Bob: One of the things, frankly, that has held back the field of gene therapy for many years is the inability to manufacture at scale. So there are many laboratories that can produce just a small amount of a potential gene therapy that would be used for research purposes. And then in order to be able to use it for clinical purposes, you would obviously need to scale up pretty substantially from laboratory scale to what we will call commercial scale, or clinical scale.

Adam: Welcome to a special *bonus* episode of Science Will Win, a miniseries about Gene Therapy. I'm Adam Rutherford.

Now, before we get into it, just a reminder, gene therapy is a promising area but it's investigational in nature for many diseases. There is much research still to be done to understand the safety and efficacy of these potential therapies. Remember, you should always discuss treatment options with your healthcare provider.

Now, there's so much to talk about when it comes to gene therapy. There's the science, the policy and most importantly, the lives gene therapy has the potential to change.

And, well, there's way more than we could fit into just four episodes. So we wanted to pull back the curtain a bit and give you an inside look at a different aspect of gene therapy: How you actually *make* one.

Let's backtrack a little bit here. First, it's helpful to remember that we're talking about rAAV gene therapies. Here's Sonal Bhatia, Pfizer's Chief Medical officer for Rare Disease.

Sonal: What we do is, the whole concept around gene therapy, is really you're reprogramming cells so that they can start to function normally. Once you understand what is the gene that is not functioning, you can then develop it. That's the whole concept around recombinant. It is a manufactured component that you're manufacturing outside the body.

Adam: Recombinant is the 'r' in rAAV. Basically, recombinant means that it's been engineered. And this is where it gets really interesting.

Adeno-associated viruses – that's the AAV – can be engineered so that the virus' *own* genome is replaced with DNA that contains the functioning or healthy copy of the gene you want to transfer. This is known as a transgene.

The engineered DNA also contains a switch to turn the gene on in specific types of cells, where it's needed to treat a particular disease.

Sonal: So when you take the gene and put it into the vector, you're in essence, creating a delivery vehicle for this gene.

Adam: The vector, that's delivery vehicle, in this case, is the combination of engineered DNA *inside* the de-activated adeno-associated virus.

AAV is actually a naturally occurring virus and although it can infect humans, it's not known to actually *cause* disease – it's non-pathogenic for us. This means that rAAV vectors are intended to not replicate in the human body.

In simpler terms, rAAV is a custom-made virus that has been modified to deliver genetic material into human cells.

Now, the type of viral vector depends on the type of organ or tissue you want to target. So for Duchenne muscular dystrophy, for example, it's muscles. For hemophilia, it's the liver. And *how* much viral vector you're gonna need also depends on the organ or tissue you're targeting: For example, for diseases of the retina it's a much smaller amount of tissue, so you need much less vector than for a muscle disease.

Either way, the challenge remains: You need to produce enough viral vector to do the job. And in this case, that means billions and trillions of AAV vectors transporting billions and trillions of genes.

Okay, with that background, let's get cooking. Gene therapies are made using methods similar to other advanced therapies, like biologics for example. But there are a few key differences.

First, you need to gather and prepare all the raw materials so that you can create a production cell line. It's actually *inside* these production cells where the vector is produced. To do this at scale, you're gonna need a bioreactor.

Bob: Which is, you can think of it as like a big vat.

Adam: That's Bob Smith. He's the person you heard at the very beginning of this episode. Bob is the senior vice president of the global gene therapy business at Pfizer. As part of his role, he works closely with the gene therapy manufacturing team.

The bioreactor – sounds super science fiction, but basically as he said it's a big vat – is where all of these different processes take place.

So, inside of the vat, you have production cells. And these cells act like mini factories.

Bob: You have all of these cells that have been producing within themselves, these vectors. So the vector is the protein capsid or the shell of the virus with the transgene encapsulated within it. And so the challenge is you have to break open the production cell in order to harvest the vector. But when you do that, you release all the other components within the cell into that bioreactor.

Adam: The process Bob is describing there is lysing – or breaking down the cell. Doing that releases the vector the cell has been producing.

Bob: All of the components of the cell membranes, different organelles within the cell, are all kind of in a big soup within the bioreactor. And then what we do is we go through a series of clarification and purification steps, and we gradually remove all of the components of that bioreactor that we don't want. And we finally just purify out the fully manufactured vectors.

Adam: During this purification process, there are also tests for quality control, to make sure the vectors contain healthy genes and are up to standard.

This process also starts small, and then grows bigger and bigger with every batch, eventually getting to a large enough scale.

Bob: And so typically you might move from a 10-liter disposable bioreactor to a 50 liter. And then from a 50-liter, you would scale up to 250 liters. And then from 250, you would scale up to 2000 liter. Now you have to have a robust process across that spectrum of say 10-liter to 2000-liter. And so as you scale up, different attributes of the manufacturing process needs to be improved upon in order to achieve a productivity from the manufacturing process, that's appropriate to support a commercial product.

Now, the process of doing this sounds really simple as a way to describe it. It's almost like making a stew in your kitchen, but it's a very complicated process where there's a lot of strict controls in terms of temperature, pH oxygen saturation, et cetera, that are required to be consistently applied from one batch of your stew, as an example, to the next batch so that we have a uniform and consistent vector that we produce that would ultimately be administered to patients.

