

## Research Briefs

### DECREASING INFLAMMATION

Johns Hopkins researcher **Neeraj Vij** has been studying the mechanisms that lead to increased inflammation in CF airway cells. His findings demonstrating that CFTR represses the inflammatory responses in airway cells have recently been published in *PLoS One* (2009;4(2):e4664). CFTR acts to inhibit the function of the inflammatory mediator NFkappaB. This inhibition is lost in CF airway cells leading to increased inflammation. These studies hope to identify targets for drug therapy to decrease inflammation in the CF airway.

### MRSA AND LUNG FUNCTION

**Dr. Elliott Dasenbrook** and his colleagues in the Johns Hopkins Adult CF Program have recently published their results on the effect of methicillin resistant *Staphylococcus aureus* (MRSA) on lung function. This study, published in the *American Journal of Respiratory and Critical Care Medicine* (2008;178(8):814-21), investigated the effect of MRSA acquisition in over 17,000 patients in the CF Foundation Patient Registry. These investigators found that patients who have MRSA in their sputum have a faster decline in lung function than those patients who do not. It is not clear if MRSA can be eradicated once it is found in the sputum. However, these findings have led the physicians at Johns Hopkins to treat new acquisition of MRSA more aggressively in hopes of eradicating this infection.

### HOPKINS NOW A TRANSLATIONAL RESEARCH CENTER

The CF Foundation created the Therapeutics Development Network (TDN) to facilitate clinical trials and speed up the process of drug development. Johns Hopkins was one of the original 12 centers that made up the TDN. But over the past 10 years the TDN was expanded first

(continued on page 4)



**No single CFTR-targeted drug may work for all patients, says pulmonologist Pam Zeitlin, but all patients may have an alternative chloride channel waiting to be activated.**

## Alternative Chloride Channels

CFTR isn't the only kid on the block when it comes to chloride channels. CIC-2, pulmonologist Pam Zeitlin is finding, may provide a parallel pathway for chloride secretion.

**A**t the root of cystic fibrosis (CF) and its symptoms is chloride transport—or the lack of it because the cystic fibrosis transmembrane conductance regulator, or CFTR, is defective in CF patients. But pulmonologist **Pam Zeitlin** and her team have found that a chloride channel highly expressed in fetal airway epithelia may provide a parallel pathway for chloride secretion with the right stimulation.

“CFTR is very important to normal function of the airway,” says Zeitlin, “but it isn't the only chloride channel.”

Zeitlin was referring to chloride channel-2, or CIC-2, which helps secrete chloride from the lungs during fetal development. Zeitlin

studied fetal CIC-2 in mouse and rat models, and was convinced the channel might be a surrogate for the CFTR channel in humans. At birth and with the first breaths of life, CIC-2 is turned down or “down regulated,” though its function may continue with the right stimulation.

“With the right drug,” Zeitlin says, “we could activate what's left of CIC-2 in the airway and treat the CF.”

The right drug might be an agent like lubiprostone, which has been approved as a treatment for constipation. Lubiprostone, Zeitlin explains, has been shown to turn on chloride secretion in the gut and hydrate the stool. Taking that finding, Zeitlin and pulmonologist **Kelvin MacDonald** found that

(continued on page 2)

## Upcoming Clinical Trials

### TIGER-2

The Johns Hopkins CF Center is currently enrolling both children and adults in a phase 3 study of Denufosal™ (Inspire Pharmaceuticals) called TIGER-2. Denufosal™ is an inhaled drug that activates an “alternative” chloride channel on the airway surface to bypass the defective CFTR that causes CF. The TIGER-2 trial will determine if Denufosal™, inhaled three times daily, is more effective than placebo. The trial will last 18 months. Previous studies have shown that Denufosal™ improves lung function and is well tolerated. If the TIGER-2 trial proves that Denufosal™ is effective then Inspire Pharmaceuticals will apply to the FDA for permission to market this drug.

### INFANT STUDY OF INHALED SALINE (ISIS)

Researchers at Johns Hopkins will begin studying the effect of hypertonic saline in children less than 5 years old this summer. Hypertonic saline is often prescribed for older children and adults. However, it is unknown if this therapy will help younger children who have good lung function. This 18-month study will investigate if pulmonary exacerbations can be prevented by hypertonic saline inhaled twice daily. The ISIS trial is the first of several studies that will be testing drugs in younger children with CF. The goal of these trials is to intervene early to prevent permanent lung damage.

