



AMDA Conference Call Transcript

Session No. 4: Gene Therapy
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I will first described Pompe disease in the way I see the disease and that will then lead into the progress we've made.

Pompe disease is a genetic disorder and a recessive disorder which means that everyone inherits two genes when they are born, one copy of the gene from each parent (one copy from mother and one copy from the father). There are 40,000 to 50,000 genes which make us human. So if you have a problem in one of these genes you can end up with a genetic disorder.

For Pompe disease you inherit a genetic defect or a problem in the acid alpha glucosidase (GAA for short).

If you inherit a mutation in GAA on both of your genes, you will end up with a form of Pompe disease, but it depends on the type of mutation you have or inherit. Pompe disease is broken down into 3 groups of patients: Infantile, juvenile, or adult onset form which is basically broken down by age of when your first symptoms become noticeable to your relatives or your physician.

The infantile form is the type most doctors know about, because that is what they learn in medical school and the infantile form presents in the first few weeks of life with failure to thrive, poor weight gain, respiratory difficulties, cardiomyopathy, which is a disease of the heart, (the lack of the ability of the heart to pump). Typically and unfortunately children that have infantile Pompe disease die by the time they are a year old from the massive cardiac problems and or the complications of not having a good respiratory function from muscle weakness of the diaphragm and the lung muscles.

Now there are juvenile and adult onset forms which are typically just a milder form of the infantile form. The symptoms come on at a later age depending on what age you are diagnosed. Typically people are classified as juvenile or adult onset and this all has to do with the type of genetic mutation that you have inherited that determine what type of onset you will have. Obviously the more severe genetic mutation you have, the more likely you will show up as a younger patient, so obviously the infantile patients have mutations that have totally knocked out the GAA gene in one form or another whereas a juvenile or adult onset form may not have a complete knockout of the gene but a partial knockout and they have a residual GAA activity to get them thru the first few years of life, but it will eventually catch up with you as you grow older.



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The thing with Pompe disease is the GAA enzyme acts like a vacuum cleaner in your muscle cells. What it does is clean up glycogen that is accumulating in the muscle cells. Glycogen is an energy source for muscle and typically your body stores it and then gets rid of it and that is what this enzyme's job is to do. So obviously if you are missing this enzyme you won't be sweeping up that glycogen and it will accumulate in abnormal amounts in your muscles. Depending on how much accumulation you get and how rapid it is will determine whether you will be an infantile, juvenile or adult onset patient.

This enzyme is unique because it presents as a protein which you can actually give to a muscle cell. The muscle cells have little receptors on them that will grab hold of the protein and drag it into the inside of the muscle cell where it can work to break down glycogen. That is a unique feature of what a lysosomal storage disease is about in general. Pompe disease is unique where muscle is the target tissue we are talking about.

Early work by several groups of doctors: Dr. Chen's group at Duke University, and Dr. Reuser's group in the Netherlands, are obviously trying to focus on trying to genetically engineer the GAA enzyme into a form where they can deliver it to the patients. There are several ways you can do this: Obviously you need technology and you need advanced technology in order to produce a genetically engineered protein and fire it up in a laboratory in order to produce enough to give to patients. Both groups achieved that through different mechanisms that I don't want to go into during this teleconference, but both groups have shown that if you give babies this recombinant enzyme as well as adult patients although less of them have been treated than the babies. In a fair number of cases you can see pretty good improvement in muscle function which is suggested if you deliver the enzyme into the vein as an infusion that it can go out and be distributed by the bloodstream to all of the muscle cells in the body and be taken up and be able to start clearing the glycogen out.

There are several problems with that approach however; relatively speaking it is showing great promise in many of the clinical trials. Some of the problems though are in order to get the enzyme into all the muscles of your body is quite a task. It's a task because 40% of your body mass is muscle and so you are trying to correct every cell in your body and this requires a large amount of this enzyme because in reality it's not effectively taken up by muscle cells. It is not an efficient process and it is nothing like what people are used to when they take a medication where small amounts can cause big dramatic effects. With this enzyme you need a lot and that turns into production issues and that is why the clinical trials are slow to advance. It is a difficult thing to try and set out to do, first of all, to make a recombinant enzyme, and secondly, to produce the massive amounts you need to treat an adult patient when you can treat several infant patients with this same amount of enzyme. And you have to repeatedly infuse this enzyme, which is better than no treatment at all, but it would be nice to decrease the frequency of how often you might have to get an infusion and those are things that are being investigated in our trials right now and making progress. Those are some of the issues.

