[MUSIC PLAYING]

DREW FERANCHAK: Thank you very much. It's great to be in the Burgh again. Today, I'm going to talk a little bit about our research. But I'm also going to talk more broadly about cystic fibrosis and how it applies to the disease of cystic fibrosis.

So in 1989, the gene for cystic fibrosis was discovered. And it was a group effort by three independent research laboratories that combined their research efforts at the end to really speed up the discovery. And to make sure there wasn't any problem with authorship of the papers, they published each of the papers in the same journal of *Science* in September of 1989. And the work was led by these three individuals from the three laboratories-- Francis Collins, who was at the Howard Hughes Institute at University of Michigan, and Lap-Chee Tsui and John Reardon who were at SickKids Hospital at Toronto.

The gene was on chromosome 7. It was a single gene that the investigators named the cystic fibrosis transmembrane conductance regulator, or CFTR. The most common mutation of this gene resulting in cystic fibrosis was in this nucleotide binding fold. And this is a cartoon image that accompanied the article with what the investigators thought CFTR may look like with the transmembrane spanning domains, the nucleotide binding folds, the regulatory domain.

Well, as you can imagine, there are a lot of excitement when this was discovered. I remember myself I was a second year medical student right here in Pittsburgh. And so some of the headlines in some of the editorials around that time were, well, this is a milestone of major importance. It's a gene discovery that heralds the cure for common genetic disease. The cure for cystic fibrosis is in sight.

And while the editorial in the journals was a little less optimistic, it, nonetheless, was a very positive. It said "The discovery of the gene makes possible its manipulation and insertion into experimental systems, thus bringing the day of therapy and cure much closer."

Well, here we are. We're about 28, 29 years later and we still haven't discovered a cure for cystic fibrosis. However, we've made a lot of strides in the treatment of children with cystic fibrosis. And so at the beginning of the century, most children died in infancy. And here we are, now, where the medium life expectancy is in the 40s. So really there has been remarkable strides. And I'm going to talk today a little bit about some of the advances in the field that accounted for that.

So today I'd like to talk about these areas related to fibrosis by answering several questions. So I'd like to first talk about why is it so common? What is cystic fibrosis? How do we diagnose it? And what is the relationship between the sweat, the blood, and the genes? What is CFTR's function? And how does an abnormal CFTR lead to the disease of cystic fibrosis? And lastly, we'll talk about therapeutic strategies focusing on the role of alternate chloride channels in CFTR modulators.

Now I'd like to do this in the context of the patient at the bedside and the scientist in the laboratory and vice versa. And I'd like to do it also in a historical frame-- going from the dark ages all the way up to the present day. So in a way, if you put this all together, I'd like to think about this as a historico, scientifico, technological perspective, if you will.

So at the time, this was one of the editorials that accompanied this article that was not very optimistic. And it talked about the scientists' role. And it said, "Early research did not seem relevant to the cystic fibrosis problem. It was pursued in the quest for extended knowledge not practical application. And at times, legislators get impatient with scientists who emphasize such research implying that while scientists may prefer it, society does not need it."

And I'd like to focus today on the scientists and think about how do we make discoveries, how do those come about? What was the technology that led to some of these areas? A lot of times we're working in the lab and don't really realize that.

So like the movie *Apollo 13,* if you saw, that I liked that movie. And while the astronauts were truly courageous, it was really that the scientists on the ground that were the true heroes of that movie, if you recall that.

So in a quote of Francis Collins, he wrote "This is not just an academic exercise by a bunch of nerdy gene hunters. This is an effort that will transform medicine." And I think that's largely true. And we'll talk about that today in some of the subsequent discoveries that have really transformed the field.

OK. So let's start and talk a little bit at the beginning about incidence. So why is cystic fibrosis so common? The incidence is about one in 2,400-3,500 live births depending on the area. It's the most common potentially fatal genetic disease in the Caucasian population. It's an autosomal recessive. The carrier frequency is about one in 20 in the Caucasian population. So that's really astounding, if you think about that, for a disease that was fatal in infancy until relatively recently. How did that gene perpetuate in the population?

Well, one theory is that that organism-- and I know Charlie knows what that is because he's an expert in the field but-- cholera. It's existed since the beginning of recorded history. There's been seven pandemics since 1817 arising in the delta of the Ganges River. It's the greatest toll on human lives of all bacterial diseases.