### BRONCHITOL

Bronchitol™ (Pharmaxis) is a dry powder formulation of the sugar mannitol. This inhaled drug is designed to draw water into the airway to hydrate and thin the mucus. If Bronchitol™ is effective it should prevent obstruction of the small airways and subsequent infection and damage. Studies from Australia have shown that Bron-

(continued on page 4)

## Just add a little salt and water...

...what could be simpler? This approach is what is behind several new therapies described in this issue of *Partners in Discovery*. Cystic fibrosis (CF) is caused by abnormal salt and water transport into and out of cells. The defect in CFTR leads to thick, sticky mucus in the airways of CF patients. New therapies are being developed to enhance chloride transport via alternative chloride channels (Denufosal) or pull water into the airway (Bronchitol and hypertonic saline). Even more exciting is the possibility of correcting the defective CFTR with drugs such as VX-770, VX-809 and PTC-124.

We are entering a new era of CF research. The drugs on the horizon are not going to just treat the symptoms of CF, but they will actually correct the basic salt transport defect in CF. Bringing salt and water into the airway will hydrate CF mucus, prevent obstruction of the small airways and the resulting infections and prevent lung damage.



The CF Foundation has made a commitment to expedite clinical trials of new drugs via expansion of the Therapeutics

Development Network (TDN). Researchers at Johns Hopkins are committed to bringing these new therapies to the market, but we need your help. Please do your part by supporting the CF Foundation or by participating in a clinical trial.

Peter J. Mogayzel Jr., M.D., Ph.D.  
*Director, Cystic Fibrosis Center  
 at Johns Hopkins*

### Alternate Chloride Channels

(continued from page 1)

the direct application of lubiprostone in the nasal airway of CF mouse models restored nearly normal levels of chloride secretion in the nasal epithelial (*American Journal of Lung Cellular and Molecular Physiology* 295: L933-L940, 2008). Now they're beginning to look for other drugs that modulate CLC-2 expression in the lung.

"We need to take what we're learning about the properties of the channel and the mice and go back and do another trial," says Zeitlin. "We're not there yet, but we think it's very promising. We're leaving no potential stone or good idea unturned."

Zeitlin adds, "In this inherited disease, in which there are different types and combinations of mutations, it's possible that no single CFTR specific drug would work for everybody. But if everybody has an alternative chloride channel that could be activated, then that might bring quite a bit of benefit." ■

## Connecting CFTR Mutations to Clinical Outcomes

Database to have important implications for diagnosis and treatments for patients and families carrying rare mutations



Patrick Sosnay, M.D.

**P**ulmonologist **Patrick Sosnay** says he got involved in studying cystic fibrosis because it's a single-gene disorder that makes the leap between the bench and the bedside a short one.

"The scientific discoveries happening in CF get to the patient quicker than in any other condition," says Sosnay. "It's pretty easy to see how the research we do day to day can affect patients we see in clinic."

So Sosnay has gone deep in investigating that single gene, cystic fibrosis transmembrane conductance regulator, or *CFTR*, and the some 1,500 mutations associated with it. Among his questions, how do these mutations behave, and why do certain mutations result in more severe disease than other mutations?

"Lung function is the biggest determinant of how well someone with cystic fibrosis does, but there are some CF patients with normal lung function and others who need a lung transplant," says Sosnay. "We're beginning to understand that the difference is in which CFTR mutation they have. It's not the only part, but it's a big part of that difference."

Now, in collaboration with Hopkins geneticist **Garry Cutting**, Sosnay is developing a database of mutations of the *CFTR* gene to help get at the answers. Surveying some 35,000 CF patients throughout the world, Sosnay and Cutting are correlating each genetic mutation with its clinical course and outcomes—including lung and pancreatic function and severity of disease.

"Are there certain mutations where patients do worse? Or better?" Sosnay says. "We're trying to do an in-depth analysis of this."

The platform for the information will be a Web site accessible by researchers but also patients and their families. The site will represent the largest ever database of CF patients and their genotypes and phenotypes. Sosnay recommends that everyone diagnosed with CF have his or her DNA tested to determine which mutation they have. They can then go to the Web site, look up their mutation and its related clinical information.

