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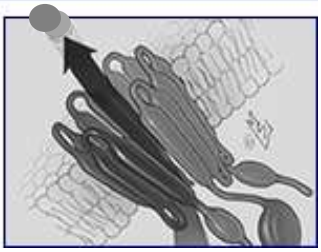

Transcript: Ongoing Research into CFTR Modulation – John Paul Clancy, MD

Dr. Mogayzel: I have the pleasure of introducing the next speaker who is going to be talking about CFTR modulation and CFTR therapies. Dr. JP Clancy is Professor of Pediatrics at the University of Alabama in Birmingham, and Director of Pediatric Pulmonary at the same institution.

DR. JP CLANCY: Good evening, and thank you all for being here tonight, and thank you, Rick, for giving such a wonderful review and introduction into several topics that we'll be spending some more time with tonight.

I'm going to focus in on one part of what Rick was discussing and really it boils down to three things, and I hope that I keep it focused enough that when you walk out of here these will be terms that make a little more sense to you.

I wanted to give you a little disclosure information. The primary things, obviously, I've received some funding through the CFFT to be a translational center. I'm very active in current Vertex clinical studies, we have contracts to perform those studies, and also to over read some of the biomarkers such as the nasal potential difference, and I am also serving a similar function with PTC in the development of their agent, and finally some grant review activities.

So I wanted to just review, we've spent a lot of time talking about new kind of molecules at this conference that do pretty interesting things and I wanted to take a step back and remind us that there are many steps between a gene and its protein that it codes for.

The CF gene was found 20 years ago on chromosome number 7, and it codes for something called the CFTR, which stands for the cystic fibrosis transmembrane conductance regulator. And just remembering the first step is that the DNA needs to be converted to a blueprint form called mRNA, which then goes out into a different part of the cell, the cytoplasm, and it is converted from mRNA into protein, and that process is called translation, and that happens in a part of the cell called the

endoplasmic reticulum or ER, and the protein then has to go through a number of processing and folding steps before it is mature. And when it becomes mature it is then transported up to the cell membrane where it does its job. And it basically works as a channel that transports chloride and also regulates a number of other ion transporters.

So what's a modulator and why is it different from other CF therapies that are used today in the clinic? Well a modulator, rather than targeting symptoms, is really trying to target the underlying defects that cause CF, and in particular, really addressing the problems with CFTR. They are trying to restore function to proteins that don't work normally. It doesn't replace them, that would be gene therapy, it actually is trying to restore some level of activity that we hope is enough to overcome the symptoms.

Again, we hope that it's going to address one of the primary causes and therefore possibly could have effects before disease manifestations. Now obviously that's a few years away, but that certainly is the long-term goal.

But as I mentioned before, there are many steps between the gene, the first level of protein production, and then the actual mature thing that goes and does what it's supposed to in the cell. And there can be defects in any one of those different areas. And because of that, there are very, very different targets. So drugs that are really addressing the early steps in creating CFTR may not have a whole lot to do with a drug that is under development for the later steps in CFTR.

So we're going to try to cover some of the agents that are being developed for these three general parts of normal gene and protein function.

So if you had to make a drug that was going to fix CFTR or try to restore activity, how could you do that? Well there's a number of ways you can go about it, and the first one would be that if you knew exactly what the protein looked like, you had what is called a crystal structure so there is no question what you are looking at, in theory you could build a drug that would fit and perhaps overcome the problem that's in that protein.

Well the problem with that right now is we don't have a fully picture of CFTR. We're getting closer, in fact, we have parts of CFTR that have been crystallized and we sort of have some long view pictures of the protein, but not quite enough to actually develop a drug based on the structure. So although that's a great principle we're not there yet.

Another approach to develop a drug or a modulator would be to really be at the bench, dissect the pathways are involved which cause the defect, identify targets in those pathways, and then one example would be to adapt therapies that we know already work in those pathways and see if they work in CF.

And some of that was what Rich was mentioning, at least in general a way that people could approach restoration of function, and people are actually adapting medications that are in the clinic right now and finding out if they actually may have restorative effects on mutant CFTR.