Adam: Of course, it's not as simple as cooking up a stew in the kitchen. These are living cells that are producing the vectors, after all. And the production process for making a gene therapy in the lab or manufacturing facility is unique in so many ways. One component to highlight is the production cell itself.

Bob: One of the challenges with producing gene therapies is the actual production process itself within the host cell by its nature, the production of the vectors within that host cell will ultimately lead to the death of that cell. So in many other types of biologic products, such as recombinant proteins, monoclonal antibodies, the blueprint to produce that therapeutic biologic within a cell is incorporated into the genome of that production cell. So it's an immortalized cell line so that you can expand that immortalized cell line into multiple cells. And then each one of those cells will produce the therapeutic protein by itself through multiple generations of that cell line.

Adam: I'm just gonna break in because this is really complex; what Bob is saying is that typically, when you're making another type of biological therapeutic product, you can re-use the production cells. You don't have to destroy them at the end of the process. You teach the production cell how to produce what you need, and then it can keep on doing that over and over again.

Bob: Whereas in gene therapies, that production cell will produce many, many vectors, but then that production cell will die. So each time we want to make a gene therapy, we kind of have to start from scratch. And that's where the challenges have been besides the fact that they're probably the most complicated therapeutic products, that we've ever researched and developed. And so they're just, by its nature, it's just highly complex.

Adam: The whole process, start to finish, from the raw material manufacturing to delivering the product to the patient, usually takes about at least 9 to 10 months. At the end, the number of doses per vat, that's the bioreactor, depends to an extent on which disease the therapy is targeting, and which cells it needs to reach.

Once the experts in the Pfizer bioreactor kitchen – so to speak – have finished this very complicated stew, the resulting gene therapy has to be frozen so that it can then be delivered to patients. And here is the next challenge – how do you get the gene therapy medicine from the manufacturing facility to a patient?

The gene therapy medicine is fragile. And so it's stored in a specialized vial at a temperature of minus 60 to minus 90 degrees Celsius. This is known as ultra-cold storage.

Bob: And then for the physical distribution, for example, from a manufacturing facility to a distribution facility, and then ultimately to a hospital or a clinic where a patient would be administered the gene therapy, we have developed unique carriers that have the ability to maintain the ultra-cold temperature with, sensors so that we know exactly where that particular carton that contains the gene therapy is at any point in time, that we know the integrity that the temperature has been maintained at the right levels, that there have been no excursions in terms of the handling of the material.

Adam: Perhaps all of this is sounding a bit familiar. Pfizer and other pharma companies also used ultra-cold storage to transport mRNA COVID-19 vaccines. That's something Bert Bruce, Pfizer's regional president for North America in the Rare Disease group, brought up when we talked to him.

Bert: So it won't be surprising to understand that some of the insights and things that we were developing for gene therapy were very transposable to the supply chain that was developed for the delivery of the COVID vaccines globally. And so if you think about the delivery and what it needs to go through in order, it's a very similar process.

Adam: And that's one of the big ripple effects of gene therapy development. The research advances and infrastructure for gene therapy were useful in the vaccine rollout, too. Here's Bob Smith again.

Bob: We started embarking on researching and developing our gene therapies in 2014. And one of the unique challenges that we knew that we had to address was how to handle these gene therapy drug products at ultra-cold temperature.

The benefit of all the work that we had done perfecting this for gene therapies was very rapidly and directly applied to the COVID vaccine and helped to enable the very rapid, commercial rollout of the COVID vaccine across the globe. So it's an area where there were some synergistic benefits of applying, you know, good science and good work that we had done for gene therapies in a clinical setting to both the clinical development, and now the commercial development and rollout of our COVID-19 vaccine.

Adam: Finally, for an approved gene therapy, you would then need to send it out to its final destination, where it can be administered to eligible patients.

But we also have to remember -- this is something a patient will potentially take just once, and the effects could be transformative for many years or even the rest of their life. This is a *very* important delivery. It's not just your average mail order prescription. And as such, it requires some special consideration.

Bob: We want to have 100% what we call error-free, frictionless delivery of the gene therapy to a patient. And so we've developed a number of technologies that have enabled the flawless delivery and physical distribution of these gene therapies for patients.

Adam: I wanna leave you with this thought; as we've explored all through this series, gene therapy has the potential to change lives, especially in the field of rare disease where there are so few treatment options.

There are a lot of challenges ahead. And fully realizing that potential will require continued scientific advancement and innovation across the entire spectrum of discovery, development, and delivery. I, for one, am extremely excited to see how this field of medicine continues to evolve.

Thanks for joining us on this special bonus episode. If you haven't listened to the rest of the season yet, please go back and do that! You'll get to know so much more about gene therapy – how it works, the challenges we face and what it could mean for patients.

Please do take a minute to rate, review and follow Science Will Win on Apple Podcasts or wherever you get your podcasts from. It really helps new listeners to find the show. Special thanks to our guests, to the Rare Disease team at Pfizer and Wonder Media Network.

Thanks for listening!