Gene therapy on brink of golden age: Here’s why

Bruce is 18 years old and for most of his life he has had to be extremely careful. During childhood, and all through high school, sports were out of the question because he has hemophilia B, a bleeding disorder, also called “Christmas disease” since it results from a deficiency of coagulation factor IX. Coagulation factor IX is also known as the “Christmas factor” and, because of a faulty gene on the X-chromosome that he received from his mother, it was useless in Bruce. Bruce’s mother is perfectly normal. In fact, all the females in Bruce’s family are normal; a classic feature of an X-linked recessive disease.

Until recently, Bruce had to receive recombinant factor IX –the protein his body doesn’t make can be synthesized by genetically modified microorganisms– through intravenous infusion several times per week. That kept Bruce from bleeding to death but he still suffered from frequent “breakthrough bleeds” because the infused factor did not render him completely normal. Had he skinned a knee, as so many school children routinely do during recess, he could have bled into the joint. Had he bumped his head, even modestly, blood could have accumulated in the connective tissue layers between his skull and brain, causing a life-threatening situation. That’s why he avoided sports, all sports, and had to be cautious in everything he did, even shaving.

But last year, Bruce got the best Christmas present ever: a permanent cure of his Christmas disease. Now, he can play soccer and basketball with the kids at the after school program where he volunteers as a big brother. He no longer needs intravenous infusions of factor IX and if he nicks himself shaving, there will be no concern; he’ll heal as quickly as anyone else, since his liver now makes working factor IX. This all happened because of gene therapy and Bruce is quite satisfied, even though he will have to be monitored for the rest of his life (just in case the gene therapy produces any long-term effects). Unlike hemophilia A, the most common type of hemophilia, where gene therapy faces slightly more difficult technical challenges, clinical trials to replace the needed gene, leading to permanent cure for hemophilia B, are well underway.

Hemophilia A will follow soon, and in the meantime gene therapy is also being tested in human patients to treat a class of conditions known as hemoglobinopathies (where red blood cells make either defective globin proteins, or not enough of them) and for certain eye diseases that lead to blindness due to inherited gene defects involving the retina.

There are reasons why these are the first areas of gene therapy success, reasons that are connected with a rather bumpy history of gene therapy that has been characterized over the last quarter century by some hefty promises interspersed with some serious disappointments. But it’s through navigating that bumpy history that researchers learned what the challenges actually were and developed a realistic idea of which conditions gene therapy could potentially cure sooner and, conversely, in which areas clinical success would likely take a lot more time.

Cystic fibrosis: Promise, failure, and finally needed insight

Though ideas for gene therapy can be traced back to the early 1970s, the field really started moving from

the realm of science fiction to science reality when the transmembrane regulator (*CFTR*) gene responsible for cystic fibrosis (CF) was cloned in 1989. Like hemophilia, CF is a recessive disease. Unlike hemophilia, CF is autosomal, not X-linked, so the disease occurs equally in males and females.But like hemophilia, CF is monogenetic, meaning the problem involves just one gene, rather than a whole bunch.

With the *CFTR* gene cloned, it seemed obvious that if you could give CF patients a normal copy of the defective gene, the disease would go away. Without at least one working *CFTR* gene, a person makes abnormally thick mucous along membranes, such as in the digestive tract. This leads to terrible symptoms but usually what kills CF patients are the effects of the thick mucous in the lungs. By the mid 1990s there were clinical studies set on using inhalation to deliver an engineered virus carrying the needed gene to the cells that needed it.

A virus is the easiest type of vector (delivery device) to be adapted for use in gene therapy because the one thing that viruses do very well is infect tissues and cells, thereby delivering genetic material. The CF gene therapy researchers used an attenuated form of adenovirus to deliver the needed *CFTR* gene to the cells lining the lungs of CF patients by inhalation. This virus had been modified to eliminate viral pathogenicity genes and was incapable of spreading throughout the body without the help of a different virus. Despite the sound scientific rational and promising results in laboratory animals, clinical trials in the 1990s and early 2000s failed, mostly because patients generated an immune response against the proteins on the viral “coat” enclosing the genetic material. Subsequent attempts to deliver the gene using vectors other than viruses also failed because they had trouble penetrating the mucous in the diseased lungs and because they stimulated inflammation.