And this is a quote from one of the scientists early on. It said, "The disease which begins where other diseases end with death." It really does happen quickly. Right? And if you were at the time looking at the journals-- this would be the headlines during one of the outbreaks showing death just coming and reaping death le cholera throughout one of these pandemics.

What is the mechanism of cholera induced diarrhea? What is it? Why is it so deadly?

Well cholera attaches to the enterocyte. It elaborates in a toxin which increases cyclic AMP, drives chloride secretion from the apical membrane. So water follows. So sodium and water follow. So there's just voluminous diarrhea that takes place in this disease. And so patients become dehydrated very rapidly. So what is that chloride channel that cholera is attacking? Well, it turns out it's CFTR. It's the chloride channels defective in cystic fibrosis.

So one of the thoughts is that perhaps over time that heterozygote carriers were partially protected from the effects of cholera. So during these worldwide pandemics of cholera, the patients that were protected were the ones that had in one abnormal copy of cystic fibrosis, of the cystic fibrosis gene. So that's an interesting thought.

And then it was tested. So going back to the lab, some scientists in 1994 published this article. And what they did was they looked at mice with the CF genotype. And so these were mice that either were normal-- and the normal mice are shown here-- the abnormal that mice had CF were here, without CFTR. Then the heterozygote group was in the middle. And they looked at fluid accumulation in the intestine in response to cholera toxin. And they showed that while the wild-type had significantly a lot of fluid, these mice really not much. They actually did-- the heterozygotes did have kind of an in-between response. And this was shown here, too.

So when the wild-type mice, in response to cholera toxin, there was a large increase in chloride secretion. And this is epithelial mounted in an Ussing chamber. And the heterozygotes or carriers that had one copy actually had much less chloride secretion. So this seemed to provide some evidence for that notion, that hypothesis that perhaps heterozygote carriers of the CF mutation were somewhat protected during cholera toxin.

OK. So let's talk about what is cystic fibrosis. Well, the initial description may have been back in the Middle Ages. This was one of the really early descriptions. And it may, in fact, be describing a patient with cystic fibrosis. It said, "Woe to that child which when kissed on the forehead tastes salty. He is bewitched and soon must die." And you could see there's the dance of death wood cut where death is taking a poor child away from his parents.

So early on, it was thought that maybe CF was-- it wasn't known what it was. And maybe could it be celiac disease? These are patients with diarrhea and fat malabsorption and malnutrition.

And Fanconi, in 1936, he described three cases of celiac disease with bronchiectasis. So celiac disease describing the fat absorption and diarrhea, malnutrition, combined with long findings.

But it was really Dorothy Andersen, probably, in New York in 1938 that really described the disease well. And she was at Babies Hospital. And she described infants with diarrhea, failure to thrive, and pneumonia. And ultimately, it was a fatal disease. And she did autopsy studies on 38 of those patients and found that their pancreas was very cystic. And so she gave the name cystic fibrosis of the pancreas. And that's probably where it first got defined.

We now know that that cystic fibrosis affects many organs-- so of course, the lungs, but the pancreas, liver, intestine, sinuses, the skin, reproductive organs. And within most of these organs, it causes a thick mucus to build up. So most of the epithelial surfaces or tubular organs become occluded with a very thick mucus that's hard to clear. And this is where it really leads to the most severe sequelae of the disease in the lungs.

So in 1945, Farber proposed, well, is this a disease of mucus production and termed the term mucoviscidosis to describe these thick secretions of mucus that occluded most of the ductal lumens in the body.

But it was Paul di Sant'Agnese that really described the disease well and started to think about what the underlying defect really is. He was born in 1914 in Rome, Italy. He arrived in New York in 1939 and worked at Columbia University and Babies Hospital.

In August of 1948, there was a heat wave. And many children were admitted to the hospital with heat stroke. A large percentage of those children that were brought into the hospital, about 50% of them, have cystic fibrosis. So why did this heatwave affect children with cystic fibrosis to a greater extent than other children?