Another way to go about this is to try and correct the underlying genetic problem in an individual, and this becomes difficult because how are you going to correct every cell in your body that has this genetic mutation which is causing Pompe disease? There are several groups working on this currently at Duke University, in Florida and in Europe. The way to do this is to



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deliver the genetic information that encodes the GAA enzyme (the blueprint), and try to deliver this into a human patient in a manner that allows that information to go into a cell and start to produce this enzyme for the body to have access to. There are several ways to do that, unfortunately they are all very inefficient right now. When you talk about delivering a gene that has to get into the cells to start producing enough enzyme for a patient is sort of another order of magnitude of difficulty. But we have been able to make some dramatic results in several animal models out of my lab, and what I have done is basically the following: We have taken a cold virus that normally would give you a cold and we have taken the genetic information out of that virus and modified it so that it looks just like a cold virus on the outside but on the inside it contains the genetic information that is missing in Pompe disease patients. We were able to make this virus, (this vector if you will) into large quantities. In fact it was easier to make this virus than the enzyme.

There are several animal models with Pompe disease that we use. There are mice that have Pompe disease that were created by several groups of scientists, Dr Reusar's group as well as Nina Raven's group which supplied the mice to me. They have been able to inject this virus intravenously into these mice and the virus hones in on liver tissue. When the virus goes into the liver it is able to start delivering the genetic information in order to make the GAA enzyme at such a high level that the liver secretes the enzyme to the rest of the body. And in so doing this the body gets enzyme into all of the muscle cells and all of the muscle cells get enzyme. They have been able to show in mice that a single injection of that vector can last for 6 months at least, so whereas right now we are injecting enzyme once a week or once every other week, this vector is able to deliver a lot more enzyme over a longer period of time after a single injection. We have also repeated this in another animal model - quails. These quails are at Duke University and they also have Pompe disease. We were pleased to see that the gene transfer works in this animal model as well. We have shown this repeatedly now for several years and we've tried to figure out some of the problems. One major problem that is holding us back from moving into a clinical setting more rapidly is the dose of virus we have to give in order to get the liver to produce enough enzyme for the entire body. If you estimate out and extrapolate from the weight of a mouse to the weight of a human the dose becomes such that we know at those high doses we would very quickly run into toxicity problems. It would be like getting a massive flu like response and what I worry about personally is to treat patients with a very limited window of success. We have a very narrow window in which to use for a dose escalation. For example, the authorities would make us start out at low doses in patients and you can only get one injection. That is the other problem with this virus is it is not like the enzyme where you can repeatedly get infused. Your body will remember you got infected with this virus and will not allow it to come back into the body again and again, so you only get one shot per patient. So what you would do is slowly increase the dose in subsequent patients. What I worry about is that we might hit a point where we can't go higher on the dose at too early of a stage where we haven't seen a good response in the patients and we would have to stop the trial prematurely. So what we are working on in my lab is to figure out how to decrease the dose or decrease the toxicity of a given dose in order to get a good response.

I have seen dramatic results with this approach and there is real hope in this treatment. I don't think that this is a pie in the sky sort of maneuver that we are doing in mice and quails, I think it is real and it is one of the more dramatic scientific maneuvers with what we are able to do



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and are hopeful for Pompe patients based on that. I see probably an evolution where we might combine the two therapies.

Here is a little vignette that I envision will eventually evolve. It will probably come to the point where enzyme will be approved for human use and the question will become will they have enough enzyme for all of the patients and what the doses will actually be. There is a serious question on production capability and how much can be made and what is the appropriate dose for a given person of a given weight for example. What they may be able to do is come up with a duo pronged approach. We might have a patient come in for a gene transfer vector to initially clean their body out of stored glycogen and then once your body is pretty much cleaned out of glycogen, we would start the patient out on ERT which can be delivered once every other week at a lower dose than you would normally have to give if you were just giving enzyme by itself. The combination of the 2 treatments might afford a more rapid response as well as a longer response as well as an ability to broaden the scope of therapy overall to all of the patients that are affected. This is my view of how I see things going.

