



AMDA Conference Call Transcript

Session No. 7: Gene Therapy

Speaker: Dr. Barry Byrne

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Paul Kessler and I began to study the approach to treating inherited muscle diseases and we really took a fairly broad view that one could use the concept of gene transfer as well as stem cell therapies for inherited muscle diseases. That really was our very first proposal in that area, to a local foundation in Maryland which funded our initial work. That initially led us to the finding that we felt we could demonstrate that it was possible to restore alpha glucosidase activity in tissue culture cells. So the first work was invitro, as it is called or in tissue culture using an adenovirus to carry the gene for human acid alpha glucosidase back into the cells that were deficient in the protein.

What was the departure for us was the work that was done with another kind of viral gene transfer agent called Adeno Associated virus (AAV). This was actually another part of my background in my graduate training so this was a great opportunity to return to that very basic virology work that I had done before.

What we found with AAV was that it went in as a vehicle to bringing the gene back into the cells. It had an unusual property in muscle cells. The DNA which was carried by the virus actually lasted in the muscle cells for (as in the initial experiment), as long as we carried out the experiments.

That was a new finding in the field of gene therapy. There had not previously been any sustained gene transfer with the DNA virus that infected non-dividing cells which was the case here. That initial work used a marker gene and was not a study done in Pompe's disease, but it was done to lay down the foundation that we had hoped to do for acid alpha glucosidase activity. So that was the start of an area of investigation about gene transfer into muscle cells which has really grown substantially over the years.

This all started in early 1995, and in the 10 years that has gone by, we have put a lot of effort into evaluating how AAV is introduced into muscle, how the transferred gene stays in muscle cells, and whether this approach would be feasible for children with Pompe's disease. As clinicians, caring for kids with inherited muscle diseases, particularly ones that affect the heart has always been our goal.

When I moved from Baltimore to Florida, it was with the intention that we would build a gene therapy center. This center would be focused on what some people call translational research. This is the kind of applied science that would bring the basic investigations to the clinic. So our center has focused on that goal and invested in facilities which could make clinical material for human studies. As well as provide the support necessary to get protocols through the regulatory review process to get the approvals needed in order to conduct these clinical trials.



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So there is a multi-level approach to doing these studies, starting with the basic science which is combined with a good idea on how to proceed to attack a problem. Designing the proof of concept studies that demonstrate the usefulness of a given approach and then importantly demonstrating that that approach is safe and effective are some of the criteria by which the FDA judges these studies. That is what we have done since I've come to Florida in 1997.

We finally reached that goal in one disease model, a form of inherited emphysema, with a lot of help from the Alfa-1-Foundation. We have been able to start a phase one trial of gene transfer into muscle, very much the same approach we anticipate using in Pompe's disease, for an inherited protein deficiency called Alpha-1-Antitrypsin deficiency. Three subjects have actually enrolled in the first trial.

There are almost sixty subjects now in a human study that have been evaluated for gene transfer for cystic fibrosis. Several patients with a type of muscular dystrophy (limb girdle muscular dystrophy), have also been studied and we are hoping to continue this work.

So that is the history of what we have been doing. I would be happy to answer questions about any specific aspects, from basic science issues, to the process of getting a program through the FDA and into the clinic, and how those studies are evaluated clinically for safety and efficacy.

I could talk about any one of these specific areas, but of course, many of the things we do in science is when we answer one question it raises two more questions and we keep finding new things about this disease. Certainly we feel like we have learned a lot from the animal models we have generated in close collaboration with Anita Rabin, back when we first started this work we felt this was a critical component. Both our lab and Nita's lab are in collaboration together to make knock out constructs which generated the mice we use today to study gene transfer in Pompe's disease. They are not entirely indicative of what we would expect in human subjects, but it has been very helpful to get a glimpse as to what can be expected after certain approaches to treatment of the disease.

Questions & Answers

Q: Mr. Byrnes from what you describe in your comments, is it my understanding that this is strictly for kids? You don't have any programs for adults?

A: The Alpha-1-Antitrypsin deficiency trial is actually for only adult subjects. The initial studies are aimed at looking at a single site of injection of AV that would lead to secretion of the protein from that site and how the therapeutic affects the rest of the body. This may be an approach which seems to work quite well in Alpha 1 disease. We had some success in hemophilia and it actually was our initial thought about how it might be approached in adults or younger patients with Pompe's. I think the immunology in Pompe's disease is a little more complicated than some of these other diseases except maybe hemophilia.



