Speaker 1:

Welcome to the Eye on the Cure podcast, the podcast about winning the fight against retinal disease from the Foundation Fighting Blindness.

Ben Shaberman:

Welcome to another episode of the Eye on the Cure podcast from the Foundation Fighting Blindness. I am Ben Shaberman, senior director of scientific outreach at the Foundation. I'm really glad that you could join us. I'm very pleased today to have with us Dr. Dave Knop. He is vice president of process development at AGTC. Welcome, Dave. Glad you could join us.

Dr. Dave Knop:

Thanks, Ben. I appreciate the opportunity to speak with you.

Ben Shaberman:

It's great again to have you. For those of you that don't know, AGTC is a gene therapy development company. They're very focused on the inherited retinal disease space. Right now, they are moving an X-Linked RP gene therapy toward a Phase 2/3 trial. They also have trials underway for achromatopsia gene therapies. And while normally, during podcasts, we talk about treatments and trials, and whether they're working or not, and who they may benefit, today, we're going to take a little different tack and talk about gene therapy manufacturing. It is not a trivial endeavor, and I'm excited to hear more about this from Dave.

But before we get started, I think it's important for people to understand exactly what a gene therapy is, what are its components. Dave, I was wondering if you could talk about that a little bit before we get into the manufacturing.

Dr. Dave Knop:

Sure. A gene therapy therapeutic is a little different than a small molecule, for example. The components that are involved with gene therapy is really targeting a broken, missing, or malfunctioning gene. You're trying to replace that with a healthy gene. In some cases, you might even be looking to repair or inactivate a malfunctioning gene. AGTC is really focused on that first version, that replacement of a broken gene. We do that using a viral delivery method. That's usually referred to as a vector. There are other approaches, but that's the approach that we take.

In specific, we use an AAV gene therapeutic. What that's comprised of is, in our case, a small human virus that the majority of the population already has had exposure to, but it doesn't cause any known disease. It doesn't cause any known pathology for which you have to get treatment for. That actually makes it a really good use for gene therapy. I'll come back to that in our discussion, I'm sure, at some point. But it's referred to as a dependovirus, [inaudible 00:03:18], meaning it needs help to make itself, which again is a very nice way of having a safe approach. But it can complicate the manufacturing, as I'm sure we'll discuss.

The viral vector actually has three major components. It's got the viral protein count, or the shell, that basically houses the gene that you're trying to deliver to the patient to fix the broken gene. It's also got the genetic component inside, which is broken into two sub-components, really. One is the so-called promoter, or the genetic element that recruits the body to go there and make that gene into a protein to try and correct the disease, and then it's got the actual gene that's being transcribed into that protein.

Ben Shaberman:

I think of these viral systems as like vast container systems with many, many containers that, in each of those shells as you call them, contain a copy, in most cases, of the gene you're trying to deliver. Is that a pretty accurate way to put it?

Dr. Dave Knop:

Exactly. You can almost think about it in a [inaudible 00:04:37], or a contemporary reference, with shipping containers that are on people's minds. You've got a shipping container that's got the cargo on the inside that you want to deliver to the destination, and the destination might be the organ that's most affected by the disease, or the cell types that are most impacted the disease, to try and correct it, and those containers have that DNA in it you're trying to deliver in them, and as you mentioned, there's many, many, many of these containers. There's billions and billions and billions of these containers inside of an actual delivery to a patient.

Ben Shaberman:

Right, so when you're injecting that tiny drop of liquid, in many cases, underneath the retina, it has those billions and billions of containers that you're trying to deliver to a large population of cells in the retina, in many cases. And then you talked about the promoter. I've heard people talk about it being like a gas pedal for the virus, so it controls the amount of protein that might be made. Is that accurate?

Dr. Dave Knop:

Yeah, exactly. So as I mentioned, you transcribe the gene into RNA, and you translate the RNA into protein, and those are the basic steps. But that promoter is what really [inaudible 00:06:08] the amount that is ultimately made of that final protein from that starting genetic material. So if you've got what is referred to as a strong promoter, it might make, let's say 1,000 copies. If you've got what's referred to as a weak promoter, you might make 10 copies, and you might make everything in between. There are cases where you might want to have a limited amount of protein made. There might be cases where you want a lot made. It really depends upon the disease and the cells you're trying to target, because there are definitely specific ways that you have to plan and attack the individual diseases to get the outcome you're looking for.