Patients with Pompe disease should be thinking to themselves that there are several opportunities that could potentially treat this disorder and I can tell them that the thousands of genetic diseases that I have encountered in my clinical practice that Pompe disease has one of the highest hopes that one or both of these therapies will eventually overtake it. This is where I am at right now.

Questions and Answers:

Q: How is the second injection tolerated? Has it cleared glycogen anymore rapidly with the 2nd infusion of the virus?

A. This is called an amnestic response. This is sort of like getting vaccinated so that your body makes an immune response to the virus because it doesn't want to get infected with it again. Your body will think this is another cold and what your body does is sends up antibodies to block the virus from coming in again so that prevents the virus from going to the cells we want which in this case is the liver, so that is the reason why we really have only one chance. It is not an absolute blockade, in fact there are maneuvers that you can do in humans that you can actually transiently deplete someone of these antibodies. So what I do in the clinic right now is to bring patients in to hook them up to a machine that runs the patient's blood through a filter to remove the antibodies that are floating around and this will last for about 24 hours. When they infuse the virus it only takes about 1/2 hour to get this vector to the liver to start working. So we can transiently deplete patient of antibodies and hopefully allow that 2nd injection to actually work.

Now there is another possibility that might work. There are actually 41 to 50 different types of cold virus out there, and that is why you repeatedly get colds. You catch a cold and then you make a immune response to that type of cold, lets say cold #31, but then your son or daugh-



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ter comes home and sneezes cold #21 on you, well your body has never seen that type before and you have to go thru the same infection and now you are resistant to #31 and #21 but you see there are 40 types out there so we can do the same thing. We can actually make the gene transfer vector out of each of these 41 different viruses and hopefully come up with a catalog of vectors. For example you come in and receive vector #21 and then maybe 5 years down the road you might need another cleanout so you come in and are given vector #31.

Q: Do you think you can clear the glycogen in a single dose?

A: Yes, if they can give enough of the virus. I have shown in mice and quail that in approximately 17 days they can clean out the glycogen in all of the muscles cells of these animals. It is pretty dramatic and the heart muscle is always the first to respond the best, followed by the diaphragm, followed by the skeletal muscles. It seems like the skeletal muscles are the toughest nut to crack.

The big question is what damage has already been done to the muscles and even if you clear out all of the glycogen is the muscle damage reversible? That is the one thing I can't tell you is how reversible is muscle damage. Let's say you have 10 years of Pompe disease where your muscles are very weak and now we go in and clean out the glycogen, will the muscles now magically regain their strength back or will it take awhile for them to rejuvenate. I don't know the answer to this; however, I do know in the mice when we clear out the glycogen when they are young they seem to get stronger rapidly. If we do the same thing when they are older they don't respond as well and that is because their underlying muscle disease has been there so long that they developed contractures and underlying changes that you don't expect to correct overnight.

Q: Is it remodeling of the muscle itself or just the loss of muscle over time?

A: I think it is both, so it all depends if different muscle groups all respond in different ways. Some groups may respond quicker than others.

Q: Does muscle tend to not to regenerate as well as you age?

A: I think so; I know I can feel it more when I exercise now that I'm older. The thing about muscle though is that it is a regenerative organ, so that is a plus. If you take away the insult which is glycogen accumulation you may in fact improve muscle at some level but the question is how much and I don't have a gauge for that.

It is logical that if someone has less muscle involvement when they got treated, they would more than likely respond since they don't have the underlying damage to the muscle to overcome.



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Q: Would combining treatment with a stem cell treatment help muscle to regenerate and maybe to prevent the disease itself?