Or the third approach, which is most recent, would be to do what's called high throughput screening. And you'll see abbreviation from time to time, HTS, and this really takes the brawn over brains approach. And the idea basically is to screen a whole bunch of compounds, find things that do what you hope they'll do, and they describe those as having hits, they validate those, confirm that they actually have the activity that they think, and then try to take that identified molecule and turn it into a medication, or excuse me, a drug that hopefully eventually becomes a medication.

And this general approach has been used by at least three, for three of the compounds that are currently in clinical studies. And the general principle is that cell lines that express the mutant CFTR, or perhaps a reporter gene that has sort of similar characteristics, may allow us to test drugs to see if they can overcome the defect that is specific to that problem, such as delta-F-508, or G551D, or a gene that has a premature stop mutation.

And this has been approached by PTC Therapeutics, and they brought the current agent, former name PTC124 into clinical studies, now it's called Ataluren, and then Vertex Pharmaceuticals, which has two agents currently in clinical trials, one is the potentiator, VX770, and the second is VX809, which is geared toward correcting delta-F-508.

So I wanted to give you a little background about how each of these agents work and then provide a little bit of background of some of the clinical research that supports their activity in human subjects with disease causing mutations.

So suppressors or stop mutations would approach what are called a class I mutation, and that is one of the very early steps in taking a gene to a protein. And one example of this is called a premature termination codon, or PTC, it just happens to be the company name, but they actually don't mean the same thing. And if you look through the, as you are reading about these genes, as you get them back from

newborn screens or diagnostic things, you'll see an X, the characteristics X, and that's basically the shorthand for stop mutation or premature termination codon.

And in that process, if we look at my very bad little picture here, what we're looking at is an example of mRNA, that's that kind of squiggly thing, and that has that genetic information that had been taken from the gene, and on it is that blue thing that is called a ribosome, and what that does is basically translates the information on the RNA to a protein.

And so at the beginning of the picture we have a stop sign right here, and this is a premature stop mutation. Basically that is an error in the genetic information that codes, instead of coding for an amino acid, making a protein, one of the building blocks, it codes for a stop sign. And what happens is as the ribosome is chugging along, it hits the stop sign and basically releases and what you are left with is what is called a truncated protein or a very short piece of protein. And it turns out that in addition to that, oftentimes this process lowers that amount of available protein and RNA.

Now if you have an agent that happens to target that process, that would be an agent that could be suppressing a stop mutation. What it does is it allows the ribosome to sort of repass that error in the message, maybe not every single time, but quite a bit, so that your ribosome is then able to go passed that stop sign, and in theory hit the authentic stop codon that's at the end of the gene. And therefore, what you will end up with is actually a full length protein. And again, the level is not as much as you would find in the absence of that stop mutation, but we hope enough to actually restore enough activity to be clinically meaningful.

And the idea of suppressing premature termination codons, or PTCs, is not a new idea. Actually it was first described in bacteria about 45 to 50 years ago, I think the earliest reference I saw was 1964. It was first shown in bacteria in yeast, and it turns out aminoglycosides, a very commonly used class of antibiotics, some of those members will actually have this effect. And if we think about how aminoglycosides work, maybe it isn't too surprising. It turns out that aminoglycosides bind to ribosomes and they can have effects on the ability of that ribosome to accurately read from the RNA.

This principle though was first applied though to try to treat genetic diseases in the mid 1990s, and the studies have been built on quite a number of preclinical research projects, including single cells, transgenic mice, and subsequently in CF

patients.

And this is a figure that I'm showing you from a study that was reported by Dr. Michael Wilschanski (Hadassah University Hospitals) from Israel, and his experience with using a topical, a nasal dropper type of study in which the medicine, gentamicin, commonly used to treat a variety of infections, was dripped into the nose of patients who either had the delta-F-508 mutation, two copies, or had a PTC. And what we're looking at is their CFTR activity, which is being measured by a test called the nasal potential difference. So at baseline before the study, this is their value and that's basically in the CF range, if you have two copies of delta-F-508 and you are treated with placebo, basically just a saline solution, nothing really happens. And then if you add, give them two weeks of gentamicin as a dropper, again, nothing much happens.