So he took this observation, went to the bedside, and collected sweat samples from all these patients thinking could it be a problem with their sweat? Does that give us a clue? And this is from the paper that he published back in 1953 in *Pediatrics* . And you could see on the top are the patients with cystic fibrosis. On the bottom are the controls. And this is just showing the sweat chloride concentrations of the patients respectively.

And you can see this is a pretty remarkable finding. Right? How lucky do you get to have findings like that, right? It's pretty dramatic.

So all the patients with CF had elevated sweat chloride. Those control patients had sweat chloride levels that were much lower. And there was almost a break point right there. Right? Couldn't be clearer than that.

Well, this is a quote from the 1500s that's probably still true today. "When a new discovery is reported to the scientific world, they say, first, it is probably not true. Thereafter, when the truth of the new proposition has been demonstrated beyond question, they say, yes, it may be true, but it is not important. Finally, when sufficient time has elapsed to fully evidence its importance, they say, yes, surely it is important, but it is no longer new." And you could probably substitute for scientific world NIH study section there. And everybody has probably experienced that if you've submitted work.

So when Paul di Sant'Agnese presented these findings, he was criticized pretty heavily at the beginning. Right? So people said, oh, that's not true. It's impossible. It couldn't be that. And so there was a quote at one of the meetings he was at by a famous sweat physiologist, said, no, it's impossible. I didn't know there was a thing as a famous sweat physiologist, but-- or you could even become famous in that field, but evidently. Well, it turned out that he was absolutely right. He discovered the sweat electrolyte abnormality in cystic fibrosis, which is still the basis of the diagnosis of the disease today.

And he also utilized antibiotics for the first time in patients with CF. So he really made important contributions to the field. To this day, the highest award for scientific achievement given by the Cystic Fibrosis Foundation is named in his honor. And so he really did a lot to help patients with this disease.

OK. So how do we diagnose it? And what is the relationship between sweat, blood, and genes? Well, the first step is what I just showed you by di Sant'Agnese is to get some sweat. How do you do that? Well, there's ways to collect sweat. And there's other ways not to collect sweat.

This is a way-- it's not very practical-- but to heat up the body in any sort of way. People have tried plastic body bags or rooms where the temperature would increase over time and that would stay at a really hot, hot temperature, or applying some heat locally. All of these were fraught with errors trying to get enough sweat to make a diagnosis.

But going back to the bench, there were some scientists that came up with an idea. And this was Gibson and Cook. And what they did was they decided, well, if we applied a drug perhaps to the skin, we can get sweat in a localized area. And so they used a technique known as tophoresis where a drug is applied to the skin and an electrical current is passed. And that helps make the drug taken up by the skin. So they used pilocarpine. It's a parasympathetic mimetic. It stimulates the autonomic nervous system to produce sweat.

And so here's a cartoon depicting that. There's a filter paper underneath this electrode with the pilocarpine. And then when sweat is produced, it's collected on this filter paper.

And that's really the basis to this day for the diagnosis. So that elevated sweat chloride level that's found in that sweat of patients with cystic fibrosis, again, that cut off level right at 60 mil equivalents has held up over all these years based on those original findings.

And a more modern way to do that, to make the diagnosis, is through genetics. And many states now have screening tests and genetic panels for this. This was an older one from Texas where I'm from showing the multiple different mutations that are tested on an average specimen. This has since been revised to test even more mutations very rapidly on a simple blood spot.

However, the screening is based on an even simpler tests known as immunoreactive trypsinogen. And so the pancreas produces this. It's one of the enzymes made by the pancreas. When the pancreas is undergoing injury, these enzymes can be measured in the blood. And trypsinogen is easily measured on a blood spot.

And so all patients with cystic fibrosis seem to have injury, even in utero, to their pancreas early on. And so this IRT, immunoreactive trypsinogen, is usually elevated in patients with cystic fibrosis early on. And it serves as a screening test for the disease.

So why is it important to screen or have a screening program? And it was this study that came out showing that differences between CF patients that were screened versus those that weren't. And you can see that-- and this is the height for age Z-score-- showing that it's really important early on for nutritional support to identify those patients, those babies, with cystic fibrosis and treat them early with enzymes an appropriate diet and so they maintain weight.

OK. What is CFTR's function? So what does that protein do? How does it cause the disease?

