

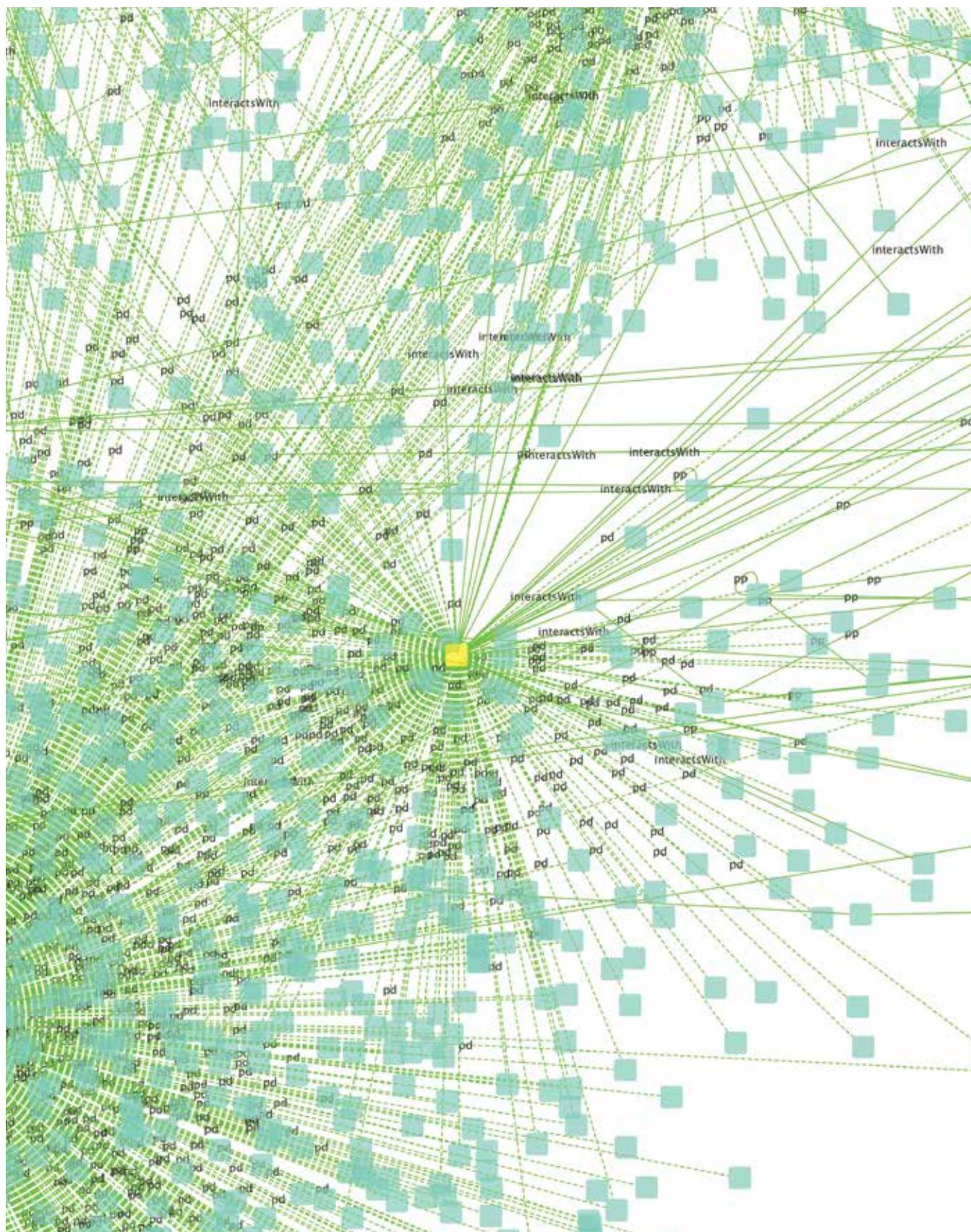
# RESEARCH HORIZONS

A Publication of the Cincinnati Children's Research Foundation

SPRING 2017



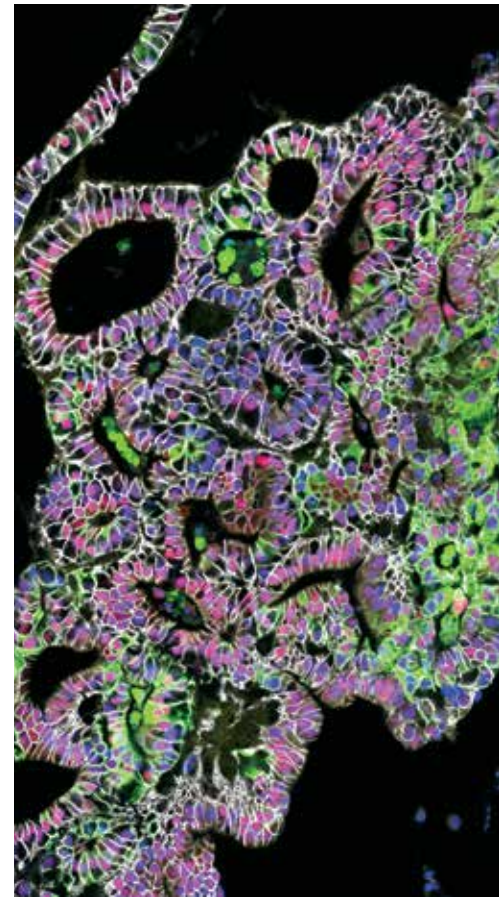
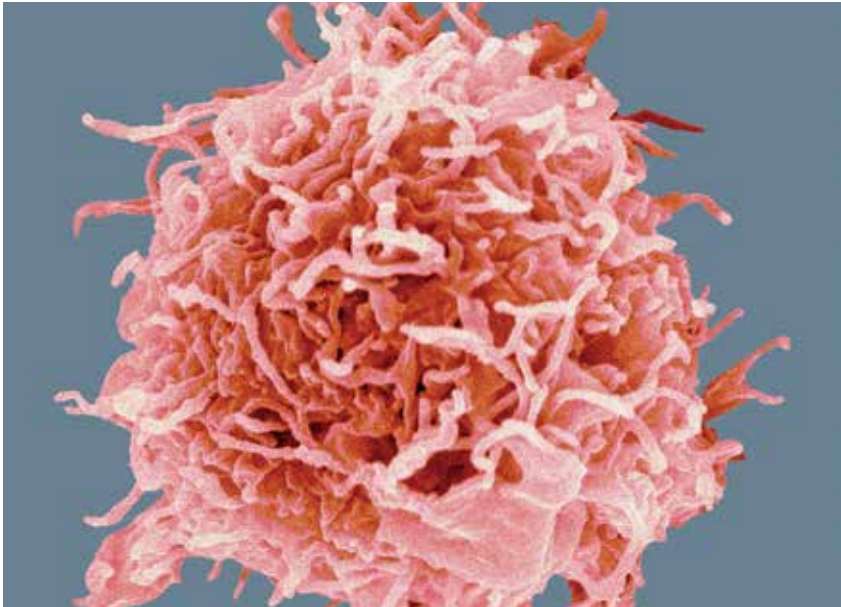




New faculty member Emily Miraldi, PhD, uses transcriptional regulatory network maps such as this, and other tools, to build mathematical models of immune cell behavior. Learn more about her work, [page 18](#).



# contents



## SPRING 2017 | Systems Vaccinology

### DEPARTMENTS

#### 02 New & Noteworthy

- >> Gene-Guided Therapy | Kumar
- >> Drug Shrinks NF1 | Ratner
- >> Leukemia Model Success | Mulloy
- >> ACHD | Veldtman
- >> Licensing Expands | Malik/Wells
- >> Organoid Part 2 | Wells/Helmrath
- >> Lung RNA Sequence | Whitsett
- >> MDS Findings | Starczynowski
- >> New Leader at CTC | Wooten

#### 28 Grants & Honors

- >> Awarded Procter Medallion | Robbins
- >> Named AAP President-Elect | Kraft

### FEATURES

#### 08 Infectious Diseases

- 08 Systems Vaccinology | Spearman & Singh
- 10 Immunity and Pregnancy | Schlaudecker and Way
- 14 New Vaccine Surveillance Network | Staat & Team

#### 16 Immunobiology

- 16 B Cell Factories | Singh & Weirauch
- 18 Attacking Infection by the Numbers | Miraldi
- 20 Blood Cell Study Stirs Debate | Grimes & Team

#### 22 The Microbiome

- 22 Fecal Metagenomics and Blood | Haslam
- 24 Battling the Dark Side of Antibiotics for Newborns | Deshmukh
- 26 Welcome to the Microbiome World | Alenghat

### About the Covers

The computer artwork on the front cover shows large plasma cells, a type of white blood cell, secreting small white antibodies. The colorized scanning electron micrograph on the back cover shows a B cell, another type of white blood cell involved in the programming of plasma cells. Scientists at Cincinnati Children's are breaking ground in understanding how the body produces the high-affinity B cells it needs to fight off dangerous infections. Read more about the research on Page 16.

# Gene-Guided Therapy Defeats Some Resistant Histiocytoses

In another advance for personalized medicine, physicians at Cincinnati Children's report using genomic profiling to reverse the course of disease for three children and one adult who were battling treatment-resistant histiocytoses.

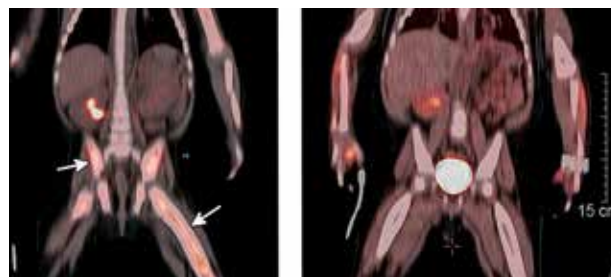
These cancerous blood syndromes cause abnormal accumulations of white blood cells that form potentially lethal tumors on vital organs. Current forms of chemotherapy work in about 50 percent of cases, but in other patients the tumors become resistant.

In a study led by Ashish Kumar, MD, PhD, researchers analyzed tumor biopsies for 72 patients with resistant disease. In 26 cases, the team detected mutations involving either the BRAF or MAP2K1 genes. This finding suggested that some patients could benefit from drugs that block the MAP-kinase cancer pathway.

Kumar and colleagues report dramatic improvements in responses to off-label uses of the approved cancer drugs dabrafenib or trametinib. Details were published Feb. 9, 2017, in the *Journal of Clinical Investigation Insight*.

Within two weeks, treatment eliminated tumors and seizures affecting a 36-year-old woman with Langerhans cell histiocytosis. Meanwhile, three infants who had endured several unsuccessful rounds of chemotherapy "are thriving now on one oral medication that put their disease into remission."

The study authors say their findings support conducting genomic profiles in all histiocytosis cases. They also suggest launching a larger clinical trial of MEK inhibitors as a potential treatment for these disorders.



These PET-CT images show disease activity in a 10-month-old child with Langerhans cell histiocytosis (LCH). The image on the right shows a decrease in activity three months after treatment with dabrafenib. Ashish Kumar, MD, PhD, directs the Langerhans Cell Histiocytosis Center at Cincinnati Children's.

## Drug Shrinks Painful NF1 Tumors

A team led by cancer biologist Nancy Ratner, PhD, reported Dec. 29, 2016, in *The New England Journal of Medicine* that selumetinib shrinks tumors in children with plexiform neurofibromas caused by the genetic disorder neurofibromatosis type 1 (NF1).

The phase 1 clinical trial of 24 children showed that the patients tolerated the drug well and, in most cases, their tumors shrank. The trial was led by Brigitte Widemann, MD, of the National Cancer Institute's (NCI) Pediatric Oncology Branch. Ratner was study co-investigator.

Ratner and her team in our Cancer and Blood Diseases Institute have studied NF1 for decades. They first reported in 2013 that blocking MEK—a critical protein in the NF1 molecular process—was effective at shrinking plexiform neurofibromas in mice. Selumetinib is an MEK inhibitor.

"Having a mouse model that predicts treatment response in humans is rare," says Ratner, "and may identify even more effective therapies."



Nancy Ratner, PhD



Jeffrey Whitsett, MD

## Cracking the Code on Lung Disease

Researchers from Cincinnati Children's and Cedars-Sinai Medical Center have deciphered the genetic code of cells involved in healthy and diseased human lungs.

After conducting what they believe to be the first single-cell RNA sequence analysis of normal and diseased human lung tissues, the scientists reported their findings Dec. 8, 2016, in the *Journal of Clinical Investigation Insights*.

Their analysis provides a genetic blueprint of cell types involved in lung disease and a window to processes driving inflammation and fibrosis. The work will help find molecular targets for diagnosing and treating childhood lung diseases, as well as adult lung diseases such as Idiopathic Pulmonary Fibrosis (IPF).

"This paper identifies a number of novel targets and molecular pathways for IPF, for which there are pharmaceutical approaches," says Jeffrey Whitsett, MD, lead investigator and Co-Director of the Perinatal Institute at Cincinnati Children's.