Ben Shaberman:

Right, right. So let's move into this topic of manufacturing. To start off, can you tell us about your role at AGTC? How long have you been with the company?

Dr. Dave Knop:

Sure. I've been with AGTC for about 19 and a half years. It'll be 20 years in March next year.

Ben Shaberman:

Wow. That's amazing.

Dr. Dave Knop:

Thank you. They keep me busy. I'm an engineer by training, and I'm always excited to work with biological systems, because it's always very fascinating to me, the complexity and the challenges, and there's never a dull day. There's always so much more to learn.

In terms of my role here, as you mentioned, and my title might suggest, process development is the definition of the processes that we use to make containers, so to speak, these viral vectors that we want to use for gene therapy, and so my team will define the way in which we make these viral vectors. They'll figure out ways to produce them, and that might encompass how fast you mix it, or how long you mix it, or what the temperature is, or what the other environmental variables might be. And then that moves into purification, which is a fancy way of saying making it amenable for use in a human or an animal background, getting rid of the stuff you don't want, keeping what you do want, which is those viral vector containers.

Once we define those processes in my team, then we transfer that to contract manufacturing organizations, or companies that really have the proper [inaudible 00:08:27] environments to meet the FDA expectations that we're trying to get to.

Ben Shaberman:

Right. And to actually make virus, my understanding from talking to you previously, is that you actually grow the virus, or make the virus in cells?

Dr. Dave Knop:

Yes, that's correct. One of the things that's interesting about AAV is it's what's referred to as a dependovirus, that aspect I mentioned earlier about it can't actually really make itself, even though everyone's had it. It needs help to make itself in cells. While that gives it a really nice safety profile for gene therapy, it also makes it really challenging to manufacture, because if you need a so called helper virus, to make the virus you actually want, you've got a very complex system on your hands.

Ben Shaberman:

Interesting. So you grow the virus in these cells. What kind of environment are you growing them in? A big container, or...

Dr. Dave Knop:

Yeah, so the process starts out pretty small. You'll take a vial of these cells that have been stored really cold. They're actually stored in liquid nitrogen, which everyone's seen in movies. It's the so called liquid that's got vapor coming off it. We'll take cells out of liquid nitrogen, and we'll put them into growth media that has many, many, many components in it to meet the nutrient needs of the cells. And we'll continue to make those into larger and larger containers until we get to that highly regulated stirred vessel, or that stirred tank that you mentioned. That'll be where we transfer the cells ultimately, and then we'll add that helper. That starts the process of making all of those viral vector containers that we were talking about earlier.

Ben Shaberman:

How big is a batch of virus, if that's an accurate way to say that?

Dr. Dave Knop:

Sure. I mean, it could be anywhere from a few liters, or a gallon, to put it in terms that people in the US are probably a lot more used to hearing. It could be anywhere from a gallon or two, up to hundreds of gallons, or thousands of liters. It really varies with your need. So for example, if you're trying to tackle a disease that has a lot of patients, you're going to have to make a lot of virus, a lot of vector in order to

try and treat that disease with gene therapy. So it really depends on the disease. It really depends on how big is the organ you're trying to treat. In our case, we're talking about eye diseases, so that's a smaller organ that requires a little bit less. Perhaps in other [inaudible 00:11:31] where you might try and target the whole body, for example, that's going to take a lot more virus in order to try and do a gene therapy approach.

Ben Shaberman:

Sure, sure. How long does it take from the beginning of the process to the end? How long does it take to actually make a batch of gene therapy?

Dr. Dave Knop:

It really varies by system, but in our system, it takes about three weeks. From the time you take that vial of cells out of that really cold storage, and you start putting it into larger and larger volumes to increase the number of cells that you have to be able to make more virus, and then you make the virus, and then you go into the [inaudible 00:12:15] processes to purify it, get rid of all the stuff you don't want, keep the viral containers you do want, that's going to be about three weeks if we go as fast as we can. And that's at about that 50 liter scale, so maybe eight to 10 gallons.

Ben Shaberman:

Okay, okay. And then once it's made, how long until the batch expires? How long can you store it?

