac-1. Integrins regulate how a cell interacts with surrounding tissues; mac-1 is present on many immune cells and when it binds with fibrinogen, it drives inflammatory events. Disabling fibrin from binding with mac-1 stopped the inflammatory process.

“The mice were significantly protected from inflammation driven colon cancer. The majority developed no adenomas whatsoever,” says Palumbo. “And the adenomas that did form were smaller and less proliferative.”

Palumbo, who is also in the early stages of exploring the hemostatic system’s influence on prostate cancer, believes these findings have powerful potential for not just treating, but preventing inflammation-driven cancers.

“What we’ve found would suggest that therapies targeting hemostatic system components could possibly prevent cancer development in some individuals,” Palumbo says. “For example, if you had patients at high risk for colon cancer, targeting these interactions could prevent them from developing the disease. Given that inflammation is such a crucial factor in the development of numerous cancers, this paradigm is likely to be relevant beyond just colon cancer.“ ■

“The case involving this family gives us an excellent opportunity to study the biology of a potentially large repository of human cells, some in people who have the mutation but have not developed leukemia. It allows us to … study biological events that might convert a cell predisposed to leukemia into full blown leukemia.”

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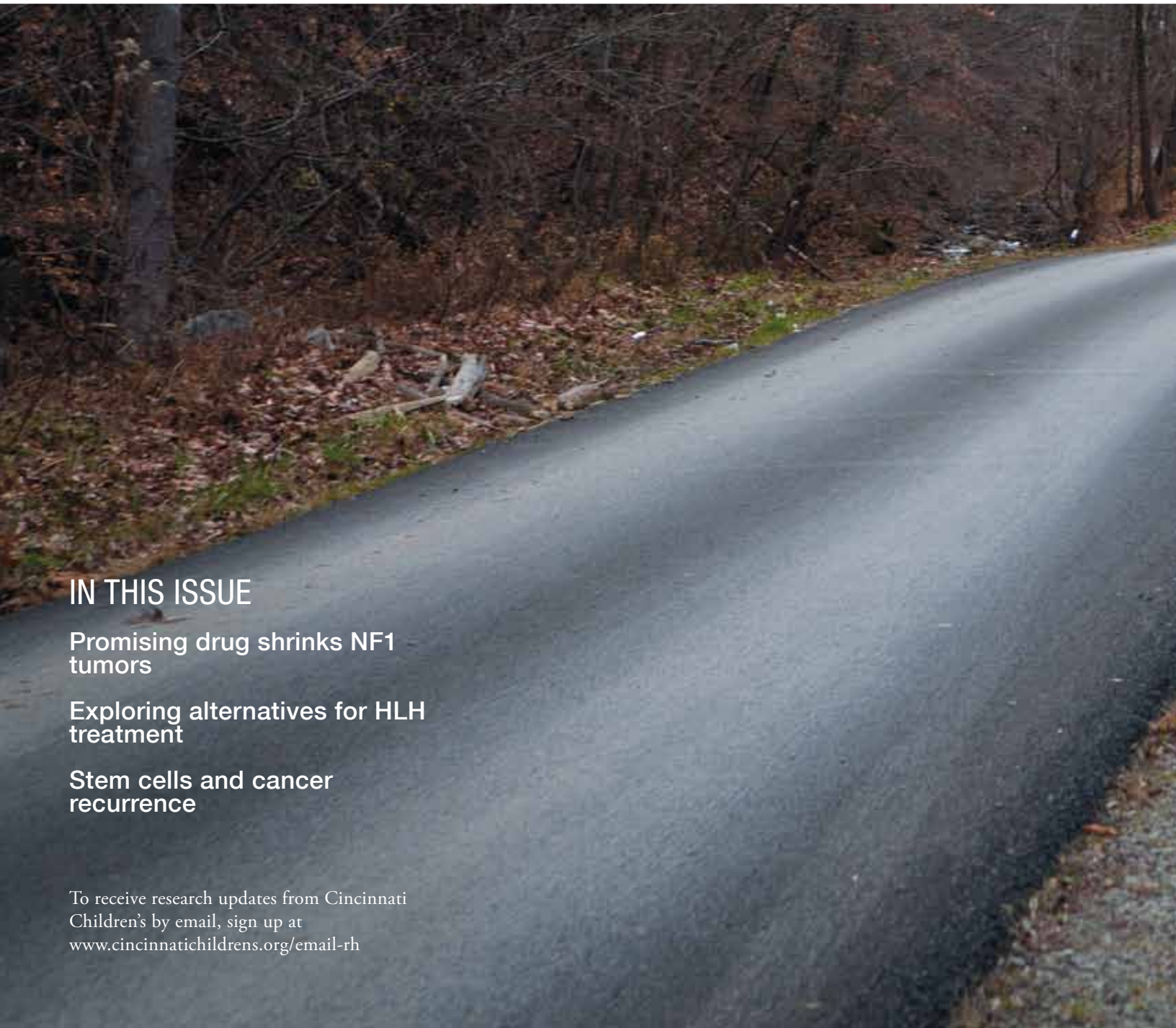

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