JOHNS HOPKINS
InsideTract

NEWS FROM THE JOHNS HOPKINS
DIVISION OF GASTROENTEROLOGY AND HEPATOLOGY

SUMMER 2015

JOHNS HOPKINS
MEDICINE

Taking Aim at a Bad Gene

With an ambitious plan to cure cystic fibrosis, Liudmila Cebotaru hopes to prevent deaths caused by its infiltration of the GI tract.

What if you could replace the faulty gene that causes cystic fibrosis with a complementary version of that gene that restores its function?

Delivered via a harmless virus, the new gene would get to work providing the instructions for restoring the function of the native protein that regulates the production of mucous, sweat, saliva and digestive enzymes—all the things that cystic fibrosis dangerously disrupts.

Liudmila Cebotaru, whose research at Johns Hopkins includes engineering that gene and the virus to carry it to its destination, never loses sight of her goal.

"We're looking for a cure for cystic fibrosis," she says.

Cebotaru says that Americans are living longer with cystic fibrosis. Antibiotics and physical therapy are helping manage the pulmonary problems that plague patients. But eventually, cystic fibrosis takes a dramatic toll on the pancreas, the liver and the intestines. Blocking ducts and poorly managing the body's water system, the disorder creates enormous—and ultimately fatal—inflammation and infection.

"It's a genetic defect," says Cebotaru, "so the only way to cure it is by fixing that gene."

Because cystic fibrosis stems from a mutation in a single gene, Cebotaru calls it a good candidate for gene therapy. She and her Johns Hopkins lab team have spent the past 10 years engineering a new gene to correct the mutation that causes roughly 70 percent of all cystic fibrosis cases.

"A lot of that time was spent searching for the gene flaw," she says. "It took years to find it."

Once they identified how the gene's mutations affected its protein function, Cebotaru and her research team engineered a virus to carry the gene to the tissue affected by cystic fibrosis.

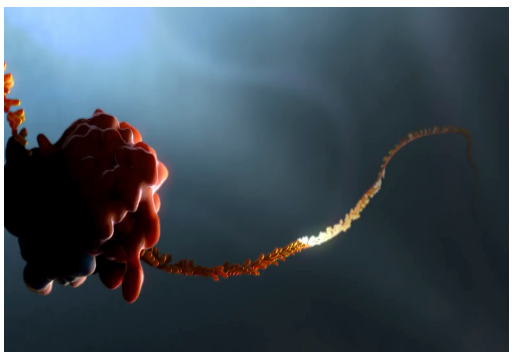
With the FDA watching closely, Cebotaru's work has shown enormous promise in animal models and it has great potential in humans, she says. After clearing a few more regulatory hurdles, Cebotaru expects the cystic fibrosis gene therapy will go to clinical trial.

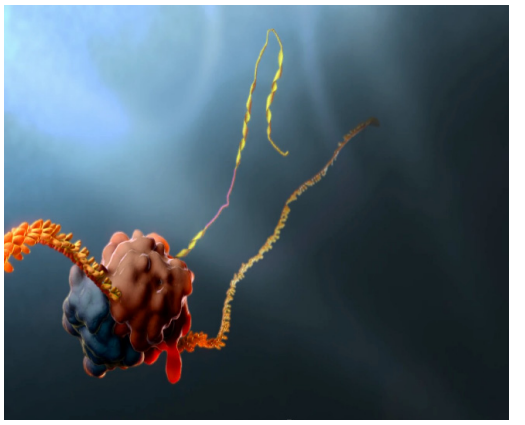
Because the lungs are the first organs to be affected by cystic fibrosis, Cebotaru says the first gene therapy will be delivered to the lungs. "We're also working on gene therapy for the pancreas and the liver," she says. "The gene is the same for those organs, but the viral vector is different."

