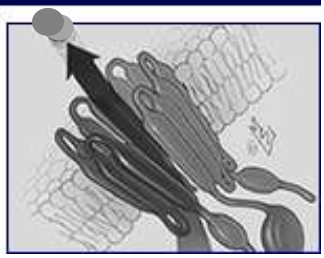
AHEAD OF THE CURVE
EMERGING CF THERAPIES 2009



Transcript: Ongoing Research into Other Emerging Therapies: Pam Zeitlin, MD, PhD

Dr. Mogayzel: I have the pleasure of introducing Pam Zeitlin who is my boss, and it is Bosses Day, so thank you for coming. She is going to talk about other targets besides CFTR that may be potential targets, and the talk is entitled "Who Needs CFTR: Alternative Activation of other Channels."

Dr. Zeitlin is Professor of Pediatrics at Johns Hopkins and Director of Pediatric Pulmonary at Johns Hopkins Hospital.

DR. PAM ZEITLIN: Thank you, Peter. So who really needs CFTR; hopefully we'll have these other therapies that we can use for patients in whom we really don't know what their mutation is doing.

Now I think I'll just skip over this slide because you can read the competing interests in your book, and we'll start with a cartoon here to try to set the stage for comparing hydration of the normal airway with what happens in CFTR, which is basically an airway dehydration situation. And on both sides you can see that there are cilia which participate in clearing mucus particles and bacteria that are trapped from the lung, mucus is generated primarily from the submucosal glands and it floats up on the top of this airway surface liquid. And the composition and depth of that liquid has to be just right for this to work.

And in order for the composition to be just right, we need a whole team of ion channels. Some of them are moving chloride, maybe another one is moving sodium, and there are other ones, as well. In cystic fibrosis we don't have CFTR, but CFTR is a master controller, as we're going to take a look at, and so the functions of the other channels can become impaired.

In the CF situation you have a hypertrophy of the submucosal glands, you have excessive production of airway mucus, it's got abnormal charges on it, it's sitting on a very dehydrated fluid layer, and it is very difficult to move compared with a normal airway.

So the master controller of this is CFTR, which I put right here in the center of things. But there are other channels that CFTR enables. And one of these is the outwardly rectifying chloride channel. It's a simple chloride channel, it moves chloride in response to agonists, but it cannot function if CFTR is not there functioning normally.

There is also other channels, among them are the PH and voltage activated channel, CLC2. And then there is the epithelial sodium channel, which requires CFTR to control it, to dampen its effect. So CFTR is enabling chloride to move out and the epithelial sodium channel is reabsorbing sodium, and it becomes very excessive in cystic fibrosis.

Now the independent channels are the calcium activated chloride channel, and possibly also the CLC2. So let's look a little closer in the airway here. So CFTR is the cyclic AMP activated chloride channel, and it's next to the outwardly regulating chloride channel. And then we have the epithelial sodium channels which are inhibited by CFTR, and we have P2Y2 receptors, which are responding to nucleotides like ATP, and they increase intracellular calcium which activates the calcium activated chloride channel.

Now here we go, back to cystic fibrosis, we'll just go over this again, as JP said, sometimes it helps to just keep looking at it in different ways. So now we have the situation where we don't have a functional CFTR in the luminal surface. And this leads to diminutions or even absence of chloride movement through the outwardly rectifying chloride channel. There may be less CLC2 activity, as well, and there is excessive epithelial sodium reabsorption. So less chloride secretion, more sodium absorption, and you have dehydrated airway surface liquid.

Now in cystic fibrosis, some of you may remember that the calcium activated chloride channel, its activity can become very exaggerated. However, as was recently shown by Robert Tarran at the UNC group, there are certain conditions in which that calcium activated chloride channel doesn't work so well. And this is when levels of 17-beta-

estradiol are high, which occurs in certain phases of the menstrual cycle, and may explain why girls, at the time of puberty and thereafter, seem to have more trouble with their cystic fibrosis. So all of this together impairs mucociliary clearance.

So what can we do? We have these other chloride channels and could they be used as targets to move chloride through? And so there are several investigational molecules that are moving through the pipeline. The first one that I'm going to talk about is Denufasol, and there is an ongoing phase III multinational trial that you've heard quite a bit about. The second one is MOLI1908, or Lancovutide, I'm not quite sure if I'm pronouncing that right, and right now this is out of an effort with Canada, Germany, although it started in the US. And then I'm going to talk about something you probably haven't hear much about, which are the prostones. And they were developed to activate the CLC2 channel by a company that originated in Japan and has now moved to the United States. And there is both an oral formulation and an aerosol in development for the CLC2 channel.