"This is especially important because there are new therapies being developed to target certain

**"Are there certain mutations where patients do worse? Or better? We're trying to do an in-depth analysis of this."**

—PATRICK SOSNAY, M.D.

mutations," says Sosnay. "So if someone has a newborn with a diagnosis of cystic fibrosis, this is a database they'll be able to go to and get information relevant to their child."

Sosnay adds that the Web site should go public this summer, and will be coupled with a cystic fibrosis mutation database at the Hospital for Sick Children in Toronto. ■

# Kelvin MacDonald, M.D.

Making a Difference in Nasal Potential Difference

**Pulmonologist Kelvin MacDonald is measuring potential alternative chloride channels in CF mouse models that may result in new therapies for CF patients.**



A respiratory therapist for 20 years before he headed off to medical school, a research bench was the last place where Kelvin MacDonald thought he'd find himself. But early on in his pulmonary fellowship at Johns Hopkins, MacDonald found himself deeply immersed in looking at phenotypes of CFTR (cystic fibrosis transmembrane conductance regulator) and CIC-2 (chloride channel-2) knockout mice, and a CF researcher was born. Now he's director of the Nasal Potential Difference Laboratory at Hopkins CF Center. Here he discusses some of his work.

## Are you surprised to find yourself in a basic science lab studying CF?

Somewhat. I thought I was going to work in critical care, but I realized in medical school that I liked making connections with families and following the progression of a disease. I enjoy caring for people with CF. But CF research was a big focus of my four-year fellowship here, and for me it turned out to be a natural. I knew my career in pediatric pulmonology would be very different if I had accepted a training position at a program not so academically focused – it would be more clinically focused. But I still get to see patients who are participating in our trials.

## So, what specifically are you working on?

Early on in Dr. [Pam] Zeitlin's lab, we worked with CFTR and CIC-2 knockout mice, and explored what happens when you crossbreed the two strains. Interestingly, children with CF have normal lung development up until the day they're born, and you really can't tell whether they have CF at birth. So we asked, what happens in lung development in mice if you knock

both of those chloride channels out. It turns out the mice don't survive. Knock out either one—the CFTR or CIC-2 channel—and they're fine.

## Where did that leave you?

It suggested that CIC-2 had properties for chloride transport like CFTR, and therefore would be a good target for CF therapy (see "Alternative Chloride Channels," page 1).

## How did you go about studying CIC-2?

Interestingly, cystic fibrosis doesn't occur in animals, so we used transgenically engineered mice. But we were challenged with trying to measure lung mechanics and the elastic properties of the lung and airways without sacrificing the mice. We collaborated with Wayne Mitzner, a world-wide recognized expert on lung pathophysiology and mouse models at the Johns Hopkins Bloomberg School of Public Health. Adapting the mouse model he developed to study asthma and chronic obstructive pulmonary disease, we've been able to demonstrate the ability to do sequential lung mechanics in mice. While this work was

going on, I started collaborating with Dr. Zeitlin on ways to measure nasal potential difference in these mouse models.

## And what did you find?

It turns out the nasal potential difference measurement of a transgenically engineered CF mouse replicates that of a person with CF. We wanted to see how a class of drugs called prostones acted on the CIC-2 channel, which is of special interest in our lab. We found that we could do this potential nasal difference on the CF mouse and reverse the basic defect of lack of chloride transport. And we could do it over and over again. Also, we could take normal mice that express CFTR, block the CFTR with a chemical, and then use prostones to stimulate the chloride secretion. So this was pretty exciting.

## How's that?

CIC-2 has been a target of cystic fibrosis therapy for more than 10 years but no one has been able to demonstrate in a pharmacologic way how to activate it. Finally, we identified a compound that did just that. But there is a problem. It turns out the prostone compound

we used, lubiprostone, is easy to wash off the cell surface so maybe it's not the best drug. We are now looking at other prostone compounds with our research partners, Sucampo Pharmaceuticals in Bethesda, MD. Currently, we're trying to collect enough preliminary data to approach the CF Foundation's therapeutics development network for support for eventual human trials in the CF community.