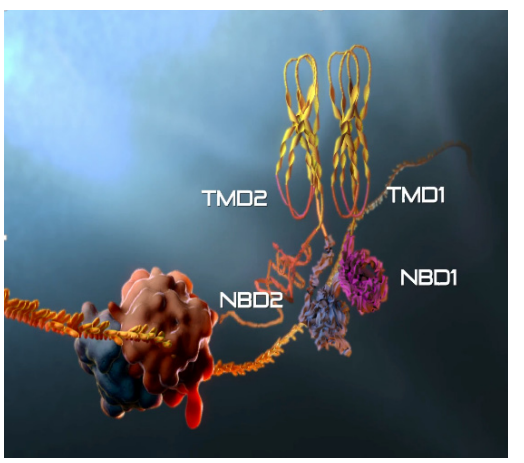
In her genetics work with cystic fibrosis, Cebotaru is also trying to solve another mystery that has long puzzled researchers. "For patients with cystic fibrosis to survive, they need a lot of antibiotics," she says. "And many patients who take a lot of antibiotics develop imbalances in gut bacteria, leading to *Clostridium difficile* infection and dangerous diarrhea."

One would expect such antibiotic regimens to wreak havoc on the gut flora of patients with cystic fibrosis. But for reasons yet unknown, says Cebotaru, when many cystic fibrosis patients get *C.diff* imbalance, they do not get diarrhea.

"I'm trying to find out why," she says. "It could teach us a lot about a problem that causes a lot of people to die around the world." ■







A string of amino acids emerges from the protein complex in the CFTR gene, whose mutation causes cystic fibrosis. Courtesy of the Cystic Fibrosis Foundation

"A LOT OF TIME WAS SPENT SEARCHING FOR THE GENE FLAW. IT TOOK YEARS TO FIND IT."

—LIUDMILA CEBOTARU

FROM THE DIRECTOR'S DESK

Tony Kalloo

One Step Beyond

One of the great joys of being on a team of the world's best gastroenterologists, hepatologists and gastro researchers is watching them constantly challenge the conventional thinking in our field.

Liudmila Cebotaru makes a bold statement in this issue of *Inside Tract:* "We're trying to cure cystic fibrosis." Engineering a gene to replace a faulty gene that causes CF is thinking big.

Amy Kim is challenging the Milan criteria, the measure that determines which patients are the best candidates for a liver transplant. She recounts a conversation with a patient who wonders how liver cancers could recur after a transplant. The question bothered her enough to devote a career to finding an answer.

When Vikesh Singh noticed that, every so often, a patient who had an endoscopic procedure involving the pancreas would develop systemic inflammatory response syndrome, he began studying data on the topic. Looking at 12 years of Johns Hopkins admissions data, Singh found a pattern that leads to both better medicine and cost savings.

In hopes of bringing relief to patients who've been told their gastric problems aren't real, Sameer Dhalla and his colleagues in our Neurogastroenterology Center are using 3-D imaging to unlock the mysteries behind the intestinal nerve system. They believe that nerves could hold the answers to questions around idiopathic gastric conditions, such as gastroparesis.

Johns Hopkins fosters a climate that encourages clinicians and researchers to ask big, bold questions. Often those challenges confirm standard practices. But sometimes they produce results that change the way we approach an issue or a condition.

PANCREATIC DISEASE

Predicting Pancreatitis

Recognizing a post-procedure reaction can save dollars and lives.

Lengthy hospital stays after pancreatic endoscopy are avoidable, says Vikesh Singh.

A journal article authored by Johns Hopkins physicians and researchers says that the absence of a systemic inflammatory response to a common endoscopic procedure could lead to shorter hospital stays and increased health care savings.

In the March issue of the journal *Pancreatology*, author and director of the Johns Hopkins Pancreatitis Center **Vikesh Singh** and his colleagues wrote that systemic inflammatory response syndrome, or SIRS, following endoscopic retrograde cholangiopancreatography, or ERCP, is an accurate, inexpensive and easy-to-obtain predictor of a severe form of a condition that frequently leads to long inpatient stays.

When patients develop or have SIRS on the second day after their ERCP, Singh says they're at high risk to develop severe acute pancreatitis, and this results in a long hospitalization.

On the other hand, patients who don't have a SIRS reaction are almost a sure bet to tolerate the procedure with no trouble. Recognizing those patients, says Singh, could reduce costs by eliminating unnecessary hospitalizations.

Studying data from 12 years of Johns Hopkins Hospital admissions for post-ERCP pancreatitis, Singh says the condition isn't common, but it is dangerous—and preventable.

Almost one in 10 outpatients develops pancreatitis after the procedure. "That's a pretty high complication rate," says Singh. Most of the cases fall into the mild to moderate range of disease severity and are hard to predict.