A: It could, now remember the underlying problem with all treatments is that you have to get every muscle cell in your body corrected or a sizable fraction, so we aren't just talking about 10 or a 100 or a 1000 cells, we are talking about 1000's and 1000's and 1000s of muscles cells be corrected simultaneously in your body. Stem cell technology is another avenue that scientists are investigating and the hope is that you can take a cell out of your bone marrow and inject it into your muscles, now those stem cells become new muscle cells, so the hope would be if you could take the stem cells from a normal person and put it into muscle of a person with Pompe disease that those donor cells would now become new muscle cells. This is a big wow, but the question is, is how efficient is this process? My opinion is biased because I am a clinician and I see patients every day and I don't take lightly to saying I think something has a high potential without really meaning it because I see these patients again and again and I don't like to give them false hope, so my feeling right now, is that stem cell technology is way far away for muscle diseases and I am not convinced that stem cells can turn into muscle cells at a level that can benefit a human. The research on this isn't even close yet. However, gene therapy technology is light years ahead of stem cell research for muscle diseases.

Q: Once you can get the vaccination for gene transmission how often do you need to go back for a 2nd shot or do you even know that yet?

A: It would depend on your age; let me put it to you this way. If you are a baby, and you have zero residual enzyme activity or if you have zero GAA enzyme activity in your body, the animal models show that a single injection keeps the glycogen down in the muscle for at least 6 months if not longer. It might actually work longer in the heart than 6 months. In the skeletal muscles they were starting to see the glycogen buildup start to creep up at 6 months, so the question would be in 6 months or maybe a year, at that point you would need to do something, either come back in for another injection with a virus that was somewhat different than the 1st, so the body would not reject it or start with ERT.

Let's take a patient who is 25 years old that presents with their first symptoms that means by definition that they have enough GAA activity in their body that kept them going for 20 years before they manifested enough symptoms to really become a problem. Now if we go into that individual and clean all of their glycogen out with a virus they may be able to go another 20 years before they reaccumulate enough glycogen before you have to do something. That is why I propose that the first people to receive gene therapy would be adults for that reason. We wouldn't have the grave worries of what are we going to do in 6 months. The babies are being treated with the enzyme because they can go in with the enzyme week after week after week without worrying about the rejection. The doctors don't worry what happens after the enzyme goes away because they can just give them another dose of enzyme.



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Q: I have a question about the initial gene treatment where you give the cleanup dose; obviously there would be immune suppression at that time?

A: Not necessarily. Everything that I have talked about and publicized is without immune suppression.

Q: I'm talking about a person with zero enzyme activity?

A: That would be a baby, by definition a juvenile or adult onset would have to have some GAA enzyme activity. A person with more GAA activity in their body would tolerate more enzyme and more likely to tolerate GAA produced from a virus than lets say a baby that doesn't produce any enzyme activity. So the baby would produce antibodies to the GAA. This is a hypothesis that I hold high but there are debates from a number of scientists whether that truly happens or whether it really matters. This is a point that is up for debate.

Let's talk a little bit about the virus. The virus may need to require some transient immune suppression. If we have to give the virus we may have to transiently immune suppress somebody, let's say like a week before we give the virus to a person and a week after we give the virus. This nothing like a tissue transplant where you are immune suppressed for life. On a relative scale this is much milder than like a tissue transplant.

Q: What does that mean actually doctor?

A: As far as immune suppress? What I'm trying to do is if I were to take this virus and inject it into myself, you or animal models, your body has evolved for million of years to fight infections and to fight them and to recognize them rapidly. Your body doesn't realize that this virus is coming in to deliver this gene that will help you. The body is saying "there is a ton of virus coming in that we have to get rid of because we are becoming infected". That triggers all kinds of alarm signals and most of you should be familiar with these alarm signals: fever, your muscles ache, you feel ill. These are all of your immune system activators getting into gear to fight off the infection and at the doses I am talking about you will really ramp up those responses to the point of severe symptoms of shock. That is what I fear that the dose of the virus that we are working on right now is very close to a dose that might induce those severe symptoms of shock. I don't want to jeopardize the future of this therapy by moving ahead too quickly.

Q: Is this the same virus and dose or is the dose getting close to what happen to the child getting gene therapy in Philadelphia?