So a major breakthrough was the ionic theory. And to talk about this a little bit, you have to understand some of the technology that went behind it. It is really amazing. It's technology that we use in our laboratory. So I'd like to take a minute and discuss that.

Really, it was these men-- Alan Hodgkin, Andrew Huxley, and the giant squid-- that together came up with this theory of the ionic theory underlying cell ion movement. And so here's the giant squid. And this is what we do in the lab to this day. That's one of the graduate students.

[LAUGHTER]

And the reason that made this possible was that the giant squid has a unique feature, which is the giant axon. it has one long axon that runs the whole body of the squid. And it's actually-- for the giant squid, it makes sense. It's able to coordinate all of its movements. And so when it tries to escape this long axon is able to send a notice so it contracts at the same time and it is able to escape prey.

But it also makes studies possible, because here is Hodgkin and Huxley's work where they can put in a catheter, a recording electrode, right into the axon. And then because it's so large, you could measure the potential difference across the membrane. And using a variety of techniques, they were able to show that this potential difference across the membrane and action potential's really the result of different conductancies of different ions. And they came up with the Hodgkin-Huxley model, which is really the next step after Ohm's law.

So really, they described the electrical properties of a cell membrane. You could think of a lipid bilayer as a simple electrical cell. And there is a conductance for each of the different ions that may be present-- a sodium, potassium, chloride-- and then really conductance for the whole cell or leak current. And you can think about that in terms of a voltage across the cell membrane, the resistance to flow, and then the flow of the ion itself based on its electrochemical gradient.

And so this is really a kind of a really brilliant observation based on those-- a lot of work with the giant squid. So for this, Hodgkin and Huxley won the Nobel Prize in 1963. Unfortunately, the squid didn't get much recognition.

But that work was then picked up years later by two men, Erwin Neher and Bert Sakmann at Max Planck Institute. And so they took those fundamental observations and decided to see if they could do this on a single cell to miniaturize that work. And they came up with this idea of patch clamp recording, where it's the same principle, but now it's applied to a single cell or a single part of the membrane.

And so they take a cell. They put a glass pipette with a recording electrode on. And if you capture one channel right here, you could actually measure the ion movement across that membrane. And it's really amazing to watch in real time. So you apply a voltage. You know the resistance. You can measure the corresponding current. And you could watch this. So when it's closed, you can watch here. Then the channel will open, open again.

And it's really one of the only technologies that I know of where you can actually see the functioning of a protein in real time right in front of you. And so if you're a geeky patch clamp scientist, you get excited when you see some channel activity like that. So Ernie and Bert, they really did a fabulous job. So they actually won the Nobel Prize in 1991 for developing this technology.

So it was really that technology which led to the ability to measure CFTR for the first time and define CFTR as a channel. And so by taking a lipid bilayer, you could take the protein, put it into a lipid bilayer, use that technology of patch clamp, and you could measure currents. And here, these are chloride currents that were measured compared to a wild-type or just a mock transected cell. So this is just a lipid bilayer without any channels in it. And it was really that observation that showed that once and for all that CFTR is, in fact, a chloride channel.

Using that same technology-- this is my mentor, Greg Fitts-- who showed that CFTR is present in liver as well and showed that it's a functioning chloride channel in the bile duct of the liver. So using that same technology, he applied the patch pipette to the membrane in response to an agonist to increase cyclic AMP, which activates the channel, so that he could activate the channel again in real time with a specific agonist. Confirming that CFTR is, in fact, a chloride channel in bile duct epithelial cells as well.

And so it turns out that these guys were absolutely right. And the name was appropriate-- the cystic fibrosis transmembrane conductance regulator. Because it turns out that not only is it a chloride channel, there's evidence now that it's a bicarbonate channel. It also regulates the function of other channels in the apical membrane, probably through binding domains, interaction with other proteins, or the act inside a skeleton.

So the name's appropriate. It's really a transmembrane regulator. Or could it be that the name was just based on the initials of their last names?

OK. So we talked a little bit about what its function. How does it cause disease? So how does the abnormal CFTR protein lead to the pathogenesis of cystic fibrosis? So the CFTR mutation is a base pair deletion in exon 10. It really is a deletion of a phenylalanine at position 508. That's right in this nucleotide binding domain region.