So Denufasol, this is INS37217. It's a second generation, chemically stable, selective P2Y2 receptor agonist. So first generation would be the ATP or the UTPs. And in this second generation it is a more complicated molecule that has a longer half life. It increases chloride secretion and mucin secretion, and improves ciliary beat frequency and mucus velocity.

It's metabolized, as I said, more slowly than the smaller molecules, and there is a lot of published data showing that it's safe in healthy volunteers and in patients with CF. So doses by aerosol between 20 and 60 milligrams are relatively well tolerated in CF patients who have mild lung function.

So the caveat about all of this is that efforts have been aimed at studying Denufasol in patients with FEV1s greater than 75 percent predicted and there have been over 8 trials that have been successful that have moved this drug along its own pipeline.

So TIGER-1 was the first multicenter trial and it was 08108, and it met its primary efficacy endpoint which was to look at change in FEV1 from baseline after 6 months of treatment when compared with placebo. And this graph shows you the significant effect on study drug here. it was a 45 mL treatment effect.

And this was a real world design. It was a broader population of patients on multiple therapies that were allowed that could at the same time have been improving FEV1. so this was a very encouraging trial and we're all now in the middle of TIGER-2. It is still currently enrolling, although I think that the company has announced they are very close to meeting or finishing the enrollment. Multiple sites, there are over 100 sites around the world, including US, Canada, Australia and New Zealand.

This is a 48 week placebo-controlled trial, and there is now an option for an additional 48 week open label period with Denufasol, which I think is really going to assist us in finishing the recruitment. Usual standard of care medications are allowed, as in a real world design, except no hypertonic saline.

Now here's the word I can't pronounce, Lancovutide, or MOLI1901. This is working on the same calcium activated chloride channel, but not through the P2Y2 receptor. Lancovutide is a 19 amino acid polycyclic peptide derived from bacteria, and it's been shown to increase intracellular calcium and then, of course, chloride secretion. And the way it works is it's thought to bind to polar heads of phospholipids in the membrane and induce a change in intracellular calcium levels, and thus that is then sensed and it activates chloride secretion.

We showed that direct application to the nose of normal or CF volunteers led to very sustained increases in chloride secretion, particularly at the higher doses of the range. It also has been shown to have a very prolonged half life in nonhuman airways on the order of days.

It is in an aerosol formulation and has since been tested and shown to be well tolerated when given for 28 days. And there was a significant increase in this small phase II trial run by the company that is now called Lantibio, where there was an increase in FEV1 compared to placebo. So this drug is still in the pipeline and we are looking forward to phase III trials in the future.

Now CLC2 is another alternative chloride channel. It is not a calcium activated chloride channel. CLC2 is one of a family of chloride channels conserved from bacteria all the way through mammals to man. This particular one is highly expressed in fetal lung. It is voltage activated and acidic pH activated. So the fluid of a developing fetal lung is slightly acidic, so it is expressed highly at a time when it

could actually be open. And it is also volume activated, so hypotonic stresses. And this is a little cartoon of its structure.

And there is a class of molecules called the prostones that can activate channel CLC2. And it has been shown to be present in airway epithelium of mice and humans, so we can test these therapies in mice and humans. And we have recently demonstrated that you can activate the channel in the absence of CFTR in mice.

Now there are two potential agonists that could be used in cystic fibrosis. The first one, lubiprostone, the GI people in the audience, if there are any of you, know as Amitiza. Lubiprostone is not absorbable, it's taken as an oral formulation, swallowed, and it activates the CLC2 that's in the intestine. Chloride secretion follows and it's being, it's an FDA approved drug for constipation, idiopathic constipation.

Now the only way you could use a non-absorbable molecule like this in the airway would be possibly to put it in directly by aerosol, but it wouldn't get absorbed. So that is not the way to go at this point in time.

So there is another one in this class of prostones called cobiprostone. And cobiprostone can be given orally and is absorbed and metabolized and it's been tested, it's still an investigational molecule for the treatment of liver disease. Liver disease unrelated to cystic fibrosis.

So some of you may remember that the CFTDN did a small phase I/phase II clinical trial of cobiprostone in cystic fibrosis where the primary outcome measure was looking for chloride secretion in the nasal PD and also there was an effort to look at sweat chloride. That trial showed that the doses that were applied were low and there wasn't really any significant absorption of this drug in cystic fibrosis or change in NPD.