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One of the challenges we see to this approach is the production of antibodies against the protein. So one of the reasons we're focusing on the younger patient population is that we see less immune resistance to the protein when high levels are generated. The same affect would not be as great in adult patients with some protein that is really only partially defective. But right now we felt like the most pressing medical need was in the infant population who would benefit from systemic gene transfer.

Adult patients are not off the list, but with the approach we are contemplating right now would focus on the smallest kids first.

Q: I have a daughter who has the disorder, she is 13, and I would like to know how many more clinical trials that might be coming up for her age. And also how many more trials before the drug can be on the market as an approved drug?

A: A general background on drug approval. It doesn't matter whether they are protein drugs or gene drugs. This is a very long process which first establishes safety then demonstrates some critical endpoints or outcomes of the treatment as to whether the drugs are effective.

So it depends on what part of the disease a person is addressing, which would influence what is on the label for so-called release or availability of the drug. This is a very long process and so far no approaches using gene therapy have been licensed or have passed the final stage of testing.

One point I might raise about the older patient, particularly in the age range of late childhood to early adulthood, since respiratory muscle weakness seems to be one of the predominant features of the disease in this population. One of the things we have contemplated, however, it is not on the docket for immediate testing is the following. We have recently published a paper about gene transfer to the diaphragm. The diaphragm is a restricted muscle group which we feel would be very responsive to the gene transfer approach and that improvement in diaphragm strength and function may be sufficient to meliorate a lot of the symptoms in the older patient and avoid the potential for toxicity related to this approach.

Q: I have a 9 year old son, is in the Expanded Access Program at University of Alabama, Birmingham, and we go every two weeks to have enzyme replacement therapy (ERT). Will ERT be a requirement before they are allowed to come into a trial for the polymer gel?

A: Now that is a very good question. Sometimes clinical studies are designed to develop a clear cut difference between one therapy over another. I think that gene transfer approaches, particularly for subjects who are already in clinical studies, have the potential to enhance these. The initial feeling is that there would not necessarily be an exclusive treatment for one over the other. If someone can distinguish the effects of one approach apart from the other approach, then that is one of the challenges looking at specific outcome measurements.



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Q. My child like so many that are on ERT is vent dependent 24/7, and has been since 1998. We haven't seen any changes in the ventilator use in the current study, but we're very young into it as he is only on his 12th treatment. We've seen a tremendous change in his heart, in his general health and well being, and he just feels better. Since his heart involvement was pretty serious, his blood pressure has been stabilized, but we haven't gotten so far into the study as to see any changes with the ventilator yet.

A. I think you bring up an important point, since there was a lot of focus on categorizing the disease into different bins, if you will, or certain types of the disease. I think our team feels pretty confident that what we are seeing is the spectrum of the disease from very severe early on-set disease, to very mild late-onset disease. It tends to correlate with the degree of enzyme activity, but we also feel that there are other factors which influence the severity of the disease in different organs systems. So it used to be thought that a child as old as 9 actually didn't have any heart involvement related to the disease, but it is clear now that that can be the case.

Q: How unique is your approach to that of Dr. Amalfitano's? You are directly targeting muscle and Dr. Amalfitano is targeting liver which leads to uptake by muscle. Do you see any particular advantage to targeting muscle directly?

A: There are two different vectors in use in those studies that are directed at the liver. We had done some early peripheral concept studies with adeno viruses and it has generally been found in the field that while there can be persistence of the targeted cells that there is the potential at least, and this has been improved upon over the years in terms of the inflammatory response to the vector itself. And that certainly has been the case with the work the Duke Group had in improving those vectors, but there has not been an example like AAV in any other class of virus other than the retro viruses for the persistence of expression. Our feeling is that the inherited diseases which are going to have a life long manifestation should use an approach of gene transfer which is going to be the most likely persistent and lead to the least inflammatory response.