And that mutation is the most common in patients with cystic fibrosis, accounting for about 60% of patients. It results in abnormal protein folding. So it makes that nucleotide binding domain unstable. The protein, then, doesn't fold normally. And because of that, it gets degraded by the prednisone pathway. And so it never makes it to the apical membrane.

Well, that's the most common mutation. There's been over 2,000 mutations now described. And there's a website at Sick Kids Hospital in Toronto. They keep an active site where any new mutations are recorded there as well. And it grows every week.

So a way to think about all these mutations-- because there are so many-- is what happens to the protein. And this is a cartoon that I like. In the wild-type conditions shown here, the proteins produced and this chloride channel, the CFTR protein makes it to the apical membrane. And it functions as a functioning channel. And you can see the different classes of mutations here. And I think it's a helpful way to look at it.

So in class I mutations, no protein is produced. And so nothing makes it. In class II mutation, the protein's produced, but then it's folded abnormally. And it's degraded by the protozoan system. This is the most common mutation, delta 508 is in this class of class II.

But then you have the class III or class IV mutations where the protein is here, but it functions at a reduced capacity. So this is a gating defect. And so the chloride channel is not regulated to the same extent. So when it opens, there's not as much chloride that's permeable. And then class V and VI results in less protein at the membrane. Either it's produced in a lesser amount or it's degraded through endocytic retrieval.

So how does not having CFTR on your apical membrane or having abnormal CFTR, how does that cause disease? Well, one of the thoughts is that an abnormal or absent CFTR protein leads to defective chloride transport. That chloride transport is the driving force for secretion in many epithelium, especially ductile epithelial. And that results in thickened inspissated secretions in those organs, at a ductal lumen obstruction, which leads to epithelial cell injury, organ injury, and, finally, organ failure.

And that's pretty consistent with what we know about the clinical manifestations of the disease. That many of these organs, in fact, become filled with inspissated secretions and then leads to end organ injury and failure.

So in the liver, it turns out that that's probably true. And when you look at patients with cystic fibrosis liver disease, they have focal areas of scarring, focal areas of fibrosis. And this is H&E stain showing bile duct areas that are filled with this inspissated material. And under just an H&E stain, it looks pink. It's been characterized as eosinophilic type of material. And that's one of the hallmarks of CF liver disease-- that biliary obstruction with this thick eosinophilic-like material.

So there does seem to be some evidence that this duct clogging, plugging or clogging hypothesis is correct. If you at the causes of death in CF, of course, lung disease is number one. Transplant complications are now number two. But liver disease is third on the list. And it may be as now as patients are surviving longer and their overall lung function is improving, now we're seeing manifestations that we may have not seen before, such as the liver disease.

So with the lung disease, there's variable pulmonary function. And we can measure that with PFTs or force expectory volume. Infection also seems to play a really important role in the pathogenesis of the lung disease. And so colonization with staph aureus pseudomonas, those patients have a much worse outcome. And it's a constant struggle to fight those infections in the lung.

The liver disease, in contrast, only affects about 20% of patients with CF even with the same mutation. And that's still unknown why that happens. But it begs the question are there other genetic modifiers or environmental factors that are playing a role. So even patients with the same, for instance, the delta F508 mutation, not all of them will develop liver disease, only about 20%.

The liver disease also develops early with the onset of about median about 14 years. And then it's progressive. There doesn't seem to be a genotype phenotype correlation. And there's no evidence that infection, direct infection of the bile ducts, such as cholangitis plays a role.

So if we think about it, any of these steps along the pathway can be influenced by genetic modifiers. Likewise, environmental factors may also affect many of these steps along the mechanism of disease pathway.

So if we wanted to find treatments, we could really target any of these areas. However, probably to make the biggest impact, we'd want to start at the beginning. So target those early steps. Can we correct the CFTR protein itself? Or can we correct the defective chloride transport? And so let's talk about both of those strategies next.

First, let's talk about the role of alternate chloride channels. Can we correct chlorine transport? So if we restored normal chloride transport, perhaps we can alleviate some of these other downstream effects of the disease.

And so our lab has focused on that aspect. Can we find alternate chloride channels that we can target for treatment? Is there any rationale for that? Well, I think there is.