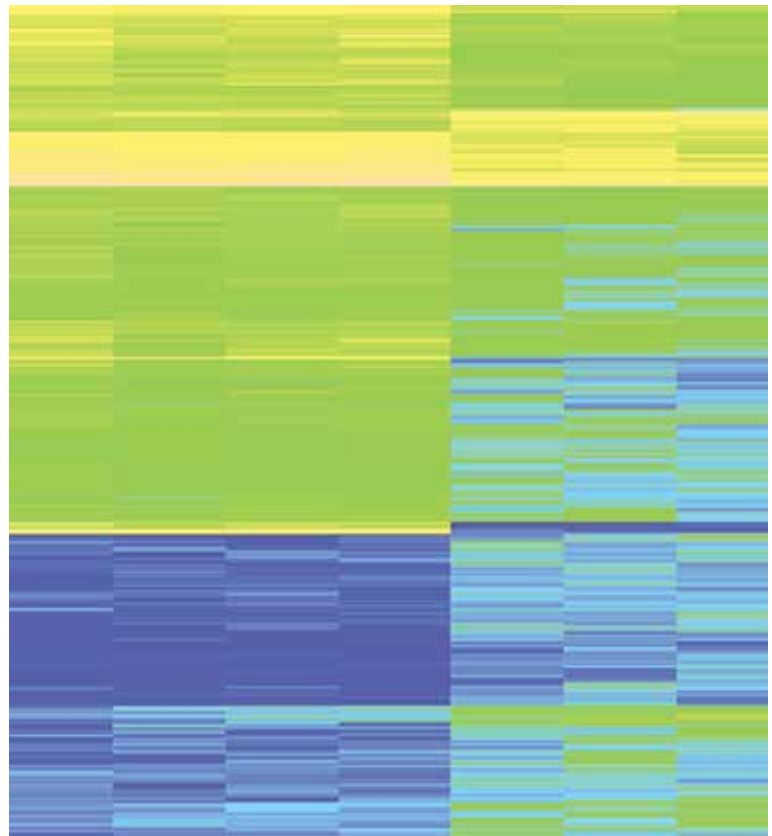
## Study Finds Cause of Deadly Blood Cell Disorders

Researchers here have identified a mechanism that controls blood-cell function, and several possible molecular treatments for myelodysplastic syndromes (MDS). These syndromes are malignant disorders in which bone marrow does not produce enough healthy blood cells. MDS can lead to acute myeloid leukemia.

The findings were reported online Dec. 26, 2016, in *Nature Immunology*. A team led by cancer biologist Daniel Starczynowski, PhD, found that overexpression of the protein TRAF6 in blood cells can result in MDS. TRAF6 is an immune sensor of pathogens.

"We found that TRAF6 overexpression in murine hematopoietic stem cells results in impaired blood cell formation and bone marrow failure," says Starczynowski, a member of the Division of Experimental Hematology and Cancer Biology.

Starczynowski and colleagues also identified therapeutic approaches that can be tested against TRAF6 and other proteins responsible for MDS.



This heat map shows exon-level microarray expression analysis of bone marrow cells from 6-month-old wild type mice and Vav-TRAF6 mice.

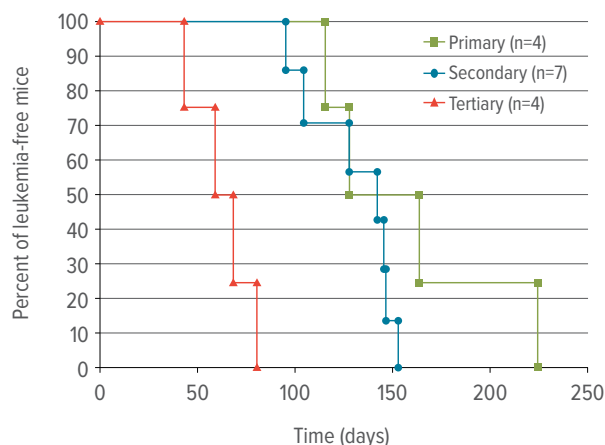


# Model for Infant Leukemia a Success

Researchers from the University of Chicago and Cincinnati Children's have created the first mouse model for a deadly form of infant leukemia. Their achievement was published Nov. 14, 2016, in *Cancer Cell*.

The model replicates a genetic flaw—MLL-AF4 fusion—that results in pro-B acute lymphoblastic leukemia (ALL), responsible for nearly 70 percent of infant and 10 percent of childhood and adult ALL. People with the disease produce vast numbers of dysfunctional blood cells. Its prognosis is among the worst for any acute leukemia.

Michael Thirman, MD, University of Chicago, has long studied the disease, but was unsuccessful creating a mouse model that mimicked it. James Mulloy, PhD, and Shan Lin, both of our Division of Experimental Hematology and Cancer Biology, worked with Thirman to finally produce a mouse model with leukemia identical to humans—an accomplishment crucial to developing and testing new drug therapies.



Survival curves for mice receiving MLL-Af4 transduced human CD34+ cells

# Accreditation Awarded to Program for Adults with Congenital Heart Defects



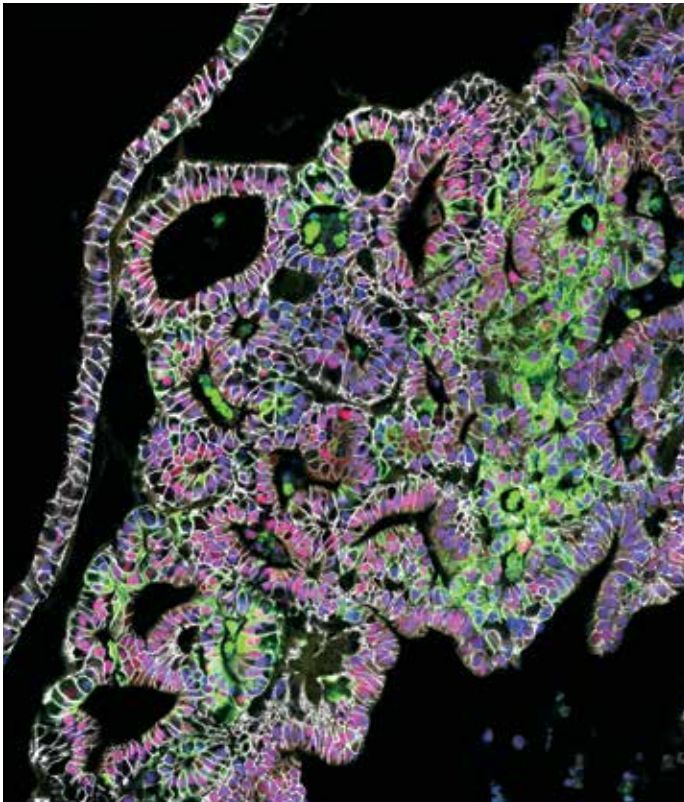
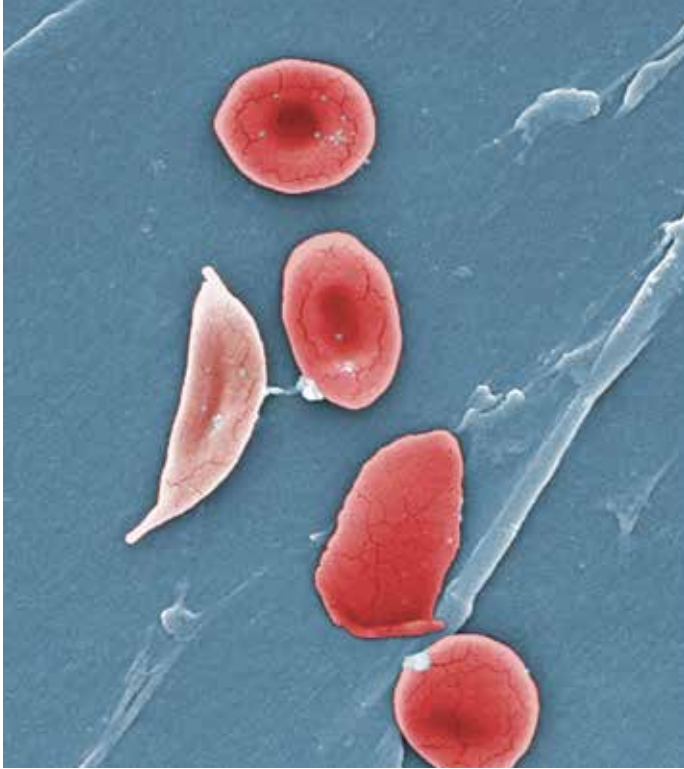
Gruschen Veldtman, MD

More than 1 million American adults now live with congenital heart defects. Half should be seeing a congenital heart expert regularly, but only 10 to 20 percent do so. The Adolescent and Adult Congenital Heart Disease (ACHD) program at Cincinnati Children's recently was accredited by the Adult Congenital Heart Association to provide this specialized care.

"Patients treated for congenital heart disease need to remain under the care of specialists trained in congenital heart disease, to ensure that problems are addressed quickly and effectively," says Gruschen Veldtman, MD, Director of the ACHD program at Cincinnati Children's.

The ACHD program is one of only a few in the nation, and will be a regional, cooperative endeavor, says Andrew Redington, MD, Executive Co-Director of the Heart Institute. "We will collaborate with all the adult care systems in the region, and are particularly grateful to work with colleagues at the University of Cincinnati Medical Center."

# Licensing Expands Breakthrough Discoveries



Cincinnati Children's has signed licensing agreements with two companies to advance promising technologies developed by our researchers.

## **Sickle cell gene therapy**

An agreement with gene therapy company Calimmune, Inc., will commercialize sickle cell gene therapy discovered by Punam Malik, MD, Director of the Cincinnati Comprehensive Sickle Cell Program.

Calimmune will combine Malik's proprietary gamma-globulin gene therapy, proven to have anti-sickling properties, with the company's Select+™ technology, which improves grafting of stem cells in the bone marrow. After the genetically modified stem cells are given back to patients, Select+™ helps increase the modified cells in the patient's system.

"Our current clinical vector has been optimized to efficiently correct hematopoietic stem cells in patients suffering from sickle cell disease," says Malik. "This collaboration allows us to create long-term or permanent solutions for patients with these diseases, who face shortened life expectancy and reduced quality of life."

## **Organoid technology**

On another front, STEMCELL Technologies Inc. will broaden the availability of technology for generating gastrointestinal organoids from pluripotent stem cells (PSCs).

Our agreement grants STEMCELL a license to use methods developed by our Pluripotent Stem Cell Core and the laboratory of James Wells, PhD, Division of Developmental Biology. The technology enables scientists to create organoids, three dimensional cell "mini organs," from PSCs in their own laboratories.

"There is a tremendous opportunity to use these new organoid models to advance studies in human development, as well as for applying them in disease modeling, drug screening, and for developing therapeutics," says Wells.

# Stem Cell-Generated Tissue Promises Health Breakthroughs

Scientists at Cincinnati Children's have had two recent breakthroughs using pluripotent stem cells to generate human tissues.

## **Stomach organoid progress**

In a Jan. 4, 2017, online report in *Nature*, researchers describe growing tissue in vitro that replicates the corpus/fundus region of the stomach. This comes just two years after the same team generated tissue that mimics the stomach's antrum region.

Investigators now can grow both parts of the stomach to study disease, potential treatments, and to understand health in ways never before possible, says James Wells, PhD, principal investigator and Director of our Pluripotent Stem Cell Facility. "Now that we can grow both antral- and corpus/fundic-type human gastric mini-organs, it's possible to study how these tissues interact physiologically, respond differently to infection and injury, and react to pharmacologic treatments," he says.

## **Intestine organoid gets nerves**

Scientists here also reported online Nov. 21, 2016, in *Nature Medicine* that they grew human intestinal tissue with functioning nerves to recreate and study the intestinal nerve disorder Hirschsprung's disease.

Their findings describe an unprecedented approach to engineering and studying tissues in the intestine, and move science closer to using human pluripotent stem cells for regenerative medicine.

"One day this technology will allow us to grow a section of healthy intestine for transplant into a patient, but the ability to use it now to test and ask countless new questions will help human health to the greatest extent," said Michael Helmrath, MD, co-lead investigator and Surgical Director of the Intestinal Rehabilitation Program.



James Wells, PhD, (top) led a *Nature* study reporting success developing a more advanced stomach organoid. Michael Helmrath, MD, was co-leader of a project that added functional nerves to intestinal organoids (bottom).



# Quality Initiative Significantly Improves Chronic Conditions

An estimated 20 percent of children in the U.S. suffer from a chronic condition, yet only half receive the recommended care.

By redesigning the way it cares for children with active chronic conditions, Cincinnati Children's successfully improved outcomes for half of the 27,221 patients involved in its Chronic Care Model from 2012-15.

The study, published in the March issue of *The Joint Commission Journal on Quality and Patient Safety*, was led by Jennifer Lail, MD, of the James M. Anderson Center for Health Systems Excellence.

Cincinnati Children's implemented a plan to help specialized clinical teams apply quality improvement principles, with each team focusing on specific chronic conditions, including juvenile arthritis, asthma, chronic kidney disease, food allergy, cardiomyopathy (heart muscle dysfunction) and sickle cell disease.

We developed condition-specific patient registries and data collection tools, classifying patients into defined risk groups, planning and coordinating care before- and after-visits, and providing self-management and caregiver/parent support for patients and their families.



Jennifer Lail, MD



Andrew Wooten, MS, MBA

## New Leader for Technology Commercialization

Andrew Wooten, MS, MBA, has been named Vice President of the Center for Technology Commercialization (CTC) at Cincinnati Children's. His role is to identify medical center discoveries with promising commercial potential and help them advance to the marketplace.

Wooten formerly served as founding Executive Director of the Innovation Development Center at Baylor University. He has held positions in industry, academia, and as a biotechnology entrepreneur. He has a BA in chemistry from Berea College, an MS in biotechnology from the University of Georgia, and an MBA from Mercer University.

"I'm excited to join a world-class clinical and research institution with a rich history of transformational commercial successes," Wooten says. "My hope is to build on a great foundation and elevate Cincinnati Children's pediatric innovation programs to even greater levels of success as a leader in improving child health."