A: The dose that was given in Philadelphia was also given to other people at the same dose, without any problem and was given at the same dose to 30 or 40 other people in other trials. However, you go much higher than that dose and you run into problems. It is unclear what happened to the Philadelphia case but when you start hitting those doses that are causing toxicities that would be a problem. I also think that patient had underlying medical problems, actually underlying liver disease that made him especially sensitive to this. This dose would be the upper limit of how high of a dose I would go and the animal models that showed good re-



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sponses is just below that dose but very close to that dose. You can go lower than that dose and clear glycogen out of just the heart muscle but not in skeletal muscle.

A juvenile or adult patient is not classically affected by heart problems so they would target the skeletal muscles. With skeletal muscle you need a higher dose of virus to achieve an affect.

Q: Why do you think the skeletal muscle is so much more resistant? Is it the number of receptors that skeletal muscle might have that differs from the heart muscle?

A: That is a good question and up for debate and I don't know the answer to that. It could be a receptor problem and they are looking at that, it could be muscle cell glycogen could be less receptive and accessible than cardiac muscle. It could be that the heart muscle is more vascular and has a better blood flow so it gets more enzyme per liter of blood that flows past it. There are a number of observations that demonstrates that skeletal muscles are more resistant but the exact mechanism is unclear. We are trying to figure that out.

Q: If you try to maximize the virus input with gene therapy and if you reach a level of toxicity, trying to clear out the glycogen with the initial dose. Once you go over the edge and the body sort of jumps on the virus and overpowers it, it would obviously shutdown what you are doing so the body can recover. If you tried to do this again the body would quickly try to kill the virus immediately, does that go back to what you were saying about having a different virus ready to inject?

A: What I mean by shutdown, I am referring to a clinical trial being shut down. Let's say I'm injecting patient #1, #2, #3 and in each patient the dose is increased in order to find that magic dose that gets a good response. I'm afraid that in patient #13 I would start running into problems with the patient's remaining stable and that the BP might start dropping in response to the virus, the patient would get an high fever, shock like symptoms and at that point the FDA would say this is getting perilously close to where there is going to be more damage to the patient than good. This would be called the MTD or the maximally tolerated dose. This now becomes an ethical issue you wouldn't do this to cure let's say baldness, but for a patient with Pompe disease which everyday robs you of another day of movement and the ability to live your life. I view that as an equivalent to cancer, since it is a lethal disorder. When I go to the FDA I will present it in that guise. We aren't dealing with some mild disorder we are talking about a disease that is robbing people every day so the standards should be elevated some. However, I will not risk people's lives and I am not ready to run a trial yet, because there is that risk and I am not willing to subject people to that risk unless I am absolutely confident that it will work. I couldn't run a dosing study due to FDA regulations and what it might do to the patients on this study. I won't take advantage of the patients that would willingly do this dosing study unless there are signs of efficacy. I have room to go slow because we now have an enzyme replacement therapy coming along as well.



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Q: It sounds very attractive what you were saying about combining the 2 therapies together.

A: In fact Genzyme is very well up to date with what I am talking about here and it definitely perks up interest presented as a guise of combining therapies to conserve drug and to get therapy out to a greater number of patients. And it may be easier to produce overall.

Q: My son in only 19 months old and he is getting a tremendous amounts of enzyme for as little as he is and when he is lets say 20 years old and 150 lbs with no enzyme activity the amount of enzyme needed would be huge.

A: It is producing this enzyme at that large scale that people are having troubles understanding. This is why opening up trials to patients is so slow, people are demanding enzyme and there isn't any available. It is not like aspirin where you can mass produce this stuff, recombinant enzyme is something that is very complex and regulated and not easy to make. The virus is fairly easy to make and the same virus has been used to vaccinate military recruits over the years. People used to catch the adenovirus when they entered boot camp and it would take out a whole unit so they vaccinated military recruits with large doses of this virus. They were able to produce enough of this virus to treat millions of people. So you can scale the virus up if it shows that it can work.

Q: Have the people that received the enzyme actually gained weight?

A: Babies that have responded to the enzyme have gained weight. They start out at what is called failure to thrive, and they aren't gaining weight, but when they respond to enzyme they have gained weight and strength, their ability to suck on a bottle improve and they gain weight. They gain weight mostly because they feel better and they are feeding better.