Dr. Dave Knop:

What's interesting is you can put the vector, or the gene delivery vehicle, into different formulations of salt, and water, and so on, and you can store it in very cold freezers less than or equal to minus 65, which is about minus 85 Fahrenheit, and you can keep it in there for years at a time. It can be stored pretty stably for a long period of time, which is great that we're able to do that, because then you take out a vial and you treat a patient, as opposed to having to make a new batch every time you want to treat a few patients.

Ben Shaberman:

Right. That's great that it can be... You go to all that trouble to make it, you want it to last, so that's great. Are there certain pitfalls, or challenges, that you really have to be aware of when making something like AAV? What kind of things can go wrong if you're not careful?

Dr. Dave Knop:

One of the analogies I like to use is, people are very familiar with small molecules, things like aspirin. They've taken aspirin many times in their life. You can make aspirin fairly straightforward, mixing a couple chemicals together in [inaudible 00:13:58] conditions and you can recover that pretty easily. By contrast, with AAVs, it's much harder to produce because, as we were discussing earlier, the cells, whether they're mammalian, or insect, or otherwise, they have this really complex media they're growing in that has all these different nutrients that they need in a specific ratio. You can get the ratio wrong and then you don't get the outcome that you want, because the cells don't grow correctly. If the cells do grow correctly and you're not careful, you can accidentally contaminate mammalian or insect cell cultures with bacteria or fungus, and then you have to throw out the whole batch, because it's something that's obviously not what you want, and it can cause big problems if it actually were given to

a person. There's very rigorous testing that you also have to do to show that the material is [inaudible 00:14:51].

That kind of contamination is also one of the really big problems that can go wrong, but then there's a lot more subtle things that can happen. For example, on the purification steps, where you're trying to recover what you want, and get rid of what you don't, one of those conditions can be off by a little bit, and you might get a very different outcome than you want. You might not remove what you're trying to get rid of, so cell parts, for example, might stick around when you don't want them to, or you might lose your viral vector containers you're trying to keep because there was a slight change in the condition that wasn't caught until it was too late. So there's a myriad of things that can go wrong along the way.

Ben Shaberman:

Okay. But you said there's rigorous quality control, so you're making sure that whatever ultimately goes into the patient's eye is pretty pure and the right formulation.

Dr. Dave Knop:

Yes, absolutely. So along the way, while we're doing the process, we're testing for various aspects to make sure the process is proceeding as expected, but then when you get that final product, there's a whole long panel of assays that the material has to pass in order to be allowed into use with people. That list is growing every year, but I think we're up to something like 40 assays that are done to release it. That's a whole other aspect of the manufacturing scenario that's very rigorous and complex, that all those assays have to be developed and rigorously applied to the FDA's expectation in order to have the material available.

Ben Shaberman:

Thanks, thanks. Well, thanks for this discussion of the manufacturing process. It's been really fascinating, Dave, and I think it gives... At least, it gives me a greater appreciation for what goes into something like a gene therapy that is obviously not something trivial to make, but it's so important to do it right for clinical trials. Obviously, you want a treatment to work, and it needs to be made correctly for it to work, so I appreciate all that you do to make sure that that process is done correctly, and can ultimately hopefully help patients. I want to remind our listeners that if you have a question, you can send it in via email to podcast@fightingblindness.org. Again, that's podcast@fightingblindness.org.

Dave, this has been a really interesting discussion. I appreciate you taking time out of your day when you're normally working on making gene therapy to talk with me, and to share all this great information with our listeners.

Dr. Dave Knop:

My pleasure. We certainly appreciate everything that the Foundation Fighting Blindness does to enhance the lives of patients who are battling these types of diseases, and we're hopeful that we'll be able to provide them some really good therapeutic options in the future.

Ben Shaberman:

Well, our constituents are very excited about the progress AGTC is making in gene therapy, and we appreciate your support as well. You're great sponsors of some of our programs, so thank you for that.

So listeners, thanks again for joining us for another episode of Eye on the Cure. We're always delighted to have you, and stay tuned for the next episode in a couple of weeks. Take care.

Dr. Dave Knop:

Thanks.

Speaker 1:
This has been Eye on the Cure. To help us win the fight, please donate at foundationfightingblindness.org.