But Singh and his Johns Hopkins co-authors say that SIRS is a reliable predictor of which patients are likely to develop severe acute post-ERCP pancreatitis and, just as importantly, which patients are not.

ERCP uses endoscopy and X-rays to diagnose and treat problems of the biliary and pancreatic ductal systems. The endoscopist accesses the system through the major duodenal papilla. Most patients tolerate the procedure with very few problems. "But to get where you're going, there can be a fair bit of trauma to the pancreas," Singh says. "In some patients, that trauma can cause some edema, which can block the pancreatic duct."

When patients begin to show a systemic reaction to ERCP, Singh recommends the prophylactic insertion of a rectal suppository of a nonsteroidal anti-inflammatory and/or placement of a pancreatic stent to prevent the duct blockage that leads to pancreatitis.

Using SIRS as a predictor of post-ERCP pancreatitis has great implications for how long a patient needs to stay in the hospital. Singh says that patients who develop severe post-ERCP pancreatitis often require hospital stays of 10 or more days. "For so many reasons, that's what we're trying to avoid," says Singh. "This is a simple marker that gives us a lot of information."

A SIRS diagnosis, says Singh, requires only a combination of a few vital signs and a laboratory measure that's likely already in place.

"You can usually look at the vital signs that have already been done," he says. "Patients are probably getting a leukocyte count anyway as part of their routine daily labs."

Singh has done other research on SIRS and its predictive power.

"The negative predictive value of SIRS is almost perfect in this case," says Singh. "If you don't have that systemic inflammatory response after ERCP, we can be pretty sure you won't develop severe pancreatitis either." ■

To see a video of Vikesh Singh discussing treatment options for pancreatitis, please visit http://bit.ly/pancreatitis_treatments.

LIVER TRANSPLANT

Beyond Milan

Amy Kim says not all liver cancers are created equal.

There's more to cancer than the size of the tumor. **Amy Kim** believes that the Milan criteria—the formula adopted around the world in 1996 to determine the need and suitability for a liver transplant in patients with hepatocellular carcinoma (HCC) or cirrhosis—might be due for an update.

Twenty percent of patients who have cancer-related liver transplants have recurrences within two years of their transplant. Kim believes the technology and expertise exist to improve on the Milan criteria.