In the midst of all of this, in 1999, during a University of Pennsylvania gene therapy trial targeting a different genetic condition (more rare than CF), an 18-year old subject, Jesse Gelsinger, died after having adenovirus particles injected into his liver, probably because of an immune response. Jesse’s death generated enormous ethical controversy because his condition, ornithine transcarbamylase (OTC) deficiency, had previously been controlled by a low-protein diet and medications since his birth and because it was only a phase I trial. The injected viral particles were not intended to cure him. Instead, they were injected only to test the safety of the procedure, which would allow testing to proceed to phase II, where the goal would be actual treatment that ultimately might have helped Jesse and others with OTC deficiency.

But it did not work out that way. As stated at the time by Georgetown University bioethicist, LeRoy Walters, “I think it’s a perilous time for gene therapy, ‘Until now, we have been able to say, ‘Well, it hasn’t helped many people, but at least it hasn’t hurt people.’ That has changed.” Thus, reporting on the case for the New York Times, Sheryl Gay Stolberg wrote:

> Every realm of medicine has its defining moment, often with a human face attached. Polio had Jonas Salk. In vitro fertilization had Louise Brown, the world’s first test-tube baby. Transplant surgery had Barney Clark, the Seattle dentist with the artificial heart. AIDS had Magic Johnson. Now gene therapy has Jesse Gelsinger.

This, plus the failure of the CF gene therapy trials in the same era, put a damper on things; however, the research continued. By studying the immune reactions to the adenovirus, researchers came up with new ways to tinker with the delivery system. Ultimately, this led them to develop a fat-like molecule that could form spheres around the genetic material that they wanted to deliver, similar to the various kinds of spherical carriers that transport cholesterol in the blood that have given rise to the popular terms “good cholesterol” (uses one type of carrier) and “bad cholesterol” (uses a slightly different carrier). This, and other non-viral vectors, has made gene therapy for CF a candidate for clinical trials once more, but the knowledge base today is orders of magnitude greater than it was 20 years ago.

Importantly, experience over the past 20 years has also taught investigators developing gene therapy for other diseases that each type of vector (viral and non-viral) has benefits and drawbacks, leading to an overriding pearl of wisdom: the formula is different for each disease. While this means that the pathway to successfully using gene therapy for specific diseases many be long and complicated, it also means that there are many diseases that could ultimately be permanently cured using this approach.

Blood diseases, eyes, and brain

Just as vaccination began first for smallpox, expanded to a handful of other diseases, and has since grown to prevent many more, the same is happening with gene therapy.

For some diseases, vaccine development was easier. With smallpox vaccination, for instance, Dr. Edward Jenner successfully used a cowpox virus back in the year 1796, even though he had no idea why it worked nor what he was administering. The 20th century saw rapid development of vaccines for measles, polio, and various other common infectious diseases, thus saving millions of people. However, for certain other infections, like Ebola and flu virus, success has only been partial and full protection against these diseases has not been realized. Yet.

An entire generation since AIDS first presented itself to medical researchers, a promising vaccine against human immunodeficiency virus (HIV) finally beckons. It has been a complex task because the way HIV causes disease, and importantly the way it incorporates into human cells, makes it a much tricker prospect, than for example, the measles virus.

A similar complex scenario applies to gene therapy. One of the great hopes is that it will be used some day to cure cancer, but that’s going to be a complex task, and rest assured it will not cure all cancers all at once. Instead, there will be different gene therapies for different types of cancer, which really constitutes a wide range of different diseases. Currently the promise and success are with diseases that lend themselves to having genes knocked out or introduced in limited anatomic sites, or in a limited number of tissues, since this allows both for targeting and concentrating the genetic modification as well as avoiding, or limiting the risk of an immune response.