# To Build Better Vaccines

Infectious Diseases, Immunobiology forge partnership to apply systems biology concepts to vaccine development

by Tim Bonfield

Cincinnati Children's has a rich history of leadership in the world of vaccine development. Roots reach back to the world-famous polio vaccine developed by Albert Sabin, MD, in the late 1950s. Beginning in the late 1980s, research led by David Bernstein, MD, MA, and Richard Ward, PhD, produced the life-saving rotavirus vaccine (Rotarix), approved for use in the U.S. in 2008.

The work to improve existing vaccines and develop new ones continues. Cincinnati Children's serves as one of nine national Vaccine Treatment and Evaluation Units. Researchers here are making strides in vaccine research to combat norovirus, cytomegalovirus, respiratory syncytial virus and other dangerous diseases.

Now, Paul Spearman, MD, the new Director of Infectious Diseases at Cincinnati Children's, and Harinder Singh, PhD, who

became Director of Immunobiology here in 2013, are working to take vaccine development to new levels.

## BRINGING 'OMICS TO RESEARCH

"We plan to jointly appoint key recruits to work with existing faculty to build up our strength in the arena of systems vaccinology," Spearman says. "We will be applying the tools and techniques of systems biology to advancing knowledge about vaccines and vaccine development."

Their goal: to further integrate expertise in genomics, transcriptomics, lipidomics, metabolomics and other emerging fields into the established vaccine world of virologists, immunologists and infectious disease specialists.

"Partly, this is about expanding basic discovery," Spearman says. "But we also are pursuing a translational focus. We seek to develop new vaccines against new pathogens and we are applying systems vaccinology approaches to make existing vaccines more effective."

Singh agrees that vaccine research is a natural shared mission for their teams.

"Many advances have been made in vaccine development, yet in other ways our knowledge remains limited," Singh says. "We know that in some people, vaccines induce good responses. Their bodies produce adequate supplies of antibodies that work effectively against the targeted pathogen. However, in others, the same vaccine can elicit a poor response. We still do not know enough about why this variation occurs."

Singh has devoted years to studying the inner workings of B cells, the antibody-producing element of the immune system. Now, Singh and colleagues are discovering more about precisely







how B cells produce the high-affinity antibodies the body needs to defeat invading pathogens (See story page 16).

This work is just one example of how scientists here are using emerging technologies such as single-cell RNA sequencing to open new doors for vaccine development. More projects lie ahead as the collaborative effort grows.

#### A GROWING TEAM

Spearman, a long-time expert in HIV vaccine research, brought five scientists with him from his previous post at Emory University. They include assistant professors Jason Hammonds, PhD, Jaang-Jiun Wang, PhD, and Karnail Singh, PhD. Together they represent a significant increase for Cincinnati Children's in expertise in HIV biology, HIV vaccine development, and design of virus-like particle vaccines.

Meanwhile, recruiting continues for several more positions.

Emily Miraldi, PhD, an expert in computational biology, joined Cincinnati Children's in January (See story page 18.) Another four open faculty positions across the two divisions remain to be filled.

"We're excited about the potential steps forward in systems vaccinology that these new recruits will bring," Spearman says.

#### WORKSHOP PLANNED

As systems biology wields growing influence on infectious disease and immune system research, many scientists are seeking more information. To that end, Cincinnati Children's has invited several influential investigators to speak at a systems immunology workshop, to be held Sept. 21-22. (For more details, see page 32.) ■

## 6 THINGS TO KNOW ABOUT THE NEW DIRECTOR

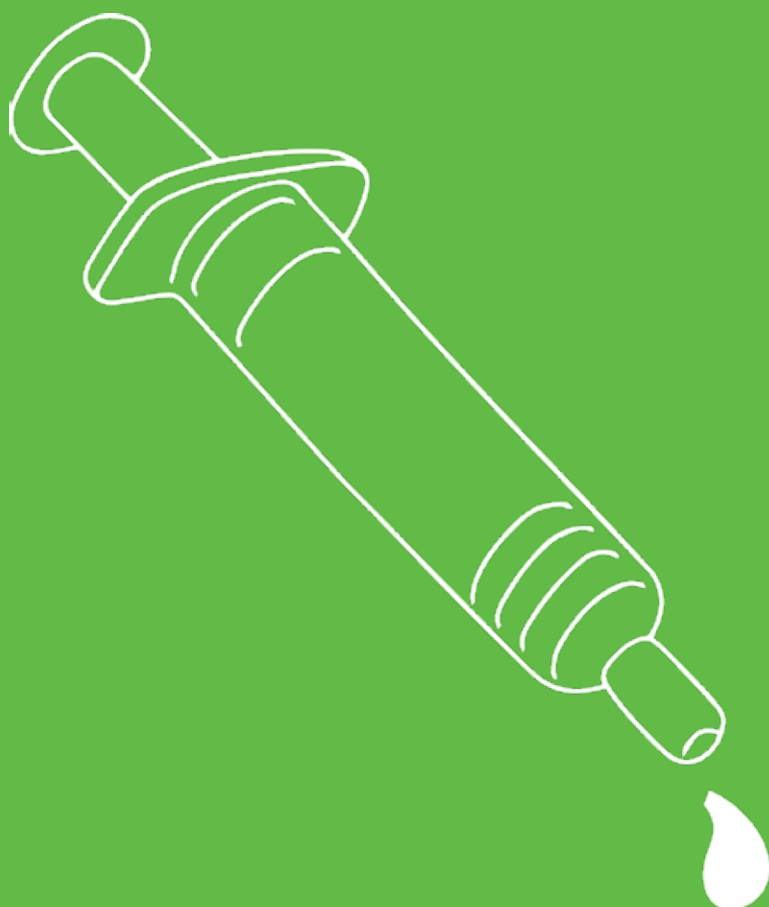
- Paul Spearman, MD, joined Cincinnati Children's in September 2016 and became the Albert B. Sabin Professor and Director of Infectious Diseases.
- Previously, Spearman directed the infectious diseases division at Emory University for 11 years, where he played a leading role in a project to expand pediatric research. He also co-led the Vaccine and Treatment Evaluation Unit (VTEU) there.
- His laboratory studies fundamental aspects of HIV biology, including HIV assembly processes. His recent vaccine research includes early development of a next-generation mucosal HIV vaccine based on parainfluenza virus type 5 (PIV5) priming and virus-like particle boosting.
- Spearman is President-elect of the Pediatric Infectious Diseases Society. He also serves on the Board of Scientific Counselors for NCI.
- Beyond the lab, Spearman continues his clinical practice in infectious diseases.
- Spearman has several personal connections to this region. He was born in Kentucky and raised in Kent, Ohio. He trained in internal medicine and pediatrics at Ohio State, together with his wife, Becky.



# Staat and the 'Fab 5' Give Vaccine Research Their Best Shot

CDC-funded New Vaccine Surveillance Network and a cohort study track vaccine effectiveness and babies' health

by Tom O'Neill



**T**itus White was working the room pretty well. He is a charmer, wise well beyond his eight weeks. The doctors were enamored. Moments later, he got his first dose of the rotavirus vaccine.

Titus doesn't know it yet, but he will provide important insights into the interwoven nature of health and vaccination. Both are rooted in the ongoing success stories of the Division of Infectious Disease's Epidemiology and Surveillance Program. It includes Immunization Safety, the New Vaccine Surveillance Network and a separate cohort study at Cincinnati Children's called PREVAIL. That's short for the Pediatric Respiratory & Enteric Virus Acquisition and Immunogenesis Longitudinal Cohort, which focuses on the first two years of a baby's life.

Both projects—a combined \$9.6 million—are funded by the Centers for Disease Control and Prevention (CDC). Call it high-level research meets ground-level care.

## HEALTHY CHILDREN, ONE PIECE OF THE PUZZLE AT A TIME

Last year, researcher Mary Allen Staat, MD, MPH, of the Division of Infectious Diseases, received a five-year, CDC grant for enhanced surveillance of new, vaccine-preventable diseases, as one of seven network sites across the U.S.

From clinics to emergency departments at Cincinnati Children's, Staat's team enrolls children seen for acute gastroenteritis or acute respiratory infection, and then they collect stool, blood and respiratory samples.

The goal is to match up the children's health with their vaccine histories, looking for insights into particular age-groups and risk factors for disease. Among current vaccines, Staat focuses on rotavirus and influenza, while gaining a better understanding of the epidemiology of norovirus and respiratory syncytial virus, with plans for potential vaccines and treatments in the future.



This approach has transformed not only the study of vaccine success rates for children treated for acute gastroenteritis and respiratory infections, but also how data is collected and shared. Cincinnati Children's has the most ambitious data-collection system among the network's seven hospitals.

"We want to fully understand the epidemiology as well as the innate factors in babies that make them susceptible to gastroenteritis and acute respiratory infections," Staat says. "I look at us as an important piece of the puzzle in vaccine research."

Back at the clinic, Titus' mother cradled him as he got his vaccine. One of the licensed rotavirus vaccines in the U.S. was developed and tested by Cincinnati Children's researchers a decade ago and has since spared millions of children worldwide from severe diarrhea and dehydration, which can be fatal.

#### STATISTICALLY SIGNIFICANT PROTECTION

In a study published in *Clinical Infectious Diseases* in December 2015, the vaccine surveillance network collected stool samples from 3,865 children who had been hospitalized or were treated at an emergency department for acute gastroenteritis during the 2012 and 2013 rotavirus seasons.

Of those children, 502 (13 percent) had rotavirus. But among the larger group as a whole, researchers found in their vaccine histories that the two existing rotavirus vaccines, RV5 and RV1, provided "statistically significant, genotype-specific protection."

The network enrolls about 1,000 children each year. All are local residents of their respective hospitals. Another type of resident provides some perspective here: medical residents.

"To think," says Staat, "we used to have hundreds of children in the hospital

and ED with dehydrating diarrhea and vomiting due to rotavirus. We now have residents who have never seen kids with rotavirus."

Staat recalled that as a trainee herself in 1987, she was so optimistic to hear from researchers that a vaccine was "just around the corner."

It wasn't. It took nearly two decades to study and license.

"So it's very gratifying," she says, "to see a vaccine to completion and then study its effectiveness over time."

Staat has nicknamed her core team the "Fab 5," in part because it's an all-women research group, but also because they reflect a wide range of scientific backgrounds.

Staat focuses on rotavirus epidemiology of infectious diseases. Elizabeth Schlaudecker, MD, MPH, also of the Division of Infectious Diseases, is devoted to the science of infant infection and maternal immunization.

Ardythe Morrow, PhD, of the Global Health Center, is Director of the Center for Interdisciplinary Research in Human Milk and Lactation and will lead the PREVAIL cohort study. Monica McNeal, MS, is a virologist in the Division of Infectious Diseases and her team processes and tests the thousands of specimens collected.

And Emily DeFranco, DO, works on maternal fetal medicine in the University of Cincinnati's Department of Obstetrics and Gynecology. She will lead the efforts to enroll mothers and infants for PREVAIL.

#### THE SCIENTIFIC BEAUTY OF UNOCCUPIED OFFICES

In addition to the Fab 5, about 20 of Staat's staff at Cincinnati Children's contribute to the surveillance network and the PREVAIL cohort study. From behind her desk, Staat motions toward her door.

"Those offices over there without staff in them, they're out enrolling people," she says. "They come in at 7 am to enroll kids



The Fab 5 (from left): Elizabeth Schlaudecker, MD, MPH; Mary Allen Staat, MD, MPH; Emily DeFranco, DO; Ardythe Morrow, PhD; and Monica McNeal, MS.

who've been admitted. We have an evening crew too. It's very labor-intensive."

Staat says she's heartened, but also somewhat surprised, that the CDC continues to fund programs that evaluate vaccines' effectiveness after they have gained FDA approval. But she emphasizes their importance because vaccine strains can change over time, so they help researchers better understand the factors associated with infection.

Staat was among the authors of another 2015 study, published in *JAMA Pediatrics*, which showed that severe rotavirus gastroenteritis was virtually absent among U.S. children who had a genetic polymorphism that inactivates FUT2 expression on the intestinal epithelium.

After controlling for vaccination and other factors, children with the non-secretor FUT2 polymorphism appeared

statistically protected. "If you were a secretor, you were more likely to be infected," Staat says.

That was a significant revelation. One co-author of that study, Morrow, is now leading the cohort study PREVAIL, funded by an additional \$3.6 million CDC grant in 2016.

It is designed to understand the natural history of infection with common pathogens in the first two years of a baby's life. Cincinnati Children's was the lone hospital to receive funding for PREVAIL, in which the center will enroll 240 mothers and infants and follow them for two years.

#### THE INTERSECTION OF 'OOHS,' 'AHHS' AND 'OLIGOSACCHARIDES'

Research staff makes home visits to teach families how to collect nasal and stool

samples and do temperature readings of their babies. They also collect milk from breastfeeding mothers.

The impact of breast milk on developing autoimmune systems has been the focus of Morrow's research for decades.

"Human milk has a major role in protection against respiratory and gastrointestinal infections of infancy," she says. "Protection involves both acquired and innate immune systems, including maternal antibodies, complement, innate defense proteins, and oligosaccharides."

Titus the charmer doesn't know big words like "oligosaccharides," but he did get a lot of fawning "oohs" and "ahhs" from the Fab 5 when they gathered in the sun-drenched lobby of the Clinical Sciences Pavilion here.