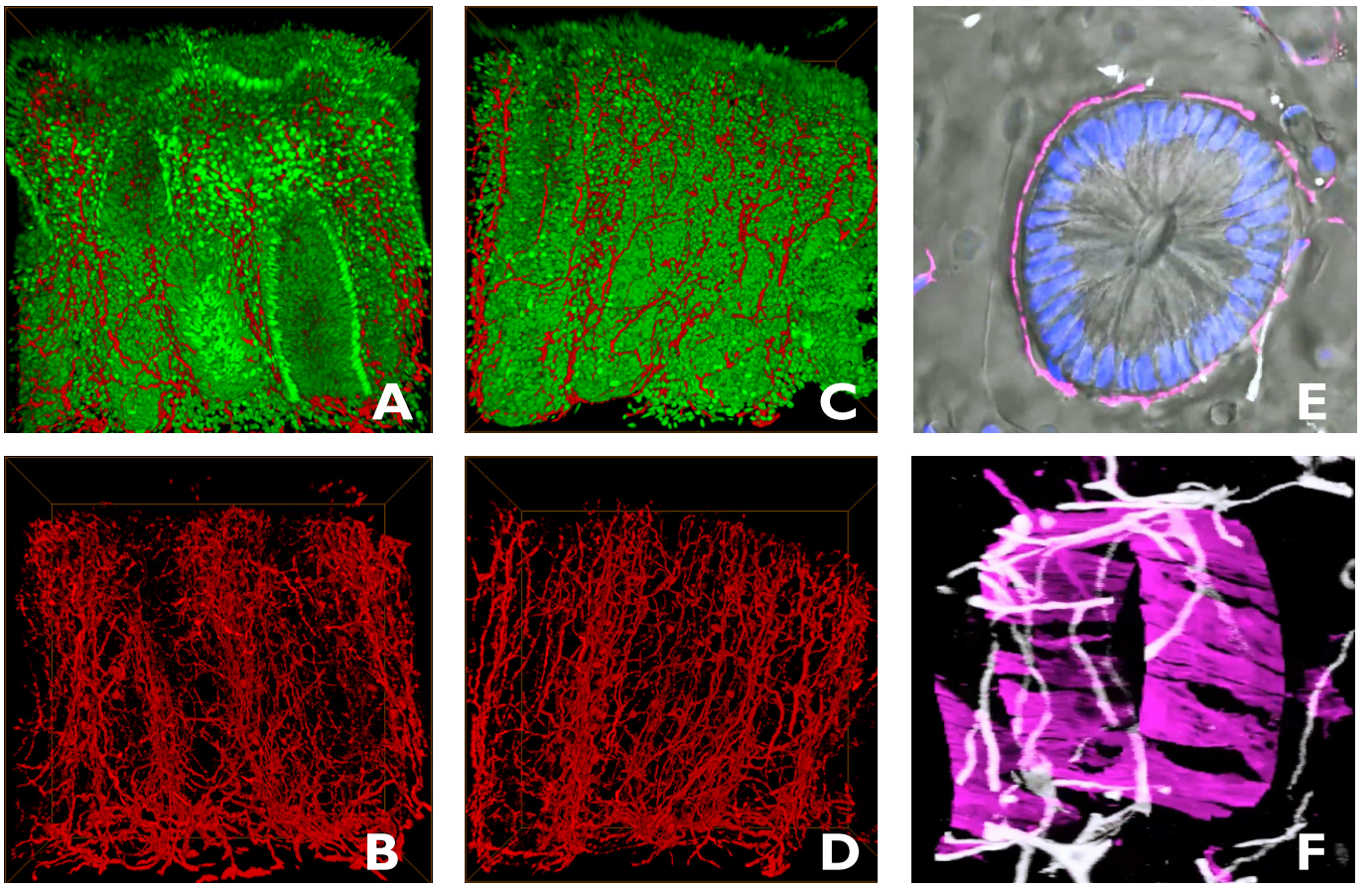
"I'm interested in identifying those recurrences sooner and managing them better—even before we do the transplant," says Kim.

She recounts a memorable experience during her transplant fellowship at Yale, when a patient asked her how a liver cancer could return after a transplant. "The simplest answer I could offer was that it had already metastasized. But it made me wonder how the cancer, specifically HCC, could metastasize when transplants are limited to patients with very small tumors and that there wasn't even microscopic evidence of vascular invasion.

"The patient's question boggled my mind for months."

The Milan criteria states a short list of guidelines to assess whether patients are good liver transplant candidates.

(continued on back page)



Mapping the Nerves

A. A mucosal pinch biopsy from an asymptomatic patient's rectum shows cells in green and nerves in red. **B.** The same biopsy shows nerves only. **C.** A similar biopsy of an asymptomatic patient's left colon shows both cells and nerves. **D.** Part of the nerve network of the left colon. **E.** The image stack reveals the network of cells, proteins and nerves in a colon biopsy sample. **F.** The same sample, converted to a 3-D, full-thickness view of the nerve system.

A Lot of Nerve

Taking a 3-D look at the enteric nervous system.

Working to solve problems like irritable bowel syndrome and gastroparesis, **Sameer Dhalla** and the team at the Johns Hopkins Center for Neurogastroenterology are venturing into the uncharted cartography of the enteric nervous system.

By modifying their endoscopic biopsy and sample staining techniques, the center's physicians and researchers are producing three-dimensional images of the nerve networks in patients' GI systems.

"Standard techniques used in GI histology can't show us the nerves that we think hold the clues to some of these idiopathic conditions," says Dhalla. "Sliced sections in 2-D under a standard microscope are great for identifying cancer or overt inflammation. But information about nerves is lost in this process.

"Nerves in the GI tract don't reside in a plane. They're interconnected and have depth. To begin to understand their role in health and disease, we had to find a way to see these nerves in three dimensions."

Dhalla's mentor, neurogastroenterolgy director Jay Pasricha, along with Johns Hopkins GI postdoctoral fellow Ya-Yuan Fu, had previously developed specialized sectioning and staining techniques to highlight nerves in 3-D from large surgically removed samples of the intestines. "My hunch was that these same techniques might work when applied to the superficial biopsies we routinely obtain during endoscopy, and thus could be more readily incorporated into clinical practice in the future."