Now the question then is why treat muscles with specific gene therapy as opposed to secretions from the liver. I think the liver has several advantages as does the muscles, so ideally one could utilize both platforms to achieve a correction. The focusing only on liver is very much like the protein transfer studies that are on going and we have seen in our own lab the same consequences of infused protein and the antibody production against the protein. When the protein is made within the cell which needs that protein to traffic properly to the lysosome, the interference of antibody does not occur within a cell itself. So although the potential for antibodies to help eliminate cells that are damaged and exposed their insides to the circulation, a cell that is functioning properly, that has cell specific genetic correction responds quite well to that approach. So that would be part of the rationale for treating muscle directly and to circumvent what were left and the antibody responses.



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Q: What needs to happen for this to enter clinical trials? What is the major hurdle that you are facing?

A: The steps required to proceed through the early proof of concept studies is to have a discussion with the FDA about a clinical proposal and then to actually execute that clinical proposal. It requires we choose a single agent for study (we always seems to be making slight improvements in the construct that expresses the gene), and I think we have finally selected one that meets all of our requirements. And then selecting the way in which it will be delivered and the doses it would be delivered at. Sometimes it is a little difficult to predict how the dose used in mice would translate to bigger folks, but once that is established then in working with the FDA a plan is established for the safety studies that are required.

We are fortunate that our center has been designated as one of five sites in the country that is supported by the National Center Research Resources part of the NIH core group that provides funding to clinical research centers. The program called the National Gene Vector Laboratories (NGVL) supports these five sites for vector production and toxicologic studies. So we expect to utilize that mechanism in order to get funding to proceed with the safety studies.

The NGVL also provides funding required in order to make clinical grade material for our improved clinical plan. That would be the very next step after getting the toxicology studies completed, which we have now done for three different programs both in muscle diseases and eye diseases. Finally, we have to get the permission through our institution to do the clinical study.

Q: Dr. Amalfitano said that he was pretty close to a critical threshold of toxicity for the doses that would be required for his approach to gene therapy. Are you having the same problem with your approach?

A: We have not found that yet in the toxicology studies we have done for the existing trials. We use a safety margin of 10 times the top dose in the study to see whether we can generate toxicities related to the vector itself. What we have attempted to do in those studies is to make a worse case scenario by giving the vector, instead of just into the muscle; we can make one group of animals receive a dose into the vein so that it theoretically goes everywhere. However, we haven't identified systemic toxicities related to that, other than one example which was presented at the Recombinant DNA Advisory committee meeting. When we presented the data a few months ago one of the questions that came up was, what is the consequence of getting the vector everywhere in the body, in such a way, that in males or females there would be positivity of the vector in germ cells or gonatal tissue (that would be eggs or sperm).

Those studies are most easily conducted by looking at contamination of sperm by the DNA. That's something that the FDA would not want to allow unless this risk was justified by a certain benefit. We've done studies with several types of AAV and actually are conducting a large longitudinal study that will look at several generations of animals to see whether having the vector in that fluid, results in the gene being transferred from one generation to the next. We have actually never been able to demonstrate that, but we need to prove that point conclusively to the FDA. So, that is the only toxicity we've observed and it has never resulted in any adverse



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affects that we can find. So we will keep looking. The group here has done very well to meet the challenge of manufacturing the clinical grade material in order to reach a dose that will actually matter. You have to be able to make enough of it and it is something we have devoted a lot of effort to do. We will be using a new manufacturing method that was developed just for the purpose of making more vector in an economical fashion.

Q: Can you talk a little bit more down the line, what therapy will be like? Is this therapy once a year, or once a month and how do you see this process occurring? Will it be used with ERT too?

A: Yes that is a good question. All of our gene transfer with AAV studies that have been done thus far, have been single dose experiments. There is a group here studying Parkinson's disease in which the gene transfer is done in the appropriate region of the brain. It's a single dose. We found that in cells that don't divide like the brain, the muscle, and the eye, that it is not necessary to give another dose of material to get any sustained expression. Depending on the clinical trial design, a single dose administration and then following subjects over time.

Q: As far as the funding goes, do you see any problems with funding down the road for the studies that you have? Are you funded well enough?

A: For many of the rare diseases we really rely on the cooperation of the various organizations as well as the rare disease entity within the NIH and the FDA. Then specific agencies within the NIH that have an interest in pediatric diseases, such as the Child Health Agency, and the Heart, Lung and Blood Institute have been supporting our work for a number of years because of the focus on heart disease. Traditionally two other agencies NIAMS and NIMDS have been supportive with the muscular dystrophies. The MDA has been supportive since the initial part of this work had a very intense focus on gene transfer for muscular dystrophies both limb girdle and Duchene muscular dystrophy.

