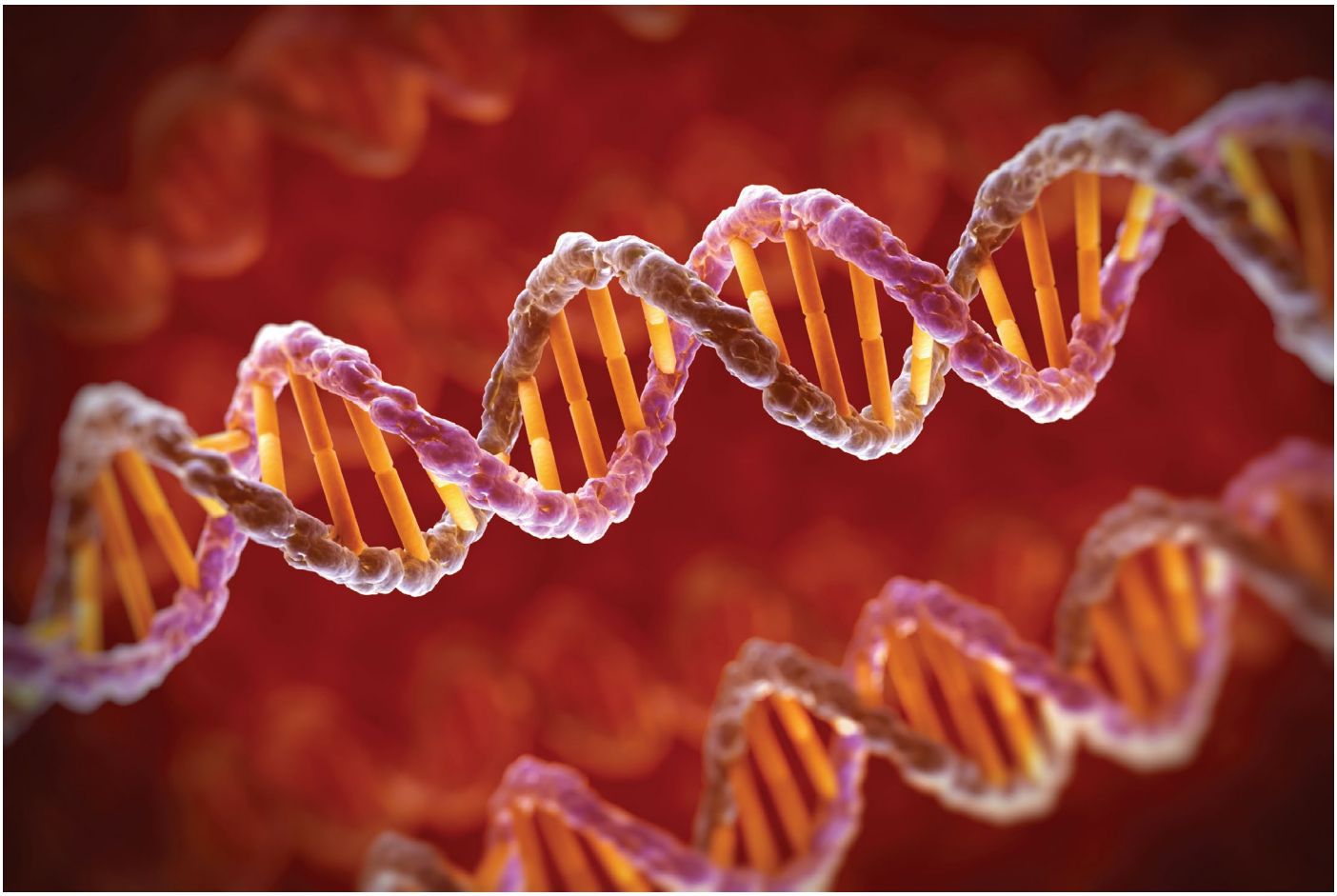

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Record number of gene-therapy trials, despite setbacks

The recent failure of a gene therapy for Huntington's disease was devastating for patients, but researchers remain optimistic.

Carrie Arnold

For decades, Claudia Testa has longed to tell her patients with Huntington's disease that she has a treatment for them. The neurologist at Virginia Commonwealth University could prescribe medications to help control the psychiatric symptoms of the deadly neurodegenerative condition, and to manage its characteristic jerking and writhing, but when it came to drugs that could slow the disease's progression, Testa had nothing. Neither did anyone else.