If you look at the CF mouse model, that the global CFTR knockout mouse turned out to be a pretty poor model to study CF because they didn't develop severe lung disease. They had very mild lung disease. However, they developed very severe intestinal disease with intestinal blockages that really looked like the human condition of meconium ileus. And so these mice died early on because they couldn't handle a regular diet. But if you were careful and fed them a liquid diet, they would get over some of those intestinal blockages.

So taking those feelings to the bench, Elaine Clark's group looked at this years ago and found that, in fact, the lung had an increased expression of alternate chloride channels, a high expression of calcium activated chloride channels, in contrast to the cyclic AMP chloride channels. However, those weren't present in the intestine.

So does that help to explain that phenotype a little bit? Well, perhaps it does that calcium activated or alternate chloride channel expression was associated with milder disease phenotype in those organs.

So can alternate chloride channels modulate disease expression in the liver? Well, first of all, where is CFTR in the liver? Well, it turns out it's not in hepatocytes. So hepatocytes don't express CFTR at all. It is found in bile duct epithelium. And in the bile duct, it's on the apical membrane.

And so here's from John Combe's group showing that there's CFTR on the apical membrane in this stain. And it turns out it's on the cells, stained here for a gamma GT label, which turned out to be cholangiocytes which are the bile duct epithelial cells.

So cholangiocytes have a prodigious capacity for secretion. And so being a Texan, I had looked that up-- prodigious means a lot. So they have a big capacity for secretion.

And they form this pattern in the liver and it looks like a biliary tree. So these ducts coalesce into larger and larger ducts until they finally lead into the large common bile duct. And it's thought that in humans, secretion by these cell types, these cholangiocytes account for about 40% to 50% of bile volume. Now, turns out CFTR is only expressed on apical membrane of these cells. And it's activated by secretin on the basolateral membrane. Increase of cyclic AMP drives chloride secretion through the channel. And it's thought that that driving for-- that chloride movement across the apical membrane is the driving force for secretion.

And so this was defined years ago by Greg Fitz's group. And looking at CFTR on the apical membrane, he described the channel. You could describe the channel in biophysical properties. And it's, again, a channel activated by cyclic AMP through PKA, PKC, dependent phosphorylation, secretin binds to the basolateral receptor on the basolateral membrane. Increase of cyclic AMP drives chloride through the membrane.

In contrast, there's other chloride channels that are present. And one predominant channel that's present is activated-- unlike CFTR, instead of cyclic AMP-- activated by calcium. And it is regulated-- unlike CFTR, which is activated by basolateral receptors-- this is activated by apical receptors. So one of the ways it's activated is through purinergic signaling. So purine, or pyrimidines, ATP, UTP, can activate purinergic receptors on the apical membrane. Increased calcium drives chloride through the channel. And so we've worked a lot on this channel in the past.

One of the ways we study it is through using chamber recordings. And cholangiocytes are a tough cell type to study, because they're hard to get to. Right? I showed you that slide. They only make up about 5% of the nuclear mass of the liver. They're intrahepatic in location. They're small in number. They're a difficult cell type.

So in the lab, we've developed some models. And so above, you can see a scanning electron micrograph of a bile duct. Here are the cholangiocytes Here they have an apical membrane. Basolateral membrane form a duct. And what we've done essentially is developed a way where we can culture those cells in culture where they will form a unified monolayer. It's polarized apical membrane, a basolateral membrane. Here they're growing on a collagen coat. So it's like filleting this open a little bit. And it's laying flat.

And so we can pop that into something called an Ussing chamber where we can measure the flecks of ions across the monolayer. So we can add agonists to either the apical or basolateral membrane. And then we can measure the short circuit current response, which is a reflection of transepithelial secretion.

In using that model, we've shown that in response to cyclic AMP, there's an increase in the short circuit current response. This is due to CFTR chloride movement across the membrane. However, there's also a big non-CFTR component. So in response to ATP applied to the apical membrane, there's even a larger increase in chloride flux across the membrane. Suggesting that perhaps CFTR isn't the predominant chloride channel driving secretion in the biliary system. Perhaps, it's this alternate calcium activated chloride channel.

And we did further studies along those lines. Again using the patch clamp technology, here's a patch pipette placed on one cell. And in the presence of a CFTR inhibitor, there's really not a lot of cyclic AMP induced current. But there is a big ATP induced current. And so in response to ATP, that channel current increases pretty dramatically, even in the presence of CFTR inhibition suggesting that this ATP is activating non CFTR chloride channels.