## So what's next?

Once we have enough preliminary data, we could start applying a topical drug in the nose and measure ion transport. That would likely escalate to an aerosol therapy. While we continue this work, we're also stepping back to an old technology called the Ussing chamber, which is used to measure ion transport across an epithelium. We're trying to see how placing the prostone compounds on sheets of human cystic fibrosis epithelial cells stimulate ion secretion in the cells—how much and for how long. So we're really getting down to the basic cellular level of how salt is moving in and out of that top airway layer.

## How does that translate into a therapy?

The "fluid" model in CF suggests rehydrating the airway is a key therapeutic goal. The adult airway's normal state is fluid absorptive, so the idea is to use chemicals to trick it back into its secretive fetal state. Hypertonic saline and the investigational drug Denufosal act this way. More importantly, the CIC-2 channel may play a role in how epithelial or outer layer cells in the airway join together, a process called tight junctions, which is disturbed in CF. CIC-2 activation with a pharmacologic agent could actually repair part of that, which could help regulate salt and water movement in the airway.

## Are you optimistic this research will result in new therapies?

If we can demonstrate that we can hydrate the airways and repair tight junctions, that will have therapeutic implications for CF as well as other airway diseases, like asthma and chronic obstructive pulmonary disease. But because people are still dying from CF, we have to cure that first. ■

# Correcting Vitamin D Deficiency



Deanna Green, M.D.

“These findings are a big wake-up call not only because they show that many children with CF are lacking vitamin D, but also because the deficiency persists even in those children who are treated with weekly doses twice or three times as high as the current recommendations.”

—DEANNA GREEN, M.D.

Existing recommendations for treating vitamin D deficiency in children with CF are too low, leaving most at high risk for bone loss and rickets, according to researchers at Hopkins Children’s (*The Journal of Pediatrics*, October 2008). They found that nearly half of the 262 children with CF in the study were vitamin D deficient, and the majority of these remained persistently so, despite getting restorative doses equal to or higher than the recommendations set by the Cystic Fibrosis Foundation (CFF).

Growing children with CF are especially vulnerable to vitamin D deficiency because a hallmark of their condition is poor absorption of nutrients and malnutrition.

As a result of the findings, Hopkins amended its treatment protocol to treat pediatric CF patients who have vitamin D deficiency with 50,000 IU daily for four weeks. However, this regime was also not effective in correcting the problem. Therefore, patients at John Hopkins now receive an extra 1000 IU of a different form of vitamin D termed cholecalciferol or vitamin

D<sub>3</sub>, each day. Patients receive twice that dose if they are vitamin D deficient.

“These findings are a big wake-up call not only because they show that many children with CF are lacking vitamin D, but also because the deficiency persists even in those children who are treated with weekly doses twice or three times as high as the current recommendations,” says pulmonologist **Deanna Green**, who led the research. “Clearly there is an urgent need to find more effective ways to restore healthy vitamin D levels.” ■

## Upcoming Clinical Trials

(continued from page 1)

chitol™ can improve lung function in CF patients. The current phase 3 trial is being held at Johns Hopkins and CF centers around the world to determine whether Bronchitol™ can be safely administered to CF patients and if it will improve lung function and prevent pulmonary exacerbations.

## DRUGS THAT FIX THE BASIC DEFECT IN CF

VX-770 (Vertex Pharmaceuticals) is designed to activate or “potentiate” abnormal CFTR that is located on the cell surface. Early studies suggest that this oral medication can improve chloride transport in patients with certain CFTR mutations. VX-809 is a “corrector” that is designed to traffic  $\Delta F508$  CFTR mutations to the cell surface. PTC-124 (PTC Therapeutics) is designed to treat patients with stop codon mutations (those that end with an X, for example W1282X). Previous short-term studies have shown promising results suggesting that PTC-124 can improve chloride transport in patients. Trials of all of these compounds will be starting this summer at Johns Hopkins and other sites around the country. ■

## Research Briefs

(continued from page 1)

to 18 and now greater than 70 centers. A core group of centers have been designated “Translational Centers” because they have specialized research capabilities needed for early phase 1 and 2 trials. Johns Hopkins has received this prestigious designation because of its unique research resources such as nasal potential difference measurements, infant pulmonary function testing and mucociliary clearance testing. ■

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