However, if you are one of these patients that carries one of these premature stop mutations, again, the baseline is here, placebo here, and when they are treated with gentamicin for two weeks there is improvement in their CFTR activity by the nasal PD. So that was really probably the most convincing first early evidence that this principle could be applied to human subjects.

The agent which has progressed to phase III clinical studies is called PTC124, and it again was developed through high put screening, and it is not an antibiotic, it is not an aminoglycoside, but it has some of the features in the sense of an aminoglycoside in that it does interact with the ribosome and it can cause that ribosome to read past stop mutations on occasion.

There have been human studies that have been performed in Israel, in the US, in Belgium and France, involving CF patients, and it has also been studied in patients with muscular dystrophy. It turns out that the whole principle of stop mutations is not unique to CF, it actually is found in most genetic diseases, if not all to some extent.

And the picture to the right is just my cartoon picture of a cell and it's just reminding us that this is one of the early steps in taking a gene down here to a protein up there, and it is really addressing the early steps in this process.

So I wanted to just show you one slide summarizing research that was published within the last year by Eitan Kerem (Hadassah University Hospitals) and colleagues in Israel, in which they investigated PTC124 in patients who had premature stop mutations. And in this study the patients were treated with either a low dose or a higher dose of the agent and they had measurements of their CFTR

activity by the nasal PD measured before they were treated and after they were treated, and each one of these little measured dots is a different patient. And what we're measuring here is their CFTR activity, and what we're hoping to see is changes in the downward direction, which would reflect more activity of CFTR.

And what we can see is at the beginning of the study, compared to at the end of a two-week treatment period, there was, in general, a reduction in their nasal PD value indicating activity of CFTR that was restored. There was a washout period and then patients were treated with a second dose, and again, in general, there was a reduction in the nasal PD, so it was statistically significant.

So providing, and several of these patients who were treated actually took their end PDs from the CF range into the lower side of the normal range. And, in fact, some of these patients went well into the normal range. So certainly very exciting and provocative findings, which have now led to a phase III study which is currently enrolling students.

I'm going to switch gears a little bit and talk about an agent which has been mentioned, too, at this meeting in the last two to three days. These agents that I want to discuss are called potentiators and these are basically small molecules that are geared to open CFTR. And they would be, the most logical target for this type of agent would be a CFTR that actually gets to the cell membrane, because you need to have CFTR available to actually be able obviously to open it. And the one that is most common that fits that bill is called G5F1D. And that mutation causes, there is a normal amount of mutant CFTR at the cell membrane, but it's not able to be activated by the normal pathways. It is found in about 3 to 4 percent of patients and what the goal of a potentiator is, is to essentially unlock that channel, help it get to a configuration which allows chloride to go through, and for it to be normally regulated. And so this a slide that was a complement of Fred Van Goor, PhD, from Vertex, which is just showing that on really the very microscopic level, and I want to show you again, I'll tell you on the right side what a potentiator is doing is working at the very end of this process, it is really trying to help a CFTR that doesn't work correctly that is already at the cell membrane.

And what we're looking at on the left side here are basically these are single channels of CFTR, and what we are looking at is the fraction of the time that they're open. And if we start here with the white on, that's actually a wild type CFTR or

normal CFTR, and it turns out that if you measure the amount of time CFTR spends open, it roughly is 40 to 50 percent. And if you look at a G551D CFTR, it spends a lot less of its time in the range of less than 10 percent. When VX770 is added though to the G551D, it actually helps restore its activity to actually essentially to wild type levels. So this kind of type of preclinical work was what supported the principle that a potentiator may have effects on CFTR in human subjects.

So this is a complicated slide that's been shown before. I won't spend a great detail on it or a great amount of time, but this just summarizes the study design for the recently completed phase II study of VX770. And it was a two-part study, the first part evaluated four doses and placebo in kind of a crossover format, and each patient was treated for two-week blocks followed by washout, then two more weeks. And the part two basically took two doses and compared that with placebo, and that was for a full one-month treatment period.

This study was completed in all adults, as one would expect everyone had the G551D mutation, and 80 percent happened to be G551D and delta-F-508, and by and large, their lung function was in the mild to moderate range.

And this is some of the original data that was presented by Frank Accurso (UCDHSC/The Children's Hospital) at last year's meeting. And I actually like looking at this because it shows it twice, and to me, I don't know, that sort of helps me confirm the effects that we think we're seeing here.