And then Amal Dutta in our lab identified the molecular identity of this channel. What is this channel? For the longest time we didn't know what it was. And in control cells in response to ATP, there's a downward reflection of this current trace. Once again, that's synonymous with chloride moving across the membrane.

And in response to transfecting the cell with siRNA to this channel, we can knock down the channel. We can see the protein expression decreases dramatically. And at the same time, the current response is abolished in response to ATP. So in response to ATP, compare to control or mock transfected cells with cells transfected with small intervening RNAs to target that channel , the ATP channels are inhibited. So that suggests that this channel, this calcium activated channel, is activated by ATP is, in fact, this TMEM16A channel which is distinct from CFTR.

And we showed that in the-- here's some rat or mouse liver sections with the antibody to the channel. And you can see it localizes in ductile like structures. And this is a stain for cholangiocytes And in fact, it is in cholangiocytes, and it's predominantly on the apical membrane of these cells.

And the next step is, well, is it present in CF cholangiocytes? And can it be a therapeutic target? Here's a stain for CFTR and TMEM16A in wild-type and CFTR knockout mice. And as expected, the CFTR knockout mouse don't express CFTR. So there's no brown staining in the bile ducts. However, they still express TMEM16A protein predominately on the apical membrane.

And Qin LI in our lab did this experiment looking at, once again, polarized preparations of cholangiocytes. And this is using confocal microscopy to measure the height of that surface fluid level. And in wild-type cells under control conditions, you could see the cells are stained green and the fluid is red. And so that's just the basal level of the apical surface liquid in response to a bile acid, UDCA in this example. The fluid secretion increases in response to ATP-- like I showed you previously-- that activates that alternate chloride pathway. There's also a large increase in the surface fluid measurements.

However, in the CFTR knockout epithelium, the CF cholangiocytes, there's much less fluid at basal levels. So that's already lower. In response to bile acid, there's not a large increase in the fluid levels. However, in response to ATP which activates this alternate chloride channel pathway, there is. And so it suggests that ATP or other purinergic analogs may be a viable strategy to increase secretion in these bile duct cells.

So we have CFTR in the apical membrane. We have this channel on the apical membrane, which we now have identified as TMEM16A and we think we can, by targeting that, we may be able to improve secretion in patients with cystic fibrosis.

So let's talk about that. Let's talk about some therapeutic strategies. How can we restore CFTR function? So we talked about how we can target alternate chloride channels. How can we restore CFTR function? That's another way to increase chloride secretion.

So going back to this pathway, if we correct CFTR, the protein at the apical membrane, we would also correct chloride transport. So we would have to correct the trafficking and transport. Those were the two things that we would need to overcome in the defects for this to work.

And going back to these class of mutations, there's a whole host of these mutations, as I showed you. Again, it is helpful to look at the different classes. Again, delta F508 is a class II mutation here. And so the protein is degraded. It never makes it to the membrane. Whereas, III and IV class mutations, it makes it to the membrane, but it has a decreased conductance.

And so depending on the mutation, different therapeutic strategies would need to be employed. So if you had a delta F508 mutation, you'd really have to correct the protein folding to get the CFTR to the membrane. If you had a class III or IV mutation, for instance, well, it already gets to the membrane, but it's not gated properly. So you'd have to restore the channel conductance properties.

So Alan Verkman's group has really been fundamental in this by utilizing some high-throughput screens to look at small molecules to see can we restore CFTR either to the membrane or can we improve its conductance? And so here's a correcter assay. Now, a corrector helps the abnormal DeltaF508 fold normally. And so these are small molecules that helps the CFTR fold into a normal pattern. It, therefore, makes it to the apical membrane like it should. The potentiator assay are small molecule compounds that actually help with the gating of CFTR. So once it gets to the membrane, that it opens and has a normal conductance again.

And using a strategy like this, able to screen lots of small molecules to see. The screen is based on a fluorescent probe that measures the anion movement at the apical membrane. And so it's a way to pick up hits of small molecule compounds that may affect CFTR trafficking or function.