Titus and his 5-year-old sister, Alyssia, are sure to be long-time contributors to

## About the New Vaccine Surveillance Network

### ESTABLISHED

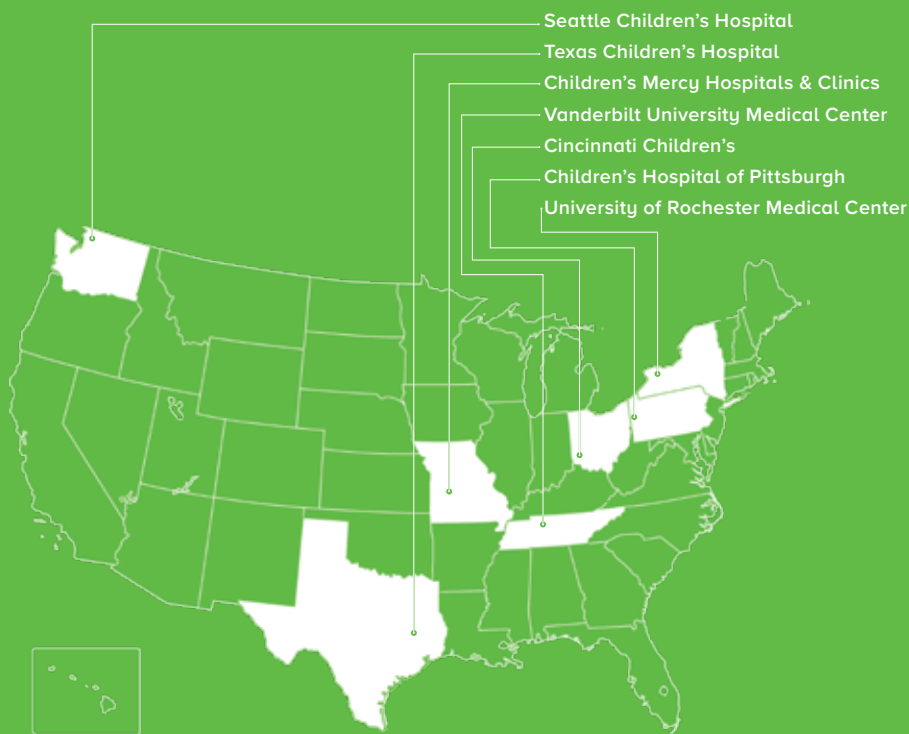
In 2002 by the National Centers for Disease Control and Prevention (CDC)

### CHARTER MEMBERS

Cincinnati Children's, Vanderbilt University and the University of Rochester

### MISSION

To evaluate the impact of new vaccines and vaccine policies





vaccine science and the ongoing work of the Fab 5.

Their mom, Megan White, will make sure of that.

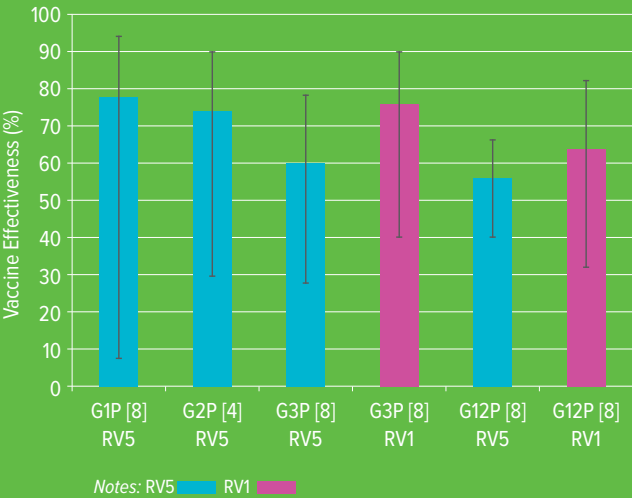
“To me it is important to be a part of Dr. Staat’s research because we are living in a time when parents are refusing to give their children vaccinations,” she explains. “There are always constant changes in viruses and making sure that the vaccine is still effective and safe puts a lot of parents at ease.”

Wisdom, not just as a mom, but as someone who works in Outpatient Registration at Cincinnati Children’s. ■

Megan White, with her son, Titus, the second of her children to be enrolled in research studies on vaccinations.



## About the Rotavirus Vaccine’s Efficacy



SECRETOR STATUS AMONG ROTAVIRUS VACCINATED PATIENTS WITH ACUTE GASTROENTERITIS

Patients	No. (%)		P Value
	Secretor	Nonsecretor	
Rotavirus positive (n = 110)	109 (99)	1 (1)	< .001
Rotavirus negative (n = 1104)	905 (82)	199 (18)	

In the figure at left, from a 2015 study published in *Clinical Infectious Diseases*, researchers analyzed stool samples from 3,865 children treated for acute gastroenteritis. They found that the two existing rotavirus vaccines, RV5 and RV1, provided “statistically significant, genotype-specific protection.”

The figure at right, from a 2015 study in *JAMA Pediatrics*, shows that severe rotavirus gastroenteritis was virtually absent in children who had a genetic polymorphism that inactivates FUT2 expression on the intestinal epithelium, suggesting that the polymorphism plays a key role in protecting children from the virus.

# For Flu Shots During Pregnancy, Earlier Appears to be Better

New research reveals that influenza vaccine follows a consistent direction, but an uneven path, as it affects the immune systems of pregnant women and their developing babies

by Tom O'Neill

The medical community widely agrees that pregnant women should receive flu vaccinations, no matter how far along the pregnancy may be. The shots can prevent dangerous complications for women during pregnancy, reduce the risk of preterm birth, and even protect newborns from illness for months after birth.

Now, after learning more about the complex ways that pregnant women and fetuses react and counter-react to each other, two researchers at Cincinnati Children's are beginning to believe that the earlier that vaccines can be provided, the better.

Emerging research led by Elizabeth Schlaudecker, MD, MPH, and Sing Sing Way, MD, PhD, both of the Division of Infectious Diseases, takes a deeper look at how the immune systems of pregnant women and fetuses reacted to the H1N1 flu vaccine.

These investigations found that serological responsiveness to influenza vaccination in the mother declined with the progression of pregnancy. The declines were consistent, but did not follow a neat, incremental pattern. They did not track with simple trimester delineations, the usual method of distinguishing pregnancy-related changes.

## A MATTER OF TIMING

"It's not the flu itself," Way says, "It's the immunological progressions that happen in the mother."

Way has studied for years how pregnancy changes the mother's own immune system, so that her body will not attack the fetus as if it were a foreign invader.

This work has helped explain why pregnant women are more likely than non-pregnant women to suffer dangerous complications from flu infections.

Schlaudecker's work explores how these concepts play out when using the H1N1 flu vaccine. Early results confirm that pregnancy-induced immunological changes in the mother are not defined by trimesters: vaccine responses are likely to be more similar between weeks 12 and 13 than between weeks 13 and 27, even though weeks 12 and 13 are in different trimesters. In fact, titers of antigen-specific IgG1 decreased by approximately 7 percent each week as pregnancy progressed to term.

"Conceptually, that makes sense," says Way. "But no one until now has gone to the lengths Liz has in this paper to show that."

Based on these findings, Schlaudecker and Way agree: Women should be inoculated against the flu as soon as they learn they are pregnant.

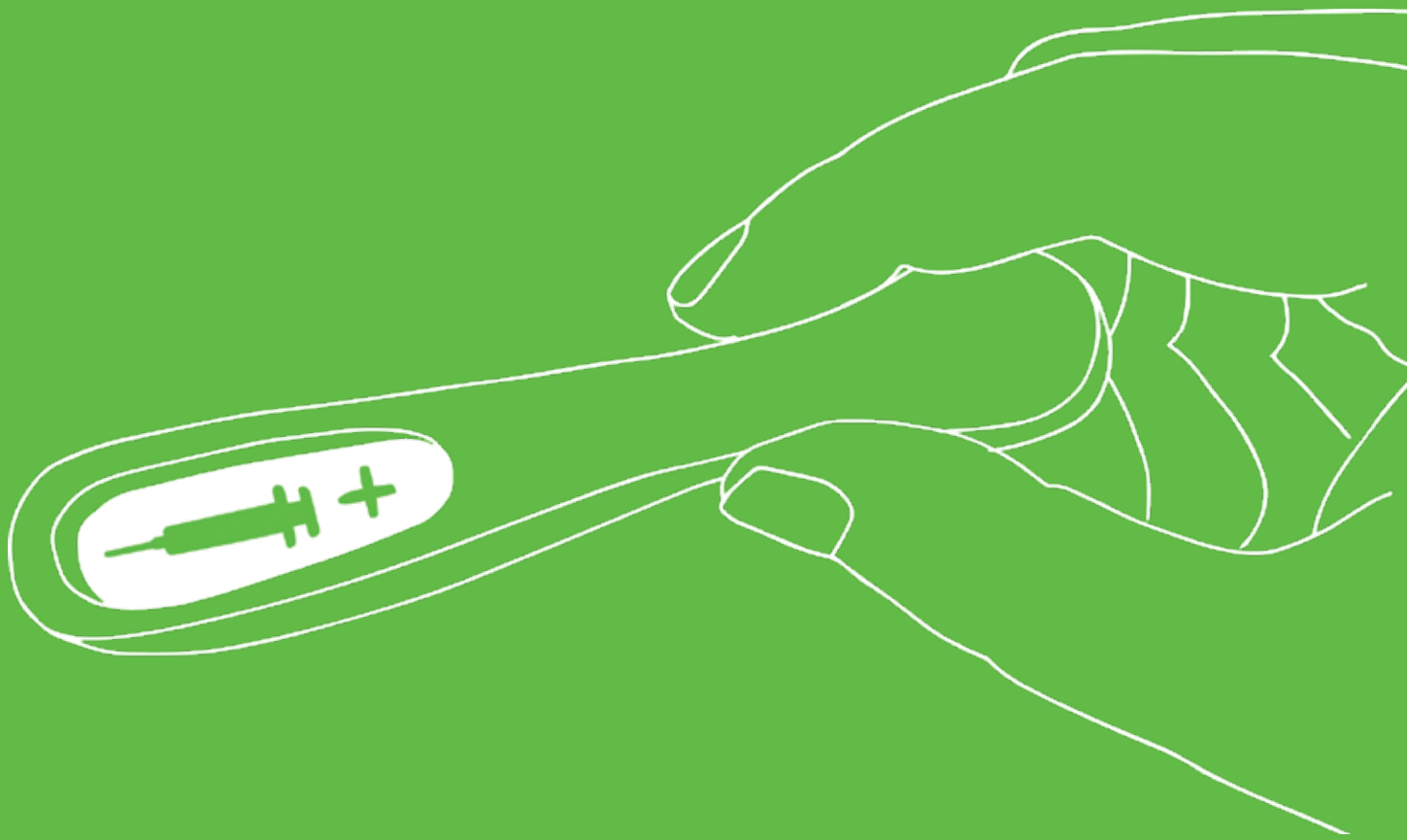
## THE KEY TO HEALTHY BABIES: HEALTHY MOMS

As science reveals more about the complex ways pregnant women and fetuses inter-react, the field of reproductive immunology is enjoying a renaissance.

"There's a lot of interest now," Schlaudecker says, "because we're finding that in several diseases that strike in the first few months of life, like respiratory syncytial virus, pertussis, group B strep, and influenza, infants are protected only if you can get the antibodies from mom."

Among the complexities: pregnancy itself makes women more susceptible to severe complications from the flu.

Schlaudecker observed this during field research in Honduras, and in re-examining medical records from the 1918 flu pandemic. However, newborn immune systems just aren't up for fighting off influenza. One of their best immunological allies is gained from breastfeeding.



“Infants clearly receive serum antibody from mom for the first four months,” Schlaudecker points out. “However, if mom is breastfeeding, that protection lasts as long as breastfeeding continues.”

#### **TWO RESEARCHERS, TWO APPROACHES, ONE GOAL**

This project is the first co-authorship for Schlaudecker and Way.

“She’s the technical and intellectual driving force of this project,” Way says. “My role is to serve as her mentor, her cheerleader. So she’s in a sense the first and senior author, although I will be listed last.”

The ongoing research also involves Cincinnati Children’s researchers from the divisions of Hospital Medicine, Biostatistics and Epidemiology, and Immunology.

“Our research is complimentary but not necessarily overlapping,” Way says. “We ask the same questions but use totally different model systems to answer them.”

Schlaudecker has focused more on clinical work in the past, using studies involving pregnant women. Way has focused on mouse models to study activity at the molecular level. The former is good at showing what is going on between the mother and fetus. The latter helps explore why.

“That’s why Sing Sing’s work is so interesting,” she says. “For some things like flu vaccines in pregnancy, we now know that immunological responses diminish, but other immunological components, like regulatory T cells, are enhanced. The path is not as linear and clear-cut as we used to believe it to be.”

Now with this project, the molecular level and family level approaches appear to have found common ground: the health of moms and babies.



Sing Sing Way, MD, PhD, and Elizabeth Schlaudecker, MD, MPH, have studied the maternal immune system for years, but along parallel paths. Now they are collaborating.



# A Peek Inside a B Cell Factory

New information about how germinal centers develop high-affinity B cells offers clues for improving vaccine responses

by Tim Bonfield



**T**he idea that our bodies fight off infection by creating tiny factories within our lymph nodes to crank out better antibody-producing B cells dates back more than 130 years, to when Walther Flemming first described the germinal center in 1884.

Now a team of experimental and computational biologists at Cincinnati Children's has managed to probe far deeper into these infection-fighting factories than Flemming could have imagined. Their work is revealing unprecedented detail about how germinal centers function, right down to how specific pairs of transcription factors interact to regulate gene activity within individual cells.

The work may sound abstract, but obtaining inside information about B cell assembly lines could have far-reaching practical value. If scientists can help improve production of high-affinity B cells, the result could be more lives saved by stronger vaccines.