And what we're looking at here is the effect of treatment with VX770 on sweat chloride values of CF patients. And what we're looking at, this is the first part of the study from measuring at day baseline before drug, 7 days, and 14 days, and looking at the sweat chloride values, the 100 value being here, and the cutoff for CF diagnosis, 60 being right here. And what you can see is that the placebo have no change, lower dose, medium and higher dose, progressive reductions in sweat chloride over the course of treatment.

The second part of the study confirmed these findings and showed it over a one-month period, took it to a little higher dose, basically showing, again, very stable, no change in the placebo group, reductions in sweat chloride values. This is actually the high dose and the 150 milligram dose down below. Again, very close, if not below the cutoff for CF diagnosis for a number of the patients.

The exciting thing about this study I think was the fact that it had effects across different types of markers, such as the sweat chloride and another test that I

eluded to before, the nasal potential difference. And this basically measures CFTR activity in your nose, and we think that that's actually a representation that is connected to the levels of CFTR that are going on in the lungs.

And what we're looking at here again is the CFTR activity that's measured by the nasal PD at baseline and the different doses. Placebo and the low dose, you really didn't have measurable effects, while the medium and higher doses began to have improvements, taking the NPD again towards the, out of the range, beginning to take it out of the range of the CF diagnosis.

The second part, again, placebo, really no changes over the courses of measurements. This is the 150 and the 250 milligram dose. And really seeing that a number of the patients had improvements that were really quite unusual to see in patients with CF.

One of the most exciting things I think is the fact that in addition to these measures of CFTR activity that we can do by sweat glands or by the nasal PD, there are complementary improvements in lung function. Again, the part one data, placebo, little effect, and then dose dependent improvements in lung function here shown as improvements over baseline FEV1, approximating 10 percent. And in part two, again showing rapid improvements in the higher dose of the study that was significant over relative to baseline values.

So we have talked about two of the types of agents that are under development and I want to take us into the third which is currently in phase II clinical trials. And that is the delta-F-508, or agents which are geared to help address the problems caused by delta-F-508.

So as we are probably all well aware, delta-F-508 is the most common cause of CF in CF. You can find at least one copy in about 80 to 90 percent of CF patients and 65 to 70 percent of all CF chromosomes. And the problem is folding. In other words, after the protein is converted from RNA into protein it gets stuck, it is not able to progress like it's supposed to through the cell and up to the cell membrane where it can perform its function.

And we can see this in a variety of ways. This is a photo micrograph that was taken from colleagues at Chapel Hill, showing where the normal CFTR resides on the top of airway cells here in green. And in the delta-F-508 expressing cells we see that this green protein is able to get to the cell membrane where it is supposed to do its job.

So if we look more closely, what is the problem, how can one little amino acid deletion cause such a dramatic effect on this protein. And what you are looking at here is a model of what we think the part of CFTR that possess delta-F-508 looks like.

And what we're looking at is basically normal and delta-F-508, sort of superimposed on each other. And we're starting up here, there is basically a blue and a red. And the point is they are very similar. When you look at, let's call, this area is called the nucleotide binding domain or NBD1, these two look very similar through many of the ways you look at it, except in a couple of places, and this is where delta-F-508 is. It turns out it is sort of on the outside of this structure and it makes some of the loops of proteins sort of stick out. There's one right here and there's one out here that really seem to deviate away from the normal.

And it is not known 100 percent for sure how this causes, but the working hypothesis is when those things are sticking out it causes destabilization and, in particular, how that little domain interacts with other parts of this very complicated protein.

And so what happens is we think that CFTR is not going to be able to sort of compact into its normal configuration. And that, it's very important that the early parts of the protein are actually able to interact in a way to compact the later parts of the protein.

So when those compacting steps don't happen, it turns out that the protein is very susceptible to being broken down. And so again, the part of the cell where it's broken down is called the proteosome but the point of it is, it doesn't look terribly different at a molecular level, but there are subtle differences that have dramatic effects on how well the cell can use the protein.