This is why Testa and so many others in the Huntington's community were avidly following the progress of a phase 3 trial for tominersen. The drug is an antisense oligonucleotide (ASO), a short, single-stranded piece of DNA designed to bind to a specific mRNA target. Tominersen, developed by Ionis Pharmaceuticals and licensed to Roche, binds to the mRNA encoding the mutant huntingtin protein and targets it for degradation by the cell. Tominersen sailed through phase 1/2 trials showing it was safe and that it lowered huntingtin levels. So when Roche announced in March 2021 that it was pulling the plug on the phase 3 trial of tominersen, Testa and others were devastated.

"This resets the timeline," she says. The field was hopeful that 2021 would be the first year they would have a real disease-modifying therapeutic, Testa says. "And now we're back to infinity."

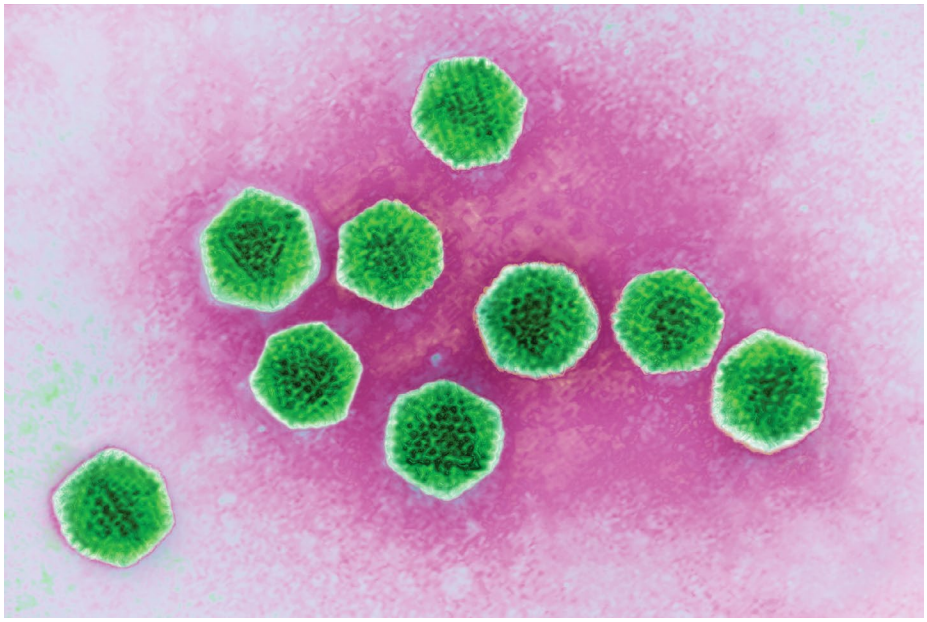
An interim analysis of the trial data showed no difference between the patients

who received an intrathecal infusion of tominersen every 16 weeks and those who received the placebo. Worse, the patients who received tominersen every 8 weeks appeared to be getting sicker than the control participants.

"They made the only decision they could possibly make. They had to stop," Testa says.

At the same time, a smaller phase 1/2 trial of an ASO for treating patients with Huntington's disease by Wave Therapeutics (from whom Testa receives consulting fees) was also shuttered after preliminary results showed the compound failed to decrease levels of mutant huntingtin. The company is now throwing its support behind a newer, potentially more effective ASO. As the Huntington's community reeled from back-to-back blows, two other biotechnology companies—Cambridge, Massachusetts–based Voyager Therapeutics, and uniQure in Amsterdam—moved forward with gene-therapy trials that use adeno-associated virus (AAV) vectors to deliver microRNAs that are designed to reduce toxic levels of mutant huntingtin protein.

This rollercoaster ride is emblematic of the gene-therapy field's recent growing pains, although safety concerns and lack of efficacy have not stopped the explosive growth in trials. Clinicaltrials.gov lists nearly 5,000 gene-therapy trials, and more than 100 trials of ASOs from around the world, more than ever before. But the long-term safety of some of these therapies remains unclear.



Adenovirus particles as seen by transmission electron microscopy (digital coloring). Credit: BSIP SA / Alamy Stock Photo.