## THE MYSTERY OF AFFINITY MATURATION

The new learning digs into the long-established concept of affinity maturation. Decades ago, experts observed that immune response after vaccination tends to improve over time. Not only do germinal centers make rising numbers of B cells, they produce cells that steadily become more accurate at recognizing the invading pathogen.

How this occurs has been a mystery.

Scientists know that B cell factories employ a highly unusual process that mixes in randomness with control, called somatic hypermutation. This allows the factories to crank out thousands of B cells, each with slight variations in the original antibodies they carry. The B cells that display the higher affinity receptors then outcompete those with lower affinity antibodies. This helps the immune system sharpen its response against viral and bacterial pathogens.

Although scientists have known that the factories favor rapid reproduction of B cells with the highest affinity for the attacker, how the

process works at a molecular level remains mysterious.

The Singh lab has now picked up interesting clues, using new experimental and computational tools.

“By separating low-affinity B cells from high-affinity B cells, and analyzing their genomes at a single cell level,” says Harinder Singh, PhD, Director of Immunobiology at Cincinnati Children’s, “we have revealed a characteristic gene expression difference that suggests how the high-affinity cells win the competition.”

The team has not yet published the specific genes involved and other details, but they say the work so far already demonstrates the value of using a systems biology approach to understanding the immune system.

#### **TECHNOLOGY SUPPORTS DEEPER DIVE**

“In vaccine research, our knowledge about producing high-affinity antibodies to particular viral or bacterial pathogens has been limited,” Singh says. “Sometimes an experimental vaccine triggers only a low-affinity response. Sometimes the response produces high-affinity B cells, but not at a high enough frequency. Meanwhile, the same vaccine can elicit widely variable responses at the individual level.”

Singh is working with computational biologist Matt Weirauch, PhD, and other colleagues at Cincinnati Children’s to employ single-cell RNA sequencing technology, as well as customized bioinformatics tools and other methods to explore these issues.

Weirauch and his graduate student Jeremy Riddell have developed a computational method that enables them to identify novel composite genomic sequences that are likely locations of pairs of interacting transcription factors.

Using B cell genomic data provided by Singh, Weirauch’s team accurately

detected the expected level of activity of a known pair of transcription factors that form a cooperative element called EICE. Singh and other immunobiologists have been studying EICE for years.

“That finding gave us the confidence to take the results seriously when the computational model also predicted an unexpected interaction among two other transcription factors,” Weirauch says.

Those findings, involving the NFAT and IRF families of transcription factors, were then confirmed by experimental work in the Singh lab by his postdoctoral fellow, Ankur Saini. The biological results demonstrate that NFATs and IRF8 molecularly cooperate to promote the germinal center response and therefore affinity maturation.

“There is a lot of exciting downstream biology that could come out of this,” Weirauch says. “The individual binding sites for these transcription factors were

known, but until now, it was not known that they cooperated with each other. I think this kind of result illustrates how strong the synergy between computational and experimental biology can be.”

#### **A PATH TO BETTER VACCINES**

By identifying specific gene networks involved in high-affinity B cell production, Singh and Weirauch’s work suggests the possibility of influencing the process. If a level of control can be achieved, it may become possible to improve vaccines so that they induce efficient generation of high-affinity memory B cells and thereby provide stronger protection.

Meanwhile, developing genetic tests based on differences in gene expression patterns could help detect which people are most likely to have poor immune responses to a standard vaccine dose, as well as others who may over-react. ■



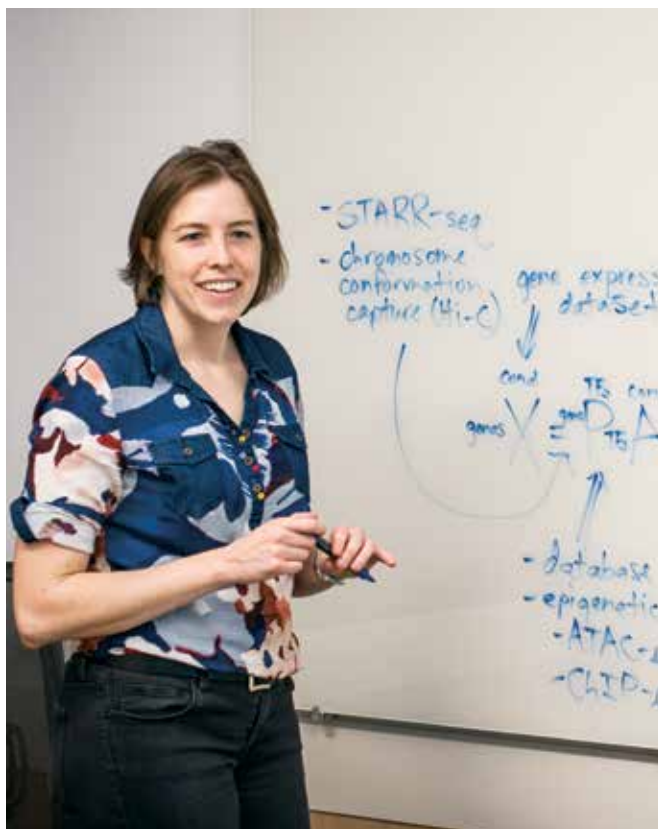
Understanding the gene networks involved in high-affinity B cell production could lead to stronger vaccines, says Harinder Singh, PhD, Director of Immunobiology at Cincinnati Children’s.

# Immuno-Engineering from the Numbers

Scientists Use Math to Back-Calculate Immune Responses

by Nick Miller

Today's mega-computers and genomic sequencing technologies let scientists analyze the basic building blocks of life in ways unimagined two decades ago. Even so, learning how to exert precise control over the body's immune system still relies on an old but useful practice—conceptualizing mathematical models on a drawing board.



Emily Miraldi, PhD

Emily Miraldi, PhD, is a computational and systems biologist who joined the divisions of Immunobiology and Biomedical Informatics in January. Coming from New York University and the Simons Foundation, the MIT-trained scientist focuses on immuno-engineering: altering the behavior of specific immune cell populations during disease without compromising the body's normal immune function.

Her team is helping develop more precise therapies to ramp up immune cells and battle cancer, or turn them down to prevent autoimmune disease, while not interfering with healthy immune function.

"There is no cure for autoimmune disease, so people are given immunosuppressive therapy," Miraldi explains. "This is akin to using a sledgehammer on the immune response, leaving patients susceptible to garden variety infections."

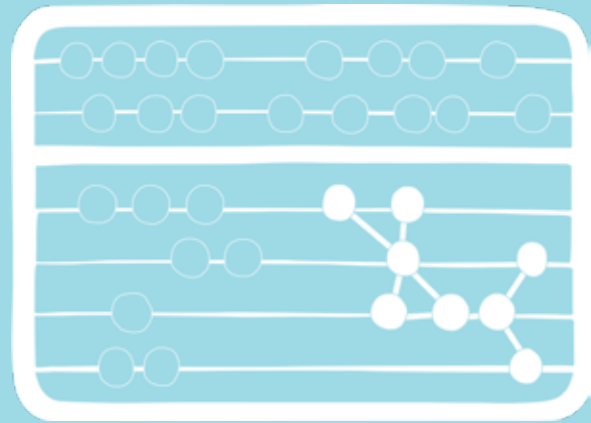
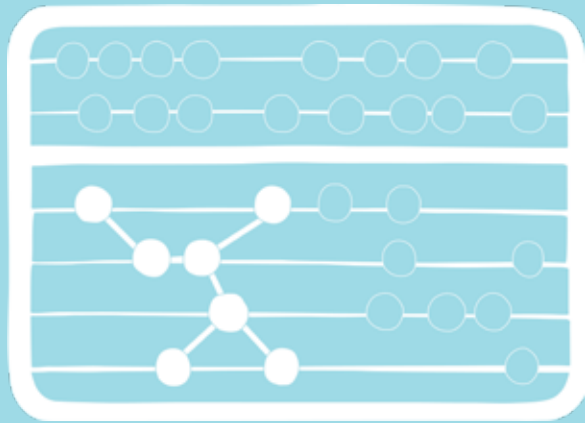
Genomics technologies provide high-dimensional snapshots of the cellular molecules (DNA, RNA, proteins, metabolites) that drive cell behavior. Mathematical modeling can stitch these snapshots together into a blueprint of how combinations of molecules work together to orchestrate responses. Miraldi and her colleagues use these models to predict the effect of molecular interventions (genetic, diet, drugs) on individual cell types.

"This takes us closer to the goal of designing therapies to target a desired cell type, while leaving other cells of the body undisturbed," she explains.

## TAG TEAM SCIENCE

Miraldi works closely with experimental immunologists who study immune cells in living biological systems, such as cell cultures and mouse models. The experimentalists provide mass quantities of multivariate measurements on how cells behave – what they do, when they do it, where they do it, and what genes are expressed. Using computational methods conceived on a drawing-board, she leverages the numbers and patterns in this





high-dimensional data to develop a mathematics-based hypotheses on the why.

The model hypotheses go back to experimentalists. After more experimental testing, new lab data comes back to Miraldi and, in a continuous feedback loop, the process repeats until answers are found.

#### TRUTH IN TRANSCRIPTION

Except for red blood cells and thrombocytes, every cell in the body has a nucleus with DNA that provides the blueprint for making any other cell in the body. An outstanding question in biology is understanding how different cell types use only those parts of the blueprint needed to make that cell type.

The biophysics of the problem is fascinating: how to organize two meters of DNA in a 6-micron nucleus to get the right gene expression patterns in the right cell? New biotechnologies make it possible to address this question on a genome scale, according to Miraldi.

Her work in recent years has focused on developing mathematical frameworks to integrate two genomic measurement types. One is RNA-seq—snapshots of all genes expressed in a cell type. The other is ATAC-seq—snapshots that provide hints about which parts of the DNA could be used for proteins called transcription factors, which bind and influence gene expression. The resulting model is called a transcriptional regulatory network. It identifies the hundreds of transcription factors that combine to control the expression of thousands of genes in a particular cell in the body.

“There are many immune cells of the body that have been studied for decades, and these new technologies combined with mathematical approaches have expanded our ability to develop a broader, more nuanced understanding—even in cell types with a rich research history,” Miraldi says. “They also provide an opportunity to benchmark the quality of my modeling approaches.”

While Miraldi uses vast sets of high-dimensional data to identify complex transcriptional networks—with hundreds or thousands of components—other researchers dive much deeper into much smaller handfuls of transcription factors, explains Harinder Singh, PhD, Director of Immunobiology.

“So while we work from the ground up to assemble networks that are small-scale, Emily is working from the other direction. The reason Emily was recruited was to enable this kind of convergence between the top-down and bottom-up approaches,” he says.

#### EXPLORING A NEW CELL

Miraldi’s most recent foray is inferring transcriptional regulatory networks in innate lymphoid cells (ILCs). The cells were discovered within the last decade, so many of the key drivers of their gene expression and behavior are unknown.

ILCs can be broken into five subtypes, each with unique contributions to host defense, whether against viruses or fungi, etc., and autoimmune disease.

The research team now has models for each of the five ILC subtypes in the small intestine, and they have proposed tens of thousands of transcription factor-to-gene relationships for the cells. Miraldi and colleagues are currently testing their hypothetical models and developing preliminary evidence to support these.

Miraldi sees the potential to break new scientific ground at Cincinnati Children’s, especially with opportunities for extensive collaboration and the blending of computational biology and immunology.

“There are dozens of labs doing cutting-edge experimental immunology, and they are eager to team up with a computational biologist to dig deeper with genomics datasets and build models,” she says. “Equally important are talented computational and mathematical biologists who want to combine forces to derive new modeling approaches as new data types and biotechnologies become available.”

# A Slightly Different Take on ‘Impact Factor’

*Nature* Paper About Blood Cell Formation Stirs Scientific Pot

by Nick Miller

Scientists call it “impact factor”—defined by the National Institutes of Health as the average frequency that articles in a given journal are cited by other publications in a particular year.

What this rather dry definition doesn’t necessarily reflect is the degree of spirited debate, newly nurtured collaboration or groundbreaking knowledge produced by a specific study or article. Using any of these standards, a paper about blood cell formation published in *Nature* last year by Cincinnati Children’s researchers is having significant impact.

“The data we have are a bit controversial and the paper has been viewed over 30,000 times,” explains H. Leighton Grimes, PhD, one of the study’s investigators and a member of the Division of Immunobiology. “The study is making a contribution and people still discuss it at scientific meetings. Some say they don’t believe our data.”

The paper has an Altmetric score of 566, which is in the 99th percentile of all research outputs rated by the organization. To gauge how much online buzz a study generates, Altmetric includes factors such as social media, online media coverage, etc.

## GENETIC TUG OF WAR

Published Aug. 31, 2016, in *Nature* (impact factor 38.138) the paper shows blood cells in mice appear to reach their final states following competitions between opposing gene regulatory networks.

As blood cells develop, they experience genetic turbulence that can be observed by turning on alternate lineage genes in individual

cells. These are termed multi-lineage states and authors of the study describe the condition as “dynamic instability.” The data show that before becoming a neutrophil or a monocyte, a cell not only goes through a readily observable multi-lineage state but also flits through a rarer bi-stable state.