Three-dimensional imaging of the superficial enteric nervous system is in its early stages, but Dhalla believes he and his colleagues are onto something.

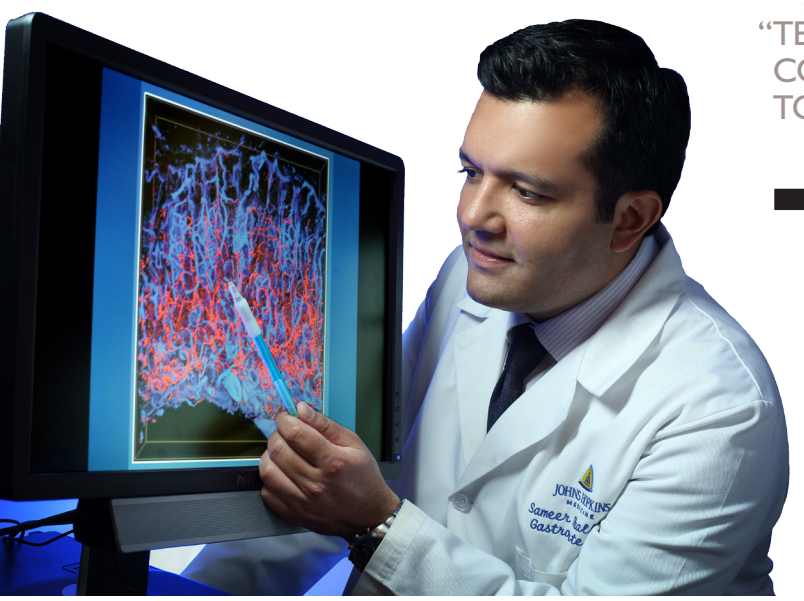
Dhalla, Pasricha and Fu performed a pilot study that provided a clue to the mysteries of idiopathic gastroparesis. "We looked at nerves in normal stomachs and compared them to nerves in the gastroparetic stomach," says Dhalla, "and we found that there were some significant differences in nerve density." These findings were presented at the recent Digestive Disease Week 2015 conference in Washington, D.C.

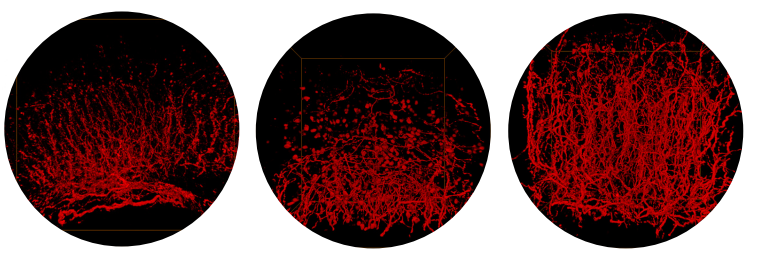
"We see patients with debilitating symptoms who've often been told the problems are 'in their head' when all their tests come back negative," Dhalla says. "More likely to me is that our current diagnostic tests are limited when it comes to the nerves in the gut … but we aim to change that paradigm."

Dhalla hopes that the 3-D imaging will lead to a better understanding of the unexplained conditions seen in the Johns Hopkins neurogastroenterology clinic in order to help tailor treatment decisions for patients. "It will take time," he says. "But we're beginning to draw an atlas of the complicated networks of nerves that drive digestion." ■

"TESTS ARE LIMITED WHEN IT COMES TO NERVES, BUT WE AIM TO CHANGE THAT"

—SAMEER DHALLA





Three-dimensional confocal imaging allows deeper photon penetration and reveals deeper tissue layers and nerves without microtome sectioning.

Beyond Milan *(continued from page 2)*

Ideal patients can't have cancer outside the liver, and there can be no major blood vessel involvement. No lesion can be larger than 5 centimeters or three or fewer lesions must measure less than 3 centimenters each.

But the criteria fails to take into account what Kim says is a vital element.

"We don't look at the tumor biology," says Kim. She says that the size of a tumor is a poor indication of a patient's health. "Essentially, the criteria says the larger the size, the higher the risk of recurrence. But that's such a generalized criteria. Each liver cancer is different."

And considering the scarcity of donor livers, she wonders if livers are going to the cases with the greatest need.