Everything stopped

In the late 1990s, gene therapy seemed just around the corner. Scientists had nearly finished sequencing the human genome, and genetic engineering allowed them to transform viruses that caused diseases such as the common cold and even AIDS into delivery vehicles for life-saving gene therapies. The idea that researchers could cure even the deadliest of human diseases did not sound so far-fetched. Then everything went horribly wrong.

In 1999, 18-year-old Jesse Gelsinger received an injection of an adenovirus vector that carried a corrected copy of his mutated gene encoding ornithine transcarbamylase, which caused a rare X-linked liver disorder. The adenovirus vector sparked an overwhelming immune response that led to his death four days later. The US Food and Drug Administration (FDA) placed an immediate clinical hold on gene-therapy trials, and overnight, the field went from sizzling to frigid.

"Everything just seemed to stop," says Roland Herzog, an immunologist at Indiana University, who was a postdoctoral fellow at Children's Hospital of Philadelphia at the time.

The problem was not the new gene to be delivered but instead the vector delivering the gene. Finding a way to safely and effectively introduce a novel gene (or gene-editing system) into the body's cells continues to be the lynchpin of any gene-therapy endeavor, says Jennifer Hamilton, a postdoctoral fellow in the lab of Nobel Prize–winner Jennifer Doudna at the University of California, Berkeley.

"Delivery is the key thing that needs to be considered for these types of approaches," she says.

The adenovirus vector's ability to elicit a robust immune response is partly the reason vaccinologists have used adenovirus to build the ChAdOx1 vaccine against COVID-19. But this immunogenicity has led researchers to turn to other viruses to deliver gene therapy. The fact that scientists would continue to rely on viruses was no surprise, according to Hamilton. "Viruses are well evolved to get genes from outside the cell to inside," she says.

Instead of adenovirus, they began experimenting with engineered lentiviruses such as HIV and with AAVs. "AAVs were a huge advance. It changed the whole landscape of gene therapy," says Mark Sands, a gene-therapy expert at Washington University School of Medicine in St. Louis.

AAV continues to be the most popular vector partly due to its multiplicity of serotypes, which are able to infect nearly every type of tissue, even non-dividing cells. Crucially, AAV, unlike adenovirus, rarely elicits a strong immune response, so researchers could give patients the high doses of the virus that are often needed to get the correct gene into an adequate number of cells.

"It's amazing that, for the most part, you can actually get away with these high doses. It tells you how benign this vector is," Herzog says.

Over time, more safety information was gathered from preclinical trials, and regulators gained experience evaluating this information, says Peter Marks, Director of the Center for Biologics Evaluation and Research at the FDA. After more than a decade, gene therapy seemed poised to take its first hesitant steps forward. This, along with the advent of CRISPR-based gene-editing tools, meant that at first a few trials launched under nervous eyes, then hundreds. The field seemed unstoppable.

Then, yet again, everything went horribly wrong.

Too high a dose

On 6 May 2020, alerts from Nicole Paulk's mobile phone woke the gene-therapy expert at the University of California, San Francisco, several hours before dawn. Colleagues had questions about the death of a participant in the ASPIRO gene-therapy trial by Audentes Therapeutics. The Bay Area biotechnology company (now part of the Japanese pharmaceutical company

Astellas Pharma) had developed a gene therapy for the rare neuromuscular disease X-linked myotubular myopathy. They delivered a very high dose of AAV: 3×10^{14} vector genomes per kilogram of body weight. The boy subsequently died as a result of sepsis.

Seven weeks later, a second boy who had received the same high dose of the same AAV gene therapy died. On 20 August, so did a third boy—all three died of sepsis. To Paulk, the deaths were a stark warning about the potential dangers of high-dose AAVs.

"I don't think anyone expected such a profound response that couldn't be controlled," she says.

The question on her mind—on everyone's mind—was whether the field was having another 'Jesse Gelsinger moment'. As in the Gelsinger case, scientists had noted severe toxicity in preclinical tests when non-human primates were given high doses of the therapy. High-dose AAVs had also caused liver and immune system toxicities in animal models and in humans given gene therapy for Duchenne muscular dystrophy.