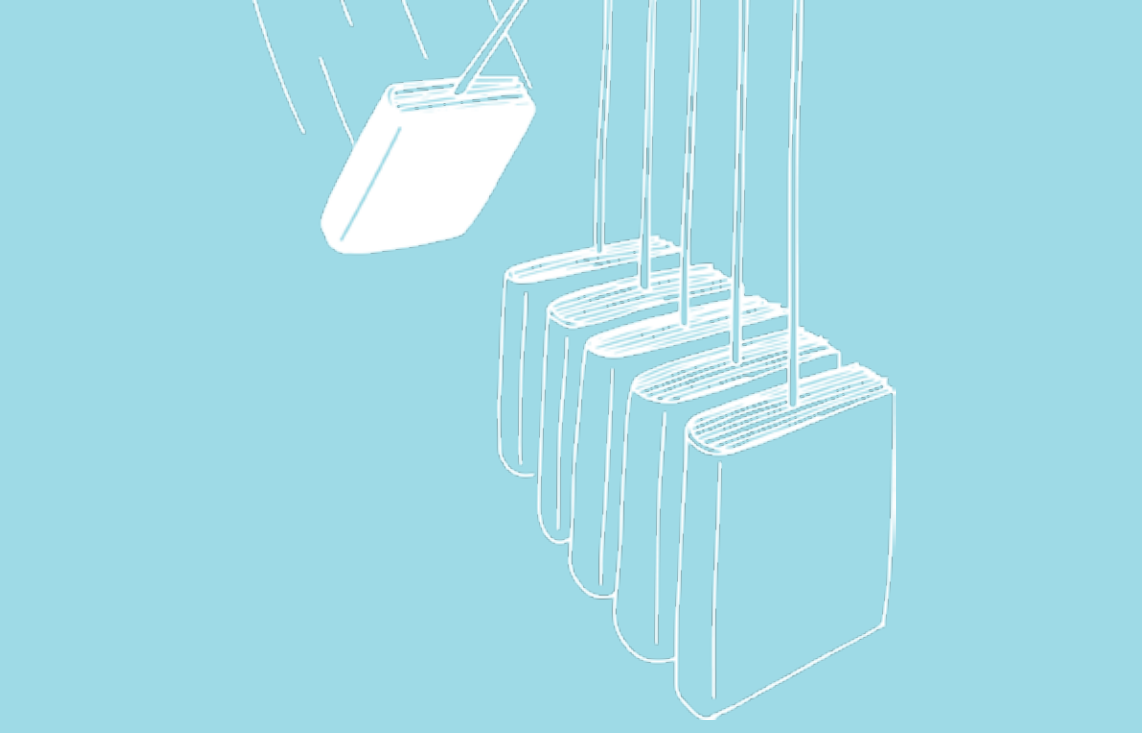
What cues blood cells to their final types remains unknown, but this research points to the competing gene networks. Although firmly grounded in the realm of basic research, the study could lead to insights about developmental miscues that cause blood or immune system disorders. The production of neutrophils or monocytes, for example, has to be precisely balanced. Having too many or too few of either can be deadly.

The paper was a collaboration between Grimes, Harinder Singh, PhD, Director of Immunobiology, and Nathan Salomonis, PhD, Biomedical Informatics. The study infers that within these genetic tugs of war that determine the fates of blood cells there are still other as yet undiscovered multi-lineage intermediates.

## DEEP VS. WIDE DEBATE

As Cincinnati Children’s researchers work to provide more evidence for understanding the necessity of multi-lineage cells as key developmental intermediates, and as the mechanism of dynamic instability, they have learned that other research groups either support the paper’s conclusions or reject them.

Key to the *Nature* paper was use of emerging single-cell RNA sequencing technology, which can identify different genes and regulatory networks within individual cells. Because the



technology is fairly new, scientists have not yet established gold standards for how to use it in varying research contexts, according to Grimes.

Some researchers favor sequencing and analyzing thousands of cells without diving as deeply into the different genes that are switching on and off. In this approach, Singh, Salomonis and Grimes sequenced only about 500 cells, and then probed much more deeply into the gene expression patterns and regulatory networks involved in pulling blood cells in one direction or the other.

“Single-cell RNA sequencing is still a relatively new tool, so you can imagine that there will be a lot of back and forth around its use,” Singh says. “This is a major conversation around sequencing thousands of cells at low depth versus fewer cells at deeper depth.”

What makes the Cincinnati Children’s paper in *Nature* even more compelling—and valid the researchers say—is that it relies on more than single-cell RNA sequencing to draw its conclusions.

“Predictions from single-cell RNA sequencing were tested using different kinds of genetic and molecular experiments,” Singh explains.

#### MORE TO THE NATURE STORY

A notable feature of the article is a new bioinformatics pipeline developed by Salomonis called ICGS (Iterative Clustering and Guide-Gene Selection).

ICGS gives researchers an automated platform to process and analyze all of the single-cell RNA sequencing data to identify the transitioning or shifting genomic states of cells.

“A number of other researchers are using bioinformatics tools that we developed to test new hypotheses,” says Salomonis. “We also are in touch with other research groups who are using data we generated in the *Nature* paper to test different hypotheses for the exact cell populations we describe.” ■



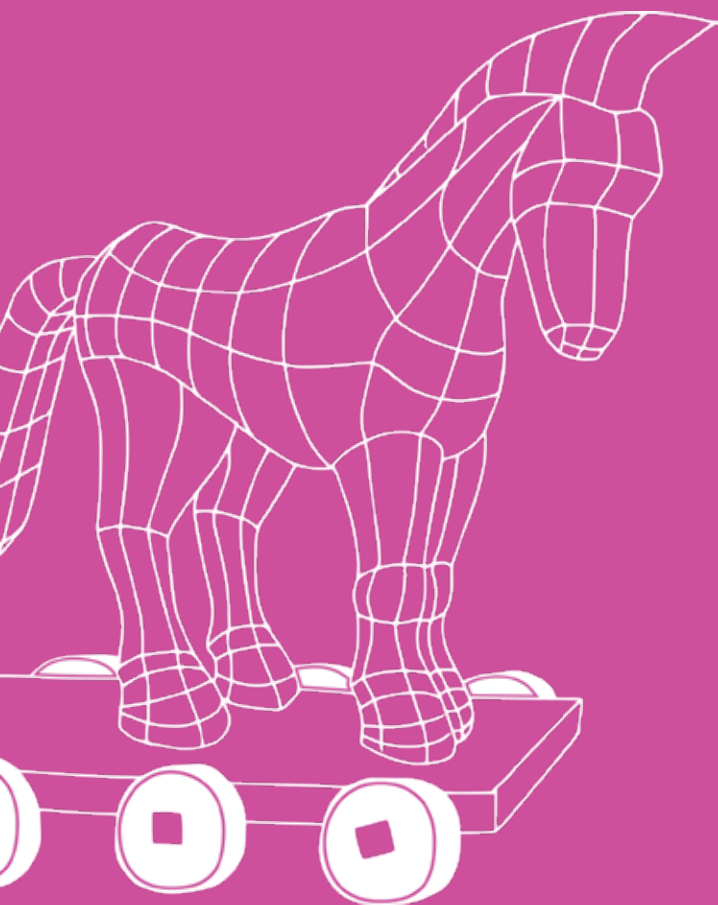
From left to right: Nathan Salomonis, PhD, research assistant Andre Olsson (with back to camera), H. Leighton Grimes, PhD, and Harinder Singh, PhD.



# Enemy in the House

## Metagenomics vs. \$10 Billion Problem: Hospital Infections

by Nick Miller



**H**ospital infections make very sick people even sicker—or worse.

Despite extensive infection control programs, hospital-based infections number more than 720,000 a year and account for around 70,000 deaths, according to the U.S. Centers for Disease Control and Prevention (CDC). A study in *JAMA Internal Medicine* by Harvard Medical School estimates the cost to the U.S. healthcare system at close to \$10 billion a year.

One problem: current blood tests generally do a poor job of detecting multidrug-resistant bacteria. But Cincinnati Children's is developing—and is close to clinically testing—a new early detection system, according to David Haslam, MD, Director of the Antimicrobial Stewardship Program here.

Haslam and colleagues have learned that some highly vulnerable children—such as children with cancer and children waiting for organ transplants—enter the hospital already carrying dangerous, resistant forms of *Escherichia coli*, *Enterococcus faecalis* or other organisms.

The new system seeks to use emerging technology to detect the organisms before a full-blown infection can develop in these children and possibly spread to others.

“Knowing that a child is carrying a resistant or potentially dangerous organism before getting the infection would be an indication to put the child in isolation,” explains Haslam. “It’s not just about one patient. It’s also an infection prevention methodology for the entire institution.”

The importance of the initiative is underscored by the fact that it is funded by grants from the CDC and the Center for Pediatric Genomics at Cincinnati Children's.

### PRECISION METAGENOMICS

Working closely with clinical fellow Heidi Anderson, MD, Haslam and his research team are combining the latest biology and computer technologies to advance the concept of precision metagenomics—the study of genetic material recovered from individual environmental samples.

In this case, clinicians collect fecal samples, cheek swabs or skin scrapes from seriously ill children admitted to the hospital. The team compares whole genome sequencing information from the patient samples to known DNA information about dangerous pathogens.

This allows caregivers to confirm the presence of antimicrobial-resistant genes. It also lets researchers to detect similarities in the genetic signatures of drug-resistant organisms among different patients—information that could be vital to detecting and preventing an outbreak.

But the potential advantages go even further. By looking at changes to an organism's DNA structure, researchers can even tell how long a resistant pathogen has lived in or on a particular patient.

This information can be plugged into electronic medical records to determine which rooms, floors and hospital areas were occupied by affected kids. Tracking this information, along with seeing how organisms in different patients are related genetically, can give care staff a powerful tool for detecting and shutting down paths of infection, Haslam and Anderson say.

"This work allows us to dig into the question of whether these infections are picked up inside an institution or whether an organism was already on a patient when they came in," Anderson says. "Infections get classified as hospital-acquired, or not, based on whether the child was in the hospital, but that doesn't determine where the pathogen came from. Was it already in the hospital or did it come in with the child?"

#### HOW THE INITIATIVE WORKS

Haslam and Anderson boil down the precision metagenomics initiative into four basic steps:

- Isolating patients colonized with harmful organisms to prevent transmission
- Identifying how prevalent a multidrug-resistant pathogen is in patients (and possibly the environment) and preventing inappropriate exposures
- Eliminating pathogenic organisms with targeted probiotics or fecal transplantation
- Choosing the correct antimicrobial treatment for a patient colonized with a multidrug-resistant pathogen who develops an infection.

The problem of hospital infections is serious enough that other hospitals and other groups within Cincinnati Children's are interested in the project and asking when they can use the technology, Anderson and Haslam say.

So far, the metagenomics analysis has been performed using samples from more than 150 severely ill hospitalized children. Haslam says the approach "works very well and we've done all the research in humans."

The research team is expanding testing to other patient populations, including healthier children, to assess which patients may benefit most from metagenomic screening. ■

## Cincinnati Children's is developing an early-detection tool for drug-resistant infections.



David Haslam, MD, (shown) worked closely with clinical fellow Heidi Anderson, MD, to compare whole genome sequencing information from patient samples to known DNA information about dangerous pathogens.

# A Vital Role for ‘Good’ Bacteria

Study connecting gut microbiome to immune system development raises questions about how antibiotics are prescribed

by Tim Bonfield



Doctors have long understood that antibiotics that protect infants from infection also can disrupt the normal growth of their gut bacteria. However, a new study reveals that the consequences of routine antibiotic use may be deeper and longer lasting than expected.

In the short term, disrupting gut bacteria makes infant mice more likely to develop pneumonia. It also makes them more likely to die from it. Longer term, continued disruption to gut bacteria appears to cause permanent immune system damage.

These are the key findings of a study published Feb. 8, 2017, in the journal *Science Translational Medicine*. The study, led by experts at Cincinnati Children's, may spark a wider conversation about antibiotics use, including the near-automatic practice of prescribing them to women before Cesarean section (C-section) deliveries.

"It is time to begin pushing back on practices that were established decades ago, when our level of understanding was different," says Hitesh Deshmukh, MD, PhD, lead author of the study. "To prevent infection in one infant, we are exposing 200 infants to the unwanted effects of antibiotics. A more balanced, more nuanced approach is possible."

In addition to Deshmukh, co-authors included Jeffrey Whitsett, MD, Co-Director of the Perinatal Institute at Cincinnati Children's; Theresa Alenghat, VMD, PhD, Division of Immunobiology; research assistants Jerilyn Gray and Katherine Oehrle; and George Worthen, MD, Children's Hospital of Philadelphia.

## TOO MUCH OF A GOOD THING?

Nearly every C-section in the U.S. involves prescribing antibiotics to mothers shortly before delivery. Up to 30 percent of newborns in neonatal intensive care units also receive antibiotics.

The treatments help prevent Group B streptococcal infections—the leading cause of deadly infections in newborns. However,



in most cases the drugs are given as a precaution, not because infections have been confirmed, Deshmukh says.

Once taken, the antibiotics act against a wide range of bacteria, be they good or bad. It turns out that commensal—or “good”—bacteria play a vital role in building a healthy immune system.

#### **LUNG DEVELOPMENT RELIES ON GUT BACTERIA SIGNALING**

Even after birth, an infant’s lungs are still forming. Their immune defenses remain under construction.

For more than two years, Deshmukh and colleagues conducted experiments in mice to define how this process works. They found that strong defenses depend on a flow of molecular signals occurring as the body reacts to waves of normal bacteria colonizing the gut.

Specifically, the presence of commensal bacteria triggers the production of group 3 innate lymphoid cells (ILC3). These sentinel cells migrate to mucosal linings in the lungs, where they produce interleukin-22 (IL-22). This vital signaling protein helps activate the immune response to infection.

The problem: when antibiotics wipe out good bacteria, they cut off that important flow of signals. As a result, the lungs build weaker defenses against future infections.