So the cobiprostone is under reformulation by Sucampo Pharmaceuticals and we're hoping that that might get to clinical trial again for CF, because an oral treatment that could go to multiple organs in the body might really have an impact in this disease.

Now we promised we would talk a little bit about the sodium channel. The sodium channel is overactive when CFTR is absent. And the sodium channel inhibitors are not as far along toward the bedside as are the chloride modulators. And there are a

couple, one is out of Novartis, from the UK side, and this is a protease inhibitor. So proteases can activate the epithelial sodium channel. So this is an inhibitor.

And then Parion Sciences, located in Durham, NC, USA has two molecules. These are small molecular weight molecules that will inhibit the sodium channel, and they have been tested in cells and in animals but are not yet to clinical trial in CF.

The goal with sodium channel inhibitors is to dampen down sodium reabsorption so you get a better airway surface liquid depth, and the goal then would be to improve mucociliary clearance.

Shorter acting sodium channel blockers have been tested in CF. You all may remember more than 10 years ago a phase III trial of amiloride by inhalation. Amiloride is a very short-acting sodium channel blocker and it in phase III was not effective. And if you combine a small or a short-acting sodium channel blocker with some other therapy, it can actually interfere with that other therapy and can even cause bronchospasm or declines in lung function. So we have to start to be very careful when we think theoretically about combining investigational agents.

Longer acting sodium channel blockers may be more desirable in CF, but these are still under evaluation.

All right, osmotic agents. Everybody knows about hypertonic saline and I think a lot of us believed that it's very effective in CF and it's been rapidly embraced in the US by CF care centers. The idea is that you are inhaling by aerosol sodium chloride and that's these little red circles that I've drawn here, and they are depositing in the airway fluid and then water is drawn through osmotically and that will increase the depth of the airway surface liquid, and now when you cough you will be much more effective at mucociliary clearance.

It has been shown to restore mucociliary clearance and be very safe, and currently is under study in younger patients now, it's under study in CF infants in the ISIS trial. But what is very interesting about hypertonic saline is that it works for a fairly long period of time after you've inhaled that dose, and that is very difficult to explain.

If the only way that it's acting is as an osmotic agent and as just salt, which the body can certainly get rid of very quickly, then how do we explain the evidence that it is

very active in improving mucociliary clearance for hours after the treatment. And the latest research is pointing to the fact that there are censors of salt by the airway that then go ahead and change gene expression, such as down regulating molecules like the epithelial sodium channel.

So I think there is a lot still to be learned about why something as simple as hypertonic saline might be a really effective treatment.

Another agent to use as an osmotic agent to improve airways surface liquid depth and clearance is a simple sugar, mannitol. So the inhaled mannitol or Bronchitol is now provided for investigation in a dry powder formulation. And its rationale, or it is thought to work by hydrating the lung and improving MCC, just like hypertonic saline. But it is more slowly permeating, it's a relatively higher molecular weight than salt, and it is longer lasting. It requires larger doses.

It is being formulated as a very unique dry powder and there is a device that is very unique just to this inhaled mannitol, and it has a requirement that you put in multiple capsules very rapidly one after another into the device and inhale this series of capsules twice a day.

So there is an ongoing phase III multinational trial that we're participating in, and it's achieved its recruitment goal as of September '09. There is also another phase III trial run by Pharmaxis for use in bronchiectasis not due to cystic fibrosis. So the companies and the physicians are looking to see whether this effect is not unique only to cystic fibrosis.

All right, so we have talked a lot about different strategies, JP was talking about correctors, potentiators, stop codon translators, I'm talking about alternative chloride channels and sodium channel blockers, how are we going to put it all together with the already complicated regimen of treatment for CF patients? The first, you know, new drug ever approved for CF was this rhDNase, what are we going to do, how are we going to combine these treatments with something that we know works.

So, for example, we're already facing that. many of our patients take rhDNase by aerosol, TOBI by aerosol, and hypertonic saline by aerosol, and for many of us what we prescribe as a typical sequence is to start first with a bronchodilator, to open up your airways, and particularly if you happen to have reactive airways, to get them

ready to take these molecules, then give the rhDNase, that helps you cough and expectorate and thin the mucus, and then give the inhaled antibiotic, TOBI.