Well it turns out delta-F-508 may be a little more complicated than just that. There is certainly clear evidence that the protein doesn't fold correctly, so very little of it, if any, gets to the plasma membrane where it's supposed to do its job. In addition, a lot of the preclinical work suggests that even when it gets to the cell membrane, it doesn't, if you can get it there by some maneuvers, it doesn't work very well, the chloride channel stays closed more than it stays open. Now there is some more recent work that suggests that hopefully that may not be as big a barrier as we thought, but clearly preclinical work so far has brought up that concern, that the protein at the cell membrane doesn't work very well.

And finally, we also know that once it gets there it doesn't stay there very long. In fact, the cell, for some reason, sees it as abnormal, tries to take it back inside, and that process is called recycling. So we really have a lot of things to address with delta-F-508.

So correctors, an example being that being developed by Vertex, VX809, the goal of these are to increase the surface density and function of delta-F-508 at the cell membrane. In other words, help, in general, it is trying to correct the folding problems that are causing the vast majority of problems with the protein.

A potentiator, as we had mentioned, is trying to increase the activity. So you could certainly see that perhaps these two might have something to do with each other. If you could correct the folding and bring some of that delta-F-508 to the cell membrane, perhaps it will be a target for an agent like VX770, which could actually help it to work the best it could.

And this has been demonstrated *in vitro* or in preclinical research studies. Again, this was performed by Vertex Pharmaceuticals, and I know this is a kind of complicated slide, but what we're looking at is concentrations of drug on the X axis, and basically activity or how much chloride is being transported on the Y axis.

And the bottom is showing increasing doses of VX809 for delta-F-508 expressing cells. And what we see is that, indeed, there is a significant improvement in chloride transport in the presence of 809 alone. Interestingly, if you add 770 on top of that you shift everything up, you essentially double the amount of activity of the delta-F-508 that is produced by 809 alone. So certainly suggesting that the two agents may be very nicely complementary.

So I wanted to summarize quickly, there are just a handful of points that I think which will be really helpful as we go forward with these agents, and as they go through phase III trials and start discussing them with our patients. CFTR modulators are trying to address the underlying cause of CF by helping to restore activity to the proteins that are dysfunctional and we believe cause the symptoms. Suppressors of stop mutations target stop mutations, those little Xs that you see on your genotype information. Potentiators, they are trying to open the chloride channel. And finally, correctors are trying to really target that primary folding defect in delta-F-508, and we hope also have effects on how it functions at the cell membrane.

And what I think this is going to take us to is that we're going to be able, at least I think our vision is that we'll be matching the genotype of patients to certain

types of treatment strategies. And I think obviously that's a cause for a lot of excitement within the field.

And the pictures you're seeing, that's where I work, Children's Hospital of Alabama, and that is Vulcan, he is the largest iron statue in the world, whatever that is worth, and he overlooks my city.

I want to thank you all for the opportunity to give you an update on the current modulator studies. I was told by Peter I have a moment, if there are any questions I'm happy to try to answer them, and I hope this was a valuable experience. And again, I really appreciate the organizers providing me the opportunity to give you an update on this information. Thank you.

MALE VOICE: When an agent such as a corrector for delta-F-508 becomes approved for treatment of CF, caused by delta-F-508, will those drugs be used in other CF causing mutations?

DR. JP CLANCY: Yes, a very relevant question. The question, I'll try to paraphrase it, is let's say that some of these agents such as a corrector for delta-F-508 becomes approved for treatment of CF caused by delta-F-508, will we be able to use those drugs in other CF causing mutations, is that essentially -- yes.

It's a fair question, and I think the answer is yes and no. It turns out, and again, I actually saw this information today, it's been a great conference and I have actually learned quite a bit, and one of these things I was really questioning about was that exact question.

My understanding, without being the person who's done this research, is that VX809 can improve some folding of some mutations that are caused by other folding defects, but it's not universal. It doesn't have effects on wild type CFTR, I don't believe it has effects on G551D CFTR; it doesn't increase the amount at the cell membrane.

So I think it's going to be really very genotype specific and it's going to be a bit tedious probably to work that information out, which are the patients most likely to benefit beyond those studied who have two copies, or arguably one copy of delta-F-508. But I think that is one of those questions that we are really going to be wrestling and trying to answer over the coming years.

Okay, thanks again.