Kim says patients can be left off the transplant list because of tumors that shouldn't disqualify them. "Even if a tumor looks really bad, very often its biology tells us it won't come back once you take out the liver."

The Model for End-Stage Liver Disease (MELD) is another numeric scoring system to assess the severity of chronic liver disease. People with cancer get extra MELD score points, bumping them higher up the liver transplant list. "Sometimes that makes sense, but there are lots of times it doesn't," says Kim.

Kim's research aims to better predict which patients are ideal transplant candidates. Along with Johns Hopkins professor of medicine and oncology **Stephen Meltzer**, Kim does research into precancerous biomarkers.

"Transplant hepatologists are bound by certain limitations," Kim says. "For instance, often, it's only when the liver is outside the body that we see things like some of the vessels already having tumor invasion. That's why I wanted to look into biomarkers."

She's also working to see if tumor cells that circulate in the bloodstream might be a predictor of post-transplant cancer recurrance.

"If we find that circulating tumor cells correlate with post-transplant liver cancer recurrence, it could improve the selection of transplant candidates," Kim says. "Donor livers would go to those with the least risk, while patients with little benefit would be spared a major abdominal surgery and commitment to immunosuppression." ■

CONTACT INFORMATION

Gastroenterology Central Scheduling
410-933-7495
The only number you need to refer any patient to any Johns Hopkins GI service

Gastroenterology and Hepatology Resource Center
www.hopkins-gi.org

Hopkins Access Line (HAL)
Your 24/7 connection to Johns Hopkins full-time faculty in any subspecialty
410-955-9444 or 800-765-5447

Online Referral Directory
Find a Johns Hopkins physician by name, specialty and more
hopkinsmedicine.org/doctor.

JOHNS HOPKINS InsideTract

CHANGE SERVICE REQUESTED

Non-Profit Org
U.S. Postage
PAID
Permit No. 5415
Baltimore, MD

Inside Tract is one of many ways the Johns Hopkins Division of Gastroenterology and Hepatology seeks to recognize and enhance its partnership with its thousands of referring physicians. Comments, questions and thoughts on topics you would like to see covered in upcoming issues are always welcome.

This newsletter is published for the Division of Gastroenterology and Hepatology by Johns Hopkins Medicine Marketing and Communications.

Johns Hopkins Medicine
Marketing and Communications
901 S. Bond St./Suite 550
Baltimore, MD 21231

Gastroenterology and Hepatology
Anthony N. Kalloo, M.D., *Editor*
Email: akalloo@jhmi.edu

Marketing and Communications
Dalal Haldeman, Ph.D., M.B.A., *Senior Vice President*
Patrick Smith, *Writer*
Justin Kovalsky, *Managing Editor*
Email: jkovals1@jhmi.edu
Max Boam, Lori Kirkpatrick, *Designers*
Keith Weller, *Photographer*

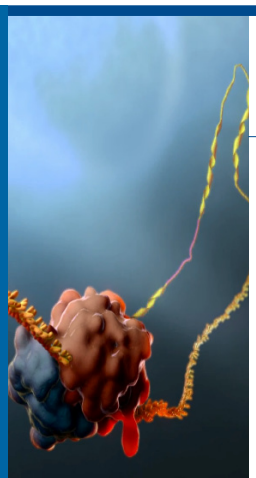
JOHNS HOPKINS InsideTract

SUMMER 2015

JOHNS HOPKINS MEDICINE

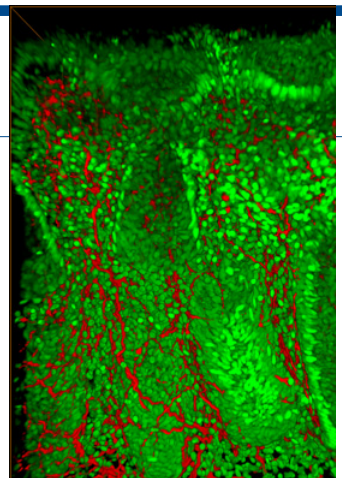
NEWS FROM THE JOHNS HOPKINS DEPARTMENT OF GASTROENTEROLOGY AND HEPATOLOGY

Inside



1 Taking Aim at a Bad Gene

2 Predicting Pancreatitis



3 A Lot of Nerve