And utilizing this technique, a number of drugs have been identified. So the first that came along was lumacaftor, or VX809 as it used to be called, which actually restored the normal trafficking of CFTR to the membrane. Subsequently, VX770 which is a potentiator drug came along. It's now called ivacaftor. And it increases the channel open probability of CFTR on the membrane. So that was pretty exciting to be able to use this screening technique to identify this.

But how does it work? And so once they were screened, they were confirmed using the same technologies I showed you in Ussing chamber studies or patch clamp studies to make sure that they actually did affect the channel as expected. And then they entered human trials. And so we'll talk about those next.

But the exciting part is now you had compounds that actually targeted specific mutations, right? And so if you had a patient with a specific mutation, you can now use a drug especially designed just for them. Right? And so either a ivacaftor, in the case of a DeltaF508 mutation, or ivacaftor, in the case of mutations that resulted in less conductance of the channel.

So this is one of the trials looking at the medication that would actually increase channel conductance once it got to the membrane. And so looking at this, the ivacaftor group did much better than the placebo group. And this was exciting.

So these were patients that were taking ivacaftor and they had noticeable improvements in their FEV1. That's a measure of their lung function. And you could see the improvement. And it was pretty dramatic in patients that were on this medication compared to placebo. And there were subsequent. So this was the first one that looked at patients with class III and IV mutations, both the G551D as well as others like the R117H mutation.

Oh. And I should mention-- so that was the correcter. The other medication, the lumacaftor, which is a-- excuse me a potentiator, the other drug which is a correcter, which actually helps CFTR fold properly and get to the membrane, lumacaftor, was also studied in human trials. However, it turns out that while it got to the membrane, it didn't really function to the same extent that normal or wild-type CFTR did. So it seemed like even though its main defect was getting to the membrane, even if it got there, it didn't function properly.

So you say yourself logically, well, why don't we combine those drugs, right? Let's get it to the membrane and try and improve its gating. So let's use lumacaftor and ivacaftor together. And that was this trial. So this was from the traffic and transport study in patients where both were used together. And it turns out that, again, compared to placebo, using these drugs-- and this was just different doses of the lumacaftor. Using them together was a viable strategy that significantly improved FEV1. And so for the first time, we had drugs now targeting the basic underlying defect in cystic fibrosis.

So if you had a patient that was homozygous for DeltaF508, there was now the combined lumacaftor ivacaftor therapy. If you have a patient with the G51D or R117 or other class III or IV mutations, you have ivacaftor as a treatment option. So really, for the first time, it gives you the idea of personalized or precision medicine, knowing what your patient's genotype is, and using a treatment that will help that specific patient.

However, that only makes up about 50% of all patients with CF, right? So if you look at patients that are DeltaF508 homozygous or have those other mutations, that's about 50%. How about the other 50? Well, theoretically, there's ways to target those patients, too. Right? So patients that have class I, V, or VI mutations or that are complex or compound heterozygous, there's new small molecules that are being developed in the same way. So using that same high throughput strategy, we're looking at new, exciting molecules that may actually target those mutations as well.

And the approach our lab is looking at is alternate chloride channels. Well, that that's a strategy that could potentially benefit all patients. Right? So a targeted channel that's already there to improve chloride conductance. So I think there's exciting things on the horizon that will really improve the lives of patients with cystic fibrosis.

And so we've come a long way already. And we can take this timeline showing the survival. And from the early ages at the beginning of the century where all patients died in childhood really up to now where we're somewhere in the mid 40s. It's predicted that patients that are born today with CF will probably live to their mid-50s and even longer based on those new treatments.

And this is the same boy who was featured on the cover of*Science.* Here he is now as an adult.

And so it's an exciting time to study CF. And I think there's going to be more and exciting treatment options that are coming along the pike.

So I want to thank all the scientists that have come before me that have studied this. It's really a work of a lot and lot of people that have really made important contributions to the field. And I don't have time today to mention everyone, even including right here in Pittsburgh, there's a lot. Your own Ray Frizzell has really contribute importantly to the field. And so I just highlighted some today, but there's so many more people that have really made important advances in the field of CF.

So with that, I want to thank the people in my own lab and our funding sources as well, all of our collaborators. And thank my old institution, and we're looking forward to our new institution. So thank you very much.