#### **DAMAGE CAN BECOME PERMANENT**

If antibiotic use is limited and early, a human infant would have some time to replenish commensal bacteria. But the process can take months, Deshmukh says, and the result may not be a normal mix of bacteria.

After about a year, human infants have completed building their immune systems. That means any construction weaknesses are likely to be permanent.

This outcome of excess antibiotic use may help explain why some people with no obvious genetic risk factors develop

asthma or other lung diseases later in life, Deshmukh says.

#### **RAPIDLY RESTORING GOOD BACTERIA MAY HELP**

The need to use antibiotics to save lives when dangerous infections strike has not changed. However, these findings do suggest re-thinking routine preventive use in newborns, the researchers say.

The good news: methods exist for restoring normal bacteria levels. In fact,

when the researchers used such methods in mice, it restored their resistance to pneumonia.

But do these mice experiments apply to humans? A clinical study has begun to evaluate the safety and benefits of limiting antibiotic use among expectant mothers and newborns, Deshmukh says. Several other next steps are planned. ■



Hitesh Deshmukh, MD, PhD, says it may be possible to protect infant immune systems from antibiotic damage.

# One Gut, a Couple Trillion Microbes, and an Enduring Mystery

Innovative research in immunobiology is fueling new insights into the regulation—and possible prevention—of disease

by Tom O'Neill

**T**heresa Alenghat's mission is to untangle the good microbes from the bad, and decipher how they regulate our cells.

From their home in our intestinal tract, trillions of microbes, collectively called the microbiota, play a key role in triggering healthy immune responses and disease development. The challenge is determining how these diverse little targets do this.

"There is a lot of the excitement about trying to determine which components

of the microbiota are beneficial, and using that knowledge to treat patients and improve health," Alenghat says.

Now, new research led by Alenghat reveals just how complicated the microbial world is, and how a better understanding of its pathways has the potential to improve treatment of a wide range of diseases. Or even, help prevent them.

Her essential tool: mice devoid of microbes.

These mice have abnormal immune and metabolic responses and can be

colonized with single or defined groups of microbes.

"For us, the big questions are: Do different intestinal microbes actually impact our cells in distinct ways? And if so, how do they do this?" says Alenghat, VMD, PhD, of the Division of Immunobiology.

## COMMENSAL MICROBES: FROM INFANCY TO INSIGHTS

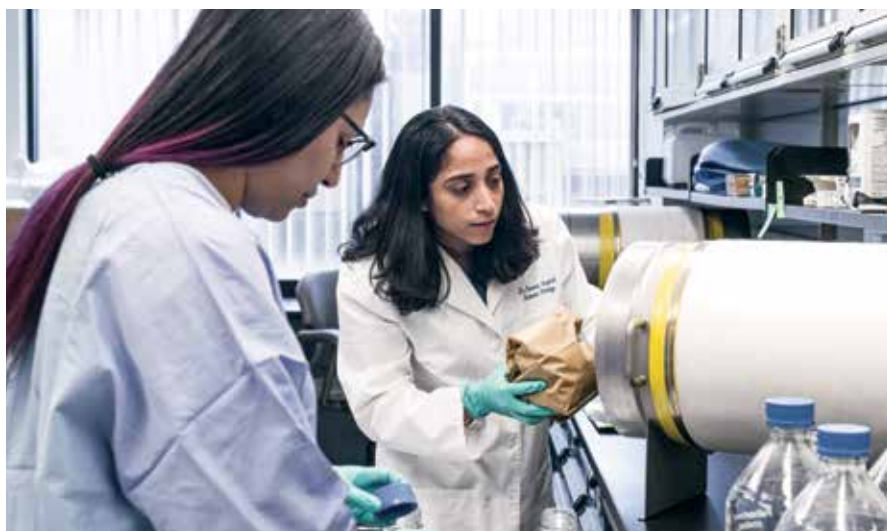
Although research into immune system response is not new, the science of analyzing the good or "commensal" microbes in mice devoid of microbes is a relatively new arena that has taken off over the last decade.

Alenghat's team was the first to bring this innovative approach to Cincinnati Children's.

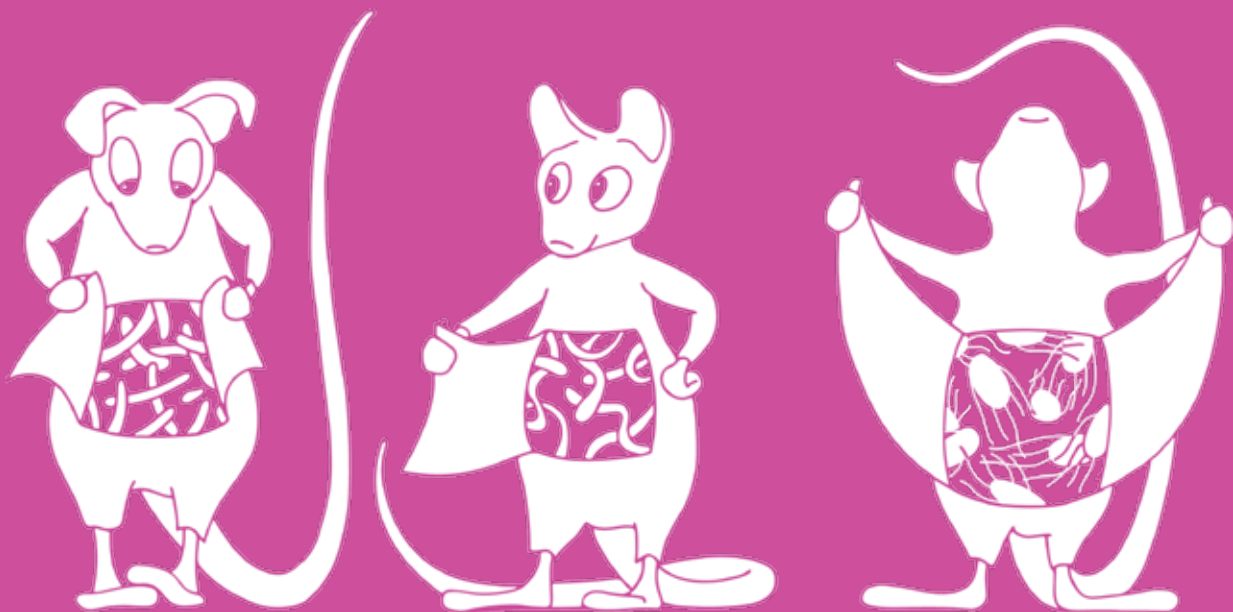
Establishing this unique tool involved support from Veterinary Services and the Research Foundation, and took about a year to complete.

"At this point we know that the microbiota are required to support health, and that the types of microbes present can change with disease," says Alenghat, a 2015 Pew Scholar.

"What we don't know is which of these microbes do what and how they do it," she adds. "With these new tools, we are now able to start exploring these questions here."



Theresa Alenghat, VMD, PhD, (in white lab coat) and Jordan Whitt, a member of the Alenghat lab, prepare supplies that need to be sterilized within specialized gnotobiotic cylinders before being brought to the germ-free room.



The diversity of these microbes is an obstacle for researchers: bacteria, viruses, fungal organisms and parasites among them. There are more than 1,000 different species of bacteria alone.

Researchers believe the microbiota could unlock the mysteries behind many diseases, among them inflammatory bowel disease, asthma, diabetes and obesity.

But the potential is even greater because microbes also regulate essential biological processes such as immunity, metabolism, neuronal development and the formation of new blood vessels.

#### **MICROBES MIGHT PLAY ROLE IN EDUCATING CELLS**

“If commensal microbes promote health and protect against disease, do they do this by educating cells in our body?” Alenghat says. “We think so. Can these educated cells then respond more effectively against infection and prevent abnormal inflammation?”

In a review published in the February 2017 issue of *Current Opinion in Immunology*, Alenghat and colleagues discussed that epigenetics may underlie how the microbiota may be so instrumental in preventing abnormal inflammation, while simultaneously promoting host defense.

Short-chain fatty acids appear to be a critical subset of metabolites that come

from the microbiota and have the potential to directly modulate the epigenetic landscape.

However, there are likely many more factors from the microbiota with this function that have not yet been identified.

#### **MICROBIOTA-EPIGENETIC “CROSSTALK”**

Building on recent revelations that the microbiota is instrumental in promoting intestinal immune health, Alenghat’s work focuses on deciphering how epigenetic “crosstalk” helps maintain healthy communication with the microbiota.

Epigenetics involves genes impacted by environmental influences, which in turn change the expression and downstream effects of other genes. This can alter the composition and function of individual cells and help program biological processes that are beneficial or trigger illness.

Alenghat and her colleagues have identified an enzyme in intestinal epithelial cells called histone deacetylases (HDAC3), which acts as a key epigenetic modifier in the intestine.

In a 2013 *Nature* study, which she co-authored while at the University of Pennsylvania, researchers found that deleting HDAC3 from intestinal epithelial cells of mice resulted in impaired

intestinal function and increased inflammation.

That original paper showed that HDAC3 is critical in integrating microbiota-derived signals to calibrate intestinal responses.

This is needed to establish normal host-microbe relationships—and with it, intestinal health.

The study set the groundwork for her current work at Cincinnati Children’s. The Alenghat lab uses her newly established models to decode pathways in our cells that are controlled by epigenetics and different components of the microbiota.

“Uncovering these new pathways will give us deep insight into how our bodies, and these commensal microbes, live symbiotically and why some microbes may be beneficial whereas others may trigger disease,” she says.

Our gut and microbiota didn’t evolve on independent, parallel roads, but as a rather busy intersection unburdened by traffic lights.

“It’s amazing,” Alenghat says, reflecting on her days as a PhD candidate in the mid-2000s, “how much more we now appreciate the intestine and microbiota in regulating diverse aspects of our body, but we are just at the beginning of understanding these complex relationships.” ■



---

## GRANTS

---

From Oct. 1 through Jan. 31, researchers at Cincinnati Children's were awarded 169 grants valued at \$94 million in total costs. Here are the recipients of grants exceeding \$1 million in total costs:

**David Bernstein, MD, MA,**

**Infectious Diseases,** received a seven-year, \$4.2 million grant from the National Institute of Allergy and Infectious Diseases, for his work with the agency's Vaccine and Treatment Evaluation Units. The project helps researchers develop new and improved vaccines and therapies against infectious diseases.

**Hermine Brunner, MD, MSc,**

**Director, Rheumatology,** received a five-year, \$1.7 million grant from Pfizer to study the efficacy, safety and tolerability to tofacitinib, as a potential treatment of juvenile idiopathic arthritis. The drug is approved to treat adult rheumatoid arthritis.

**James Cnota, MD,**

**Heart Institute,** received a six-year, \$2.4 million grant from the National Heart, Lung, and Blood Institute, for his role in the Pediatric Heart Network Prairieland Consortium, a collaboration between the cardiovascular programs at Cincinnati Children's and Riley Hospital for Children in Indianapolis.

**Biplab Dasgupta, PhD, MS,**

**Cancer and Blood Diseases Institute,** will study the biguanide sensitivity of glioma stem cells, with a five-year, \$1.9 million grant from the National Institute of Neurological Disorders and Stroke.

**Robert Frenck, MD,**

**Medical Director, Infectious Diseases,** received a two-year, \$1.7 million grant from PATH Vaccine Solutions to study development of a *Shigella sonnei* human challenge model, using a newly manufactured lyophilized lot of strain 53G that could serve as a challenge strain for all *S. sonnei* vaccine candidates. Shigella infection is an intestinal disease.

**John Harley, MD, PhD,**

**Director, Center for Autoimmune Genomics and Etiology (CAGE),** received a three-year, \$2.6 million grant from the National Human Genome Research Institute for his leadership role in "Better Outcomes for Children: Promoting Excellence in Healthcare Genomics to Inform Policy." The project is part of the eMERGE collaboration with Boston Children's, which incorporates advances of genetics, genomics and electronic medical recordkeeping.

**Michael Helmrath, MD, MS,**

**Director, Surgical Research,** will study the investigation of regional identity in human intestinal stem cells, using a five-year, \$1.8 million grant from the National Institute of Diabetes and Digestive and Kidney Diseases.

**Gurjit (Neeru) Khurana Hershey, MD, PhD,**

**Director, Asthma Research,** received a seven-year, \$5.6 million grant from the National Institute of Allergy and Infectious Diseases, for her work with the Children's Respiratory Research and Environment Workgroup.

**Todd Jenkins, PhD, MPH,**

**Surgery,** will study longitudinal assessment of teens who have undergone bariatric surgery, using a five-year, \$4.7 million grant from the National Institute of Diabetes and Digestive and Kidney Diseases.

**Brad Kurowski, MD, MS,**

**Physical Medicine and Rehabilitation,** will use a five-year, \$3.4 million grant from the National Institute of Neurological Diseases and Stroke, to study the genetic and environmental influences on recovery of severe brain injury.

---

# GRANTS

---

[Richard Lang, PhD,](#)

**Ophthalmology**, received a five-year, \$2.4 million grant from the National Eye Institute, to study the regulation of vascular development in the eye by an opsin 5-dependent clock.

[Ian Lewkowich, PhD,](#)

**Immunobiology**, will study the mechanisms of IL-17A-mediated enhancement of asthma severity, using a three-year, \$1.7 million grant from the National Heart, Lung, and Blood Institute.

[Jeffery Molkentin, PhD,](#)

**Molecular Cardiovascular Biology**, will study the paracrine hypothesis underlying cardiac stem cell therapies, with a four-year, \$1.6 million grant from the National Heart, Lung, and Blood Institute. He also received a five-year, \$1.1 million grant from the same agency for research into targeting mitochondria to treat heart disease.