Now so how is this all going to work if we're also going to add in an aerosolized activator of P2Y2 receptors, or one of CLC2, or maybe even both? Because a little of one and a little of another, why wouldn't it be synergistic? So all of this is going to have to be studied.

What we do know is that if the effect of rhDNase in combination with some of these other novel therapies that are already moving into phase III, that is under study.

And so I just have a list of references here that I believe are in your booklet, so we'll move right through to the questions.

Well I think I actually am on time and we have time for a few questions if anybody would like to ask a question.

MALE VOICE: (inaudible – TEXT QUESTION):
Do you think that protease inhibitors will be inhaled and if so will they lead to significant bronchospasm?

DR. PAM ZEITLIN: No, I don't think I meant specifically to target protease inhibitors, but I think in a lot of these aerosol trials there is always a fraction of patients who have a little bit of cough and bronchospasm before they inhale it. Say, for example, even TOBI, there is a certain fraction of your patients that do better if they take a bronchodilator first to try to relax their bronchial smooth muscle. Some of these alternative chloride channel agonists do the same thing, work on the calcium activated chloride channel. There is a small group of people that seem to have cough and a little more bronchospasm. But I don't think I was trying to target protease inhibitors specifically.

MALE VOICE: (inaudible - TEXT SLIDE): Why do you say we need to find longer acting sodium channel inhibitors, when the short acting ones are associated with cough or bronchospasm? Wouldn't that lead to more toxicity?

DR. PAN ZEITLIN: Okay, so Dr. Mogayzel says the question is really if short-acting sodium channel blocker amiloride causes bronchospasm, why wouldn't a longer acting? A longer acting may, they are chemically different, amiloride is chemically different from a protease inhibitor, which is different from a small molecule. I don't know that the small molecule blocker would in any way cause cough or bronchospasm. But the longer acting your agonist is, the better for the CF patient because they won't have to take the drug too often.

We all know our patients really have busy lives and they count up all the minutes that it takes to do their therapies. Aerosol therapies do take a little bit of time. So I think I was just trying to make that point.

Yes.

MALE VOICE: On Vertex 770, the potentiator, it looks like the effect is fairly rapid, I mean after a period of 14 days, of 28 days, you can see effects on chloride channel, effects on even pulmonary function. But when you look at some of the data on the P2Y2 agonists or Denufasol, it looks like it takes months to potentiate some effect.

DR. PAM ZEITLIN: I think that's a great question actually. I think when you are looking at the master controller, if you can help that master controller get back to the cell surface and open, because it is only 10 percent open to start with, if you can open that significantly more you may actually be able, because it's the master controller to turn on the outwardly rectifying chloride channel, you know, all that hasn't been clearly looked at in human volunteers, but because of that, because it's that controller and there are all these dominoes that are waiting to be fixed, you may be able to get that airway surface liquid hydrated for longer and then eventually hopefully that turns into lung function.

For the alternative chloride channel agonists, which are going to improve airway hydration, that is a step backward, I have to admit, even though I'm in favor of doing this bypassing CFTR. So we think it might take longer to see an effect, it's just from that hydrating portion of those other channels.

Now Denufasol may actually down regulate the sodium channel to some extent, you heard Dr. Moss say that, so that could help, but I think it still needs more study, you're right.

MALE VOICE: The Denufasol, what class of drug is it?

DR. PAM ZEITLIN: Yes, so the question is what class of drug is Denufasol. It's of the nucleotide family, but it's a more complicated nucleotide than ATP. And its target is P2Y2 receptor. So the P2Y2 receptor is found on the apical membrane of the airway epithelium, it is also sometimes found on bronchial smooth muscle, and when you activate that intracellular calcium goes up.

MALE VOICE: Just a comment on Berol's question, I have another take on that, which is that there may be a factor of the root of delivery. So Denufasol is delivered into the airway, to act it's got to see that epithelial surface get to that receptor. To get there you have got to have a lack of mucus obstruction. If you give an oral drug like the VX770, it's delivered systemically, gets into the cell right away, and perhaps that's a reason for the different kinetics, so it might be another explanation.

DR. PAM ZEITLIN: Yes, that's another good point.

Dr. Chris Goss who is an associate professor of medicine at the University of Washington is going to have a short presentation on managing patient expectations, then we're going to have two cases for you to discuss and we're going to get your opinions that we would like to work together on to get some thoughts about these cases. And we are going to sort of divide the room in two and see who can get the best responses to these questions and I guess maybe divide the desserts that way.

All right.