"Back when the original events occurred at the turn of the millennium, we did not have the same amount of experience at FDA," Marks says. "The scientific knowledge and regulatory maturation have given us more confidence. It's why these deaths didn't stop the field like it did in 2000."

In December 2020, the FDA lifted its clinical hold on the ASPIRO trials. "The exact biological mechanism that led to the patients' deaths has not been conclusively determined," says Edward Conner, Astellas Gene Therapies Site Lead. "Within the comprehensive review, Astellas has not identified clinical evidence, either direct or indirect, that immune responses contributed to the liver injury."

A detailed analysis of the company's internal findings was published. Still, scientists held their breath, and fears grew as serious adverse events appeared in other gene-therapy trials. In December 2020, Amsterdam-based uniQure reported that one person in its phase 3 trial of an AAV5-based therapy for hemophilia B had developed hepatocellular carcinoma (in March 2021, an independent investigation cleared the vector of any role). A uniQure spokesperson said that detailed analysis of the patient's liver tissue revealed a precancerous state due to several pre-existing conditions.

At the University of Pennsylvania, Denise Sabatino and colleagues published a paper in *Nature Biotechnology* showing potential liver problems that had emerged in dogs nearly a decade after they were treated with an AAV gene therapy for canine hemophilia A. In February, the team at bluebird bio announced two cases of acute myeloid leukemia, as well as one incidence of myelodysplastic syndrome (a diagnosis later revised to transfusion-dependent anemia), in participants in its trial of LentiGlobin gene therapy for sickle-cell disease. And in late April 2021, Adverum Biotechnologies announced that a participant in its trial for diabetic macular edema had developed vision loss in the dosed eye.

Unexpected integration

Despite these setbacks, and despite COVID-19, the number of new gene-therapy trials has sped up in the past year. Sands wonders if this is wise.

After Gelsinger's death, gene-therapy experts began to rely on AAVs as the vector of choice. This small virus seemed like the workhorse the field was looking for. But even as AAV was being hailed as the savior of gene therapy, Sands had noticed some long-term safety issues.

Sands found a hepatocellular carcinoma in several neonatal mice treated with an AAV-based gene therapy, used to treat a metabolic disorder. After Sands published his findings in 2001, however, his conclusions were questioned because previous trials had dosed older mice with AAVs without an apparent problem.

"There were a lot of companies that were springing up and there were a lot of people's reputations on the line," Sands says. "Everyone said these vectors were benign and here I come along and say wait a minute. There was enormous pushback."

Six years of arduous bench work finally yielded the specific sites where the AAV vector had integrated itself into the mouse genome. Sands and colleagues discovered that the viruses had inserted themselves into a 6-kilobase region on chromosome 12, altering the expression of host genes and leading to tumorigenesis. To Sands, this highlights the need for long-term safety studies on AAV-based gene therapy so the field can move forward safely.

"I am a huge proponent of AAV therapy. But my concern has always been that if [integration] is a problem for human gene therapy, it should have been studied in a systematic way for the past 20 years," he says. "If we don't understand how integration is happening, we can't re-engineer the AAVs to make them safer."

Sabatino agrees. Her work found that the AAV vectors given to treat canine hemophilia sometimes integrated into the dogs' liver cells, creating clonal expansions that have the potential to become cancerous. But these problems take years to develop in humans. Problems like these are not picked up by tests in non-human primates, Sabatino says, because these animals develop an immune response to the human gene therapy. This means their bodies fight off the virus and its accompanying genetic payload, so monitoring generally lasts for only a few months.

"This is the value of long-term animal models for follow-up. You can't track a monkey for several months and expect to find a clonal expansion," she says.

The FDA's Center for Biologics Evaluation and Research, which oversees gene-therapy trials and approvals, is aware of the potential for long-term issues with gene therapy, Marks says. The FDA is now recommending 15 years of follow-up in its draft gene-therapy guidance for industry.

"We have to have safety of these products, and that's why we are here at FDA, to make sure that this is done safely," Marks says.

Alison Bateman-House, a bioethicist at New York University, agrees that long-term animal studies are needed. Without knowing the full magnitude of potential risk, Bateman-House says, patients cannot provide true informed consent for clinical trials.

"We're really flying blind with this," she says.

Where do you want it?