[Takahisa Nakamura, PhD,](#)

**Endocrinology**, received a five-year, \$1.9 million grant from the National Institute of Diabetes and Digestive and Kidney Diseases, to study the role of hepatic RNA silencing in insulin resistance.

[Nehal Parikh DO, MS,](#)

**Perinatal Institute**, received two grants from the National Institute of Neurological Disorders and Stroke. He will study early prediction of cerebral palsy in premature infants using a five-year, \$4.3 million grant, and will use a five-year, \$2.2 million award to explore a new model to identify preterm newborns at high-risk for cognitive deficits.

[Anne Karina Perl, PhD, MS,](#)

**Neonatology and Pulmonary Biology**, received a three-year, \$2 million grant from the National Heart, Lung, and Blood Institute, to study the interstitial resident fibroblasts direct alveolar epithelial differentiation. The goal is to better understand the mechanisms of development, normal lung repair, and lung disease.

[Samir Shah, MD, MSCE,](#)

**Director, Hospital Medicine**, will study strategies to improve post-discharge outcomes for patients and their families, using a three-year, \$2.2 million grant from the Patient-Centered Outcome Research Institute.

[Jennifer D. Smith, PsyD,](#)

**Developmental and Behavioral Pediatrics**, received a five-year, \$2.8 million grant from the Health Resources and Services Administration, for her role with the Leadership Education in Neurodevelopmental and Related Disabilities program.

[Mary Allen Staat, MD, MPH,](#)

**Infectious Diseases**, will study enhanced surveillance strategies for new vaccine-preventable diseases, using a one-year, \$2 million grant from the national Centers for Disease Control and Prevention.

[Dan Starczynowski, PhD,](#)

**Experimental Hematology and Cancer Biology**, received a six-year, \$6.4 million grant from the National Heart, Lung, and Blood Institute, for his work in decoding the innate immune signaling in normal and myelodysplastic hematopoiesis.

[Takanori Takebe, MD,](#)

**Gastroenterology, Hepatology and Nutrition**, received a four-year, \$1.5 million grant from the New York Stem Cell Foundation, to study the clinical translation of organ bud transplant therapy.

[Katherine Yutzey, PhD,](#)

**Molecular Cardiovascular Biology**, received a four-year, \$1.7 million grant from the National Heart, Lung, and Blood Institute, to study the regulatory mechanisms of adult cardiomyocyte proliferation.

---

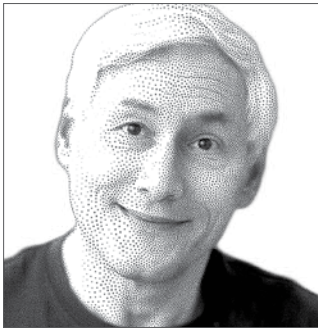
## HONORS

---

### Robbins Awarded Procter Medallion

Jeff Robbins, PhD, Executive Co-Director of the Heart Institute, and Director, Molecular Cardiovascular Biology, has received the William Cooper Procter Medallion—the highest honor bestowed by Cincinnati Children’s.

Robbins has published more than 250 peer-reviewed scientific articles. Known as the “father of cardiac transgenesis,” his work led to the development of reagents that have helped scientists understand the actions of proteins responsible for human cardiac disease and design effective therapies. Robbins’ research advanced our understanding of both normal and disease-causing cardiac proteins. Thousands of other investigators have leveraged his data and reagents to advance their own research.



Jeff Robbins, PhD

The Procter Medallion is the latest of many awards during Robbins’ 45-year career. Others include the Louis and Artur Lucian Award for research in circulatory disease; the Presidential Award from the International Society of Heart Research; and the prestigious Research Achievement Award from the American Heart Association.

#### Steven Black, MD,

**Global Child Health**, was recently named editor-in-chief of the *Pediatric Infectious Diseases Journal*.

#### Daniel Choo, MD,

**Director, Otolaryngology**, was elected to the Board of Directors for the American Cochlear Implant Alliance, which sponsors research, raises awareness and advocates for improved access to cochlear implants for patients of all ages.

#### Robert Frenck Jr., MD,

**Infectious Diseases**, received a national award from the American Academy of Pediatrics for his long-time service to the Ohio chapter.

#### Victor Garcia, MD,

**Founding Director, Trauma Services**, received the 2017 Health Care Heroes Lifetime Achievement Award from the *Cincinnati Business Courier* on Feb. 23.

#### Margaret Hostetter, MD,

**Chair, UC College of Medicine Department of Pediatrics, and Director, Cincinnati Children’s Research Foundation**, received a 2017 Career Woman of Achievement Award from the Greater Cincinnati YWCA.



---

## HONORS

---

[Alan Jobe, MD, PhD,](#)

**Director, Perinatal Biology**, has received the 2016 Mary Ellen Avery Award from the American Pediatric Society and the Society for Pediatric Research. This lifetime achievement award honors Jobe's role in building the scientific foundation for global use of surfactant and steroids. Jobe has authored more than 350 peer-reviewed publications. His career includes leading the NICHD Neonatal Research Network and the NICHD Global Network for Women's and Children's Health Research.

[John Harley, MD, PhD,](#)

**Director, Center for Autoimmune Genomics and Etiology (CAGE)**, was named a Master of the American College of Rheumatology. The honor recognizes career-long achievement in advancing rheumatology research and treatment.

[Gurjit \(Neeru\) Khurana Hershey, MD, PhD,](#)

**Director, Asthma Research**, was selected Chair of the NIAID's U19 Asthma and Allergic Diseases Centers Steering Committee.

[Jaimie Nathan, MD,](#)

**Surgical Director, Pancreas Care Center**, was honored by the Ohio/Kentucky Chapter of the National Pancreas Foundation for his efforts to expand the research work of the Pancreas Care Center.

### Kraft Named President-Elect of the American Academy of Pediatrics

Colleen Kraft, MD, Medical Director of The Health Network by Cincinnati Children's, was selected in November to be President-Elect of the American Academy of Pediatrics.

Kraft will serve as a spokesperson for 66,000 pediatricians across the U.S. She will be at the helm of the organization's policy decisions and will be charged with communicating those decisions to pediatricians and to the public.

Kraft says she wants to preserve the personal nature of the provider/patient relationship, which can get lost in a larger healthcare system. "Private practices

tend to be more familiar with individual patients' conditions. That relationship is so undervalued in this country."



Colleen Kraft, MD

Kraft's 3-year commitment to the AAP includes serving as president-elect in 2017, as president in 2018 and as past-president in 2019.

SAVE THE DATE

# 2017 Systems Immunology Workshop

This second workshop will focus on inference and validation of immune signaling and gene regulatory networks, computational modeling of immune processes, immuno-protein and cellular engineering along with applications and advances in human immunology. The meeting is intended to stimulate new research initiatives and where possible, experimental and conceptual consolidation. The bi-annual forum serves to advance programmatic initiatives at Cincinnati Children's and UC in Systems Biology. It also is catalyzing our efforts to establish a vibrant Center for Systems Immunology.

**SEPTEMBER 21-22, 2017**  
at Cincinnati Children's S1.203/204

PRESENTERS INCLUDE:

Grégoire Altan-Bonnet, PhD, NIH / Pamela Bjorkman, PhD, Caltech  
Arup Chakraborty, PhD, MIT / Ronald Germain, MD, PhD, NIH  
Alexander Hoffman, PhD, UCLA / Jeffrey Hubbel, PhD, University of Chicago  
Oleg Igoshin, PhD, Rice University  
Aly Azeem Khan, PhD, Toyota Technological Institute at Chicago  
David Kranz, PhD, University of Illinois, Urbana-Champaign  
Douglas Lauffenburger, PhD, MIT / Vincent Luca, PhD, Stanford University  
Kathryn Miller-Jensen, PhD, Yale / John Tsang, PhD, NIH



Organized by Harinder Singh,  
Andrew Herr and Emily Miraldi

For event details, contact:  
Kimberly McLay at  
[kimberly.mclay@cchmc.org](mailto:kimberly.mclay@cchmc.org)

## Research Horizons

Editorial Advisors:

Margaret Hostetter, MD  
Louis Muglia, MD, PhD  
Kristine Justus, PhD

Editorial Staff:

Tim Bonfield/Managing Editor  
Tom O'Neill, Nick Miller

Contributor:

Mary Silva

Design and Illustration: The Fairview Agency  
Photography: Julie Kramer, Michael Wilson

*Research Horizons* is published by the Cincinnati Children's Research Foundation to showcase work that changes outcomes for children.

Produced by:

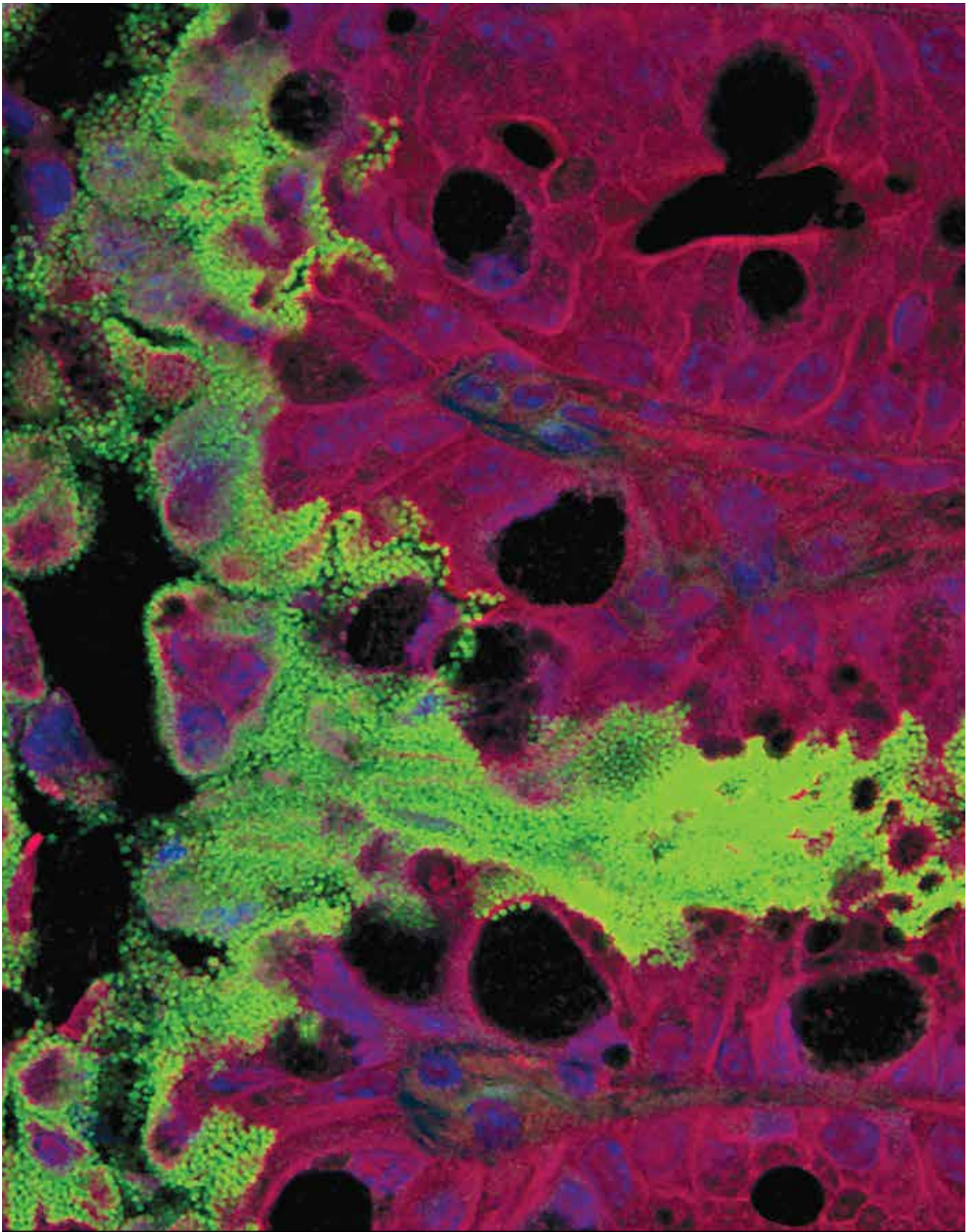
Department of Marketing and Communications  
Cincinnati Children's  
3333 Burnet Avenue, MLC 9012  
Cincinnati, OH 45229-3026  
513-636-4420

To receive this publication digitally, sign up at  
[www.cincinnatichildrens.org/email-rh](http://www.cincinnatichildrens.org/email-rh)

If you wish to unsubscribe, call 513-636-4420  
or send an email to [marketing@cchmc.org](mailto:marketing@cchmc.org).

Cincinnati Children's is a teaching affiliate of the  
University of Cincinnati College of Medicine.

© 2017 Cincinnati Children's



This confocal image shows bacteria (green) interacting with cells (red) in the intestine. It is from the lab of Theresa Alenghat, VMD, PhD, whose research is featured on page 26.





Cincinnati  
**Children's**<sup>™</sup>

3333 BURNET AVE, MLC 9012  
CINCINNATI, OH 45229-3026

Nonprofit Org.  
US Postage

**PAID**

Cincinnati, Ohio  
Permit No. 4167

## IN THIS ISSUE

Infectious Diseases  
Immunobiology  
The Microbiome

To receive research updates from  
Cincinnati Children's by email, sign up at  
[www.cincinnatichildrens.org/email-rh](http://www.cincinnatichildrens.org/email-rh)

BRV210767