Q: I was under the impression that gene therapy was going to be a cure and that you would only get one injection and maybe no other treatment would be necessary after that. It seems now that it would be used in conjunction with ERT, so it is going to be an ongoing treatment and not a permanent fix. Why is that?

A: First, I never said it would be life long, and if you understand that the gene therapy is not repairing the genetic mutation in every cell of the body. The virus that is being use is not integrated with your genes and become one with them. However, I am going to be very conservative on how long you can go before you require another injection. If you can deliver the gene to every liver cell, the liver cells don't typically turn over every day, the liver cells you have right now are the same ones your going to have in 20 years, unless you damage the liver cells some way (such as alcoholism). If I can get the gene into those liver cells, and as long as there isn't an immune response to those liver cells and they are eliminated, they could potentially last life long. I always tell people it is likely the injection would have to be repeated because I just don't know and I don't want to give false hope. One injection could last years, but until we actually do it, we won't know.



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Q: Would it be preferable to target muscle instead of the liver?

A: Preferably it would be nicer to target muscle instead of the liver, but imagine what would have to be done. You would have to inject the virus into the muscles. The virus doesn't magically diffuse to all the muscle cells so a patient would need several thousand injections just to hit a little muscle in your foot.

Q: Is there a virus that typically targets muscle?

A: Well, the adenovirus does target muscle to a certain degree, but your body is designed to prevent that from happening. You got compartments, sort of like a ship, and if one compartment floods there are others that lock down and stops the flooding. Your body is not going to allow a virus free reign to all of the cells of the body. The body is set up to have firewalls for example.

Q: Is there a virus that is given systemically that targets the liver?

A: One of the functions of the liver is to filter out the viruses. I am taking advantage of that. When the virus is injected into the vein a large amount is taken into the liver and then the liver secretes this enzyme into the muscle cells.

Q: What percentage of the Genzyme enzyme binds to the receptor?

A: Actually we don't know what receptor that the enzyme binds to for sure.

Q: What percentage is phosphorylated? Is this something that would increase uptake in the muscle?

A: Theoretically and that is all I will say. Remember, doing something in a little laboratory dish is different than doing something in a human body with about a million other variables going on that will try to defeat the results.

Most of the things I talked about in the conference have already been published and you can go look and read it for yourself.

Q: What about naked DNA or plasma DNA injected into the muscle?

A: I am not sure you would get enough muscle corrected at one time and that you could clear the glycogen from the muscle. Typically people do inject DNA into muscles of live patients done to induce a vaccine to immunize someone from something (Ebola, etc) but the response has been weak.

I have seen DNA injected intravenously in animals locally and under high pressure add a substance given to make the blood vessels a little leaky and by doing this you can get the DNA into more and more muscle groups. That is something that is being pursued, but you can do a lot of



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things to a mouse that you can't do in a human.

Q: You talked about the leakiness in the blood vessels, how permeable are blood vessels to not only the enzyme but the virus?

A: Our blood vessels are meant to keep fluid in and to regulate what passes through and that is influencing where the enzyme gets out. So the enzyme has to cross the blood vessel barrier to get to the target cells. The same thing goes for the virus when they inject it.

Q: Does this contribute to the need for higher doses?

A: Higher doses than diseases such as Gaucher disease or Fabry disease?

Remember Gauchers cells are in the blood stream so they are bathed in the enzyme when it is infused; your muscle cells are on the other side of the window looking in saying "hey please come here". If you can permeablize muscle to allow some of the gene transfer agents better access to get in you might be doing something. However, are you also doing damage to your muscle by doing this? These are questions still out there that need answering.

Q: Timing of gene therapy coming on line?

A: We are in the process of combining therapies in the animal model right now to see if the dose of enzyme can be reduced they would first have to do a trial with just the gene therapy, and then one combining both therapies. That is a tall order and would cost a lot of money, however, Genzyme is committed to doing this when the timing is right and when the data supports going forward with that. If that were to happen, it would take a year to produce the virus to a grade that could actually be given to humans and all the problems that are involved in starting up a trial. I give it 2 years minimum.

This was the end of the one hour teleconference on Gene Therapy.