Q: So, as far as funding goes it's not really blocking your findings as you go forward with a clinical trial. Do you see anything as far as a certain avenue you want to approach, or a certain type of virus that you like and want to use, and can you see implementing it in a clinical trial in the future? If so, how long and what time-frame would you be able to implement something?

A: The clinical studies that have been done thus far with AAV have had a good safety profile. So as long as we design studies which help answer some of the basic questions that are good science then we expect the NIH will get behind those too, since they continue to be supportive of gene transfer studies in general. This has been the focused of a lot of different review groups within the NIH and there is considerable expertise in that. The funding for this approach can utilize both public and private sources and it's a fairly expensive proposition even just to test the safety studies that are required by the FDA. We need to utilize all the resources that are out there, but I think the good news for rare diseases, is the voice of the organizations have been heard both in congress and the NIH who listens very carefully to the appropriations committee that decides their funding level. Efforts by the MDA and other rare disease organizations and



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NORD in particular has, I think been heard, we've see a lot of initiatives coming out of the NIH soliciting proposals related to rare diseases and I think this is a good thing.

Q: So you are saying positive findings in gene transfer with other diseases could help Pompe disease?

A: Oh absolutely, and by the same token entities that are interested in more common diseases like heart failure will be interested and are interested in the findings from rare disease programs. The advantage in the rare disease studies is that we know the very specific path of the physiology of the disease, we hope, in some of the more complex multi-factorial diseases that affect millions of people like heart failure. It is unclear what the best therapeutic approach is and we know any single gene defects to go after.

Q: Is there a possibility of getting a private corporation involved in helping to fund a clinical trial for Pompe or is it such a rare disease that it would be difficult to find a company that would be interested?

A: No one ever rules out that possibility. There have been various companies that have expressed interest in both lysosomal storage diseases and cardiovascular disease that would theoretically use this as an example, if you will. The economics of it all are complicated and I don't begin to understand what would make something a successful commercial venture, but I think the science behind it has to be the first motivator. And particularly the fact that we continue to look for successful therapeutic approaches for what is a complex problem.

Q: If I understand what you are saying, the gene therapy would be administered by injection into the muscle?

A: The study we are doing now is establishing the safety of injecting into muscle. However with Pompe disease where multiple muscles are affected we really want an approach that is more systemic in nature and there are various strategies for achieving that. By a publication recently by Dr. Chamberlain's group, in which they are focusing on Duchene muscular dystrophy used the strategy of AAV gene transfer to muscle, but takes advantage of a unique feature called VEGF which helps get the vector beyond the vasculature of the muscle cells. In other types of AAV, not the types used in that study, the vector seems to do that bit all on its own. So that's the approach we are contemplating will be most useful in Pompe's disease. And in conditions where there is mostly heart muscle involvement there are other strategies for delivering the dose to the heart muscle or to the diaphragm or specific muscle groups that might be most effective. But I think the one approach is to try to consider getting all the muscle groups at once.



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Q: I was reading today about the Myostatin blockers with mighty mice and that they were seeing that it was making them much stronger. For somebody like me, who is just beginning to have difficulty with walking, would something like that possibly be beneficial to me to have something that might work with the muscles I have and make them stronger?

A: That is a very interesting concept. For those that haven't followed this, I can give you a smidgeon of background on this protein. Myostatin is part of the family of protein's that regulate how different organs grow to a certain size. So that muscle mass is controlled by level of Myostatin expression. It influences it in reverse, if you will; more Myostatin makes you smaller and less Myostatin makes more muscle. For some of the diseases where there is an abnormal loss of cells, it may be that Myostatin causes the dividing myoblasts to become myotubes or mature muscle cells. In the case of Acid Maltase Deficiency, those cells would probably be equally affected by glycogen accumulation so it wouldn't necessarily be a long term benefit to actually promote muscle mass using the precursor cells at a faster rate than one might want. So I don't think that is probably a good strategy, but it is a very interesting approach to some of the other dystrophies or particularly those people without inherited muscle disease.