Sands and Sabatino remain optimistic about the promise of AAVs and other gene therapies. To Paulk, the challenge will be designing better recombinant vectors with improved tropism and decreased immunogenicity so that patients need less vector and have a reduced chance of a severe immune reaction.

At Affinia Therapeutics, based in Waltham, Massachusetts, Chief Scientific Officer Charles Albright says that the company is working to engineer precisely these types of improved vectors. One vector has 32 times more RNA expression, which allows use of a reduced dose; another vector was tweaked both to steer it away from the liver and again to steer it towards muscle.

"This is very advantageous," says Albright. "To detarget the liver and increase uptake into muscle—this specific combination will be very powerful if we can translate it to non-human primates."

Ongoing safety concerns about AAV-based gene therapies have led some scientists rethink how gene therapy is delivered.

In November 2020, Intellia Therapeutics began a phase 1 trial of the world's first systemically administered CRISPR–Cas9 therapy, for hereditary transthyretin amyloidosis with polyneuropathy. Instead of

packaging the gene-editing system in a viral vector, Intellia is using a lipid nanoparticle, somewhat akin to the method used in the mRNA vaccines against SARS-CoV-2 developed by Moderna and BioNTech–Pfizer. Preclinical research showed that the CRISPR–Cas9 therapy knocked down more than 97% of the misfolded transthyretin protein. The advantage to this method, according to Hamilton, is that patients can receive multiple doses over time, since their immune systems will not form antibodies against the vector. In late June 2021, Intellia published the first results of the trial, which showed a dose-dependent drop in serum transthyretin levels.

Hamilton is working to create a different type of CRISPR–Cas9 system that delivers the gene-editing system as a ribonucleoprotein complex that can enter the targeted cell, edit the genome, and then degrade. Not only does this transient dosing strategy prevent the development of antibodies against a viral vector, its short-lived time in the cell also means the immune system will not raise antibodies against Cas9, Hamilton says.

Ex vivo CRISPR has its own disadvantages, because it requires the removal of hematopoietic stem cells for editing in the lab, which are then returned as a stem-cell transplant. Trial participants must undergo treatment with a myeloablative agent such as busulfan, which destroys their own stem cells, requiring weeks of hospitalization. Ex vivo CRISPR gene editing is therefore not an option for low resource settings.

"Low-income countries in sub-Saharan Africa tend to lack the significant infrastructure required to broadly provide bone marrow transplants, let alone gene therapy, which requires the cells to be removed, shipped, genetically modified, and returned to the patient," says Betsy Foss-Campbell, Director of Policy and Advocacy at the American Society of Gene and Cell Therapy.

Equity of therapy

With an eye toward addressing this challenge, the Bill & Melinda Gates Foundation partnered with Novartis in February 2021 to bring a single-administration, in vivo gene therapy for sickle-cell disease to Africa, where most of the people with this condition live. This therapy will need to be delivered directly to the patient, without the need to modify cells in the lab, and without the need for lengthy and expensive hospitalization. If everything goes to plan, says Susan Stevenson, Executive Director at the Novartis Institute for Biomedical Research, a sickle-cell-disease gene therapy may be ready for human trials in a little over 5 years.

"It's a great model to follow. Take the treatment where the majority of the patients are," she says.

From his lab at Pontificia Universidad Javeriana in Bogotá, Carlos Javier Alméciga Díaz has been following advances in gene therapy with interest. He studies a group of hereditary lysosomal disorders that are fatal in childhood. Colombia has the world's highest rates of a mucopolysaccharide storage disease known as Morquio A syndrome. Díaz says that although Morquio A syndrome is the perfect candidate for gene therapy, getting interest from the pharmaceutical industry and securing funding remains a challenge, because the condition is vanishingly rare elsewhere in the world. What the field needs, he says, are more local solutions, developed for specific populations affected by specific diseases.

"This is not like a regular drug. This is not like an aspirin or a paracetamol that you can give to the patient and say, okay, take two pills every few hours. This is a different treatment," Díaz says.

Far from being hamstrung by recent setbacks and challenges, the gene-therapy field remains hopeful and optimistic. There are substantial challenges in terms of improving delivery and affordability, as well as monitoring long-term safety, but to the scientists, they do not seem insurmountable.

"Even with all the disappointment and pain, it's just an incredibly exciting time in this field," Testa says. ❐

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