Thus, people who have gone blind because of certain inherited enzyme deficiencies affecting the retina today are getting their site back in clinical trials, because the eyes lend themselves well to gene therapy delivered locally. It may sound scary, but specialized ophthalmologists who focus on treating retinal

conditions are very good at injecting treatments directly to the retina at the back of the eyeball. They do it with drugs all the time, so they can just as easily do it with genetic treatments. The old adenovirus vectors used in early gene therapy have evolved into what are called adeno-associated virus (AAV) vectors, and these have proven to be the right kind of delivery system for getting genes into retinal cells, or for delivering agents knock out genes. AAV gene therapy is showing promise in the retina, because the treatment is concentrated where it's needed and also does not evoke an immune response.

AAV vector also has been the vector employed in cases like that of Bruce, and others who have received gene therapy for hemophilia B. This is because AAV has been modified successfully to carry the factor IX gene and needed gene editing equipment, and also because the virus hones in on the liver. Unlike the experimental treatment that led to Jesse Gelsinger's tragic death, the AAV system as employed for hemophilia B is not injected directly to the liver. Rather, it's injected into the blood and simply has a tendency to make its way into liver cells. That's a good thing, because the liver is where the coagulation factors, including factor IX, are made normally, and that's why gene therapy for hemophilia B is working. As for hemophilia A, the problem is a lack of a different factor, factor XIII, which is much bigger than factor IX and thus is more likely to evoke an immune response. That's the reason why investigators chose to work on hemophilia B first. It's always good to gain experience doing the easier thing. But the difference in complexity between hemophilia A and B is not insurmountable, and given the success with treating the latter, the research is poised to expand to hemophilia A. There is also a hemophilia C, caused by a defect in yet another coagulation factor (XI), which affects mostly Jews, and this too could become a target for gene therapy.

As for hemoglobinopathies, these include thalassemia and sickle cell anemia. In clinical trials, gene therapy has already cured a handful of patients with one of the worse types of thalassemia, beta-thalassemia major. Since the problem gene of beta-thalassemia is the same gene that malfunctions in sickle cell anemia, patients with the latter have also begun getting the same gene therapy as of a year ago. Gene therapy is working with these conditions for a few reasons. One reason is that the genetic modification is done *ex vivo*, meaning outside of the patient. Bone marrow stem cells are harvested, genetically modified, then put back, so a virus or other vector is not delivered systemically. Also, for technical reasons, it's actually easier to knock out a gene than to add a gene. With both beta-thalassemia and sickle cell anemia, rather than adding the needed gene, the patient's hemoglobin can be improved by knocking out certain genes. But this approach does not work for another type of thalassemia called alpha-thalassemia, for which genes MUST be added, thereby adding to the technical challenge. But as with gene therapy for hemophilia A, the added layer of complexity only pushes back the treatment by a few years.

Finally, the brain is another area where gene therapy is starting to look promising. This may be surprising, since usually the brain is a the most challenging part of the body to treat, but various neurological conditions result from deficiencies localized to very limited anatomic regions. One example is Parkinson disease, where there's a deficiency of the neurotransmitter dopamine in an area called the substantia nigra. Synthesis of dopamine requires certain enzymes and by adding the gene for a needed enzyme researchers are now getting the substantia nigra, or areas near it, to produce the needed dopamine, and thus curing Parkinson in some patients. They're making similar strides curing children of rare diseases resulting from absence of the same enzymes in what same area of the brain, and they're doing it using

catheters to deliver the gene in a viral vector in a surgical procedure. Because the delivery of the treatment is local, there's no stimulation of immune reactions, so the risk is reduced.

While this is all fairly technical, the take home message here is not the specifics of each disease and treatment, but rather the idea that they are all very different. As with many treatments in medicine, as with genetic technology applied to agriculture and other areas, there is no one size fits all solution. Gene therapy, like many other areas of technology, is advancing and succeeding in increments. And like the first 18 years of Bruce's life, it must follow a very cautious pathway.

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