A hiccup in gene therapy progress?

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Credit: NHGRI

Zebrafish, roundworms, fruit flies, mice, rats, rabbits, dogs, cats, pigs, and monkeys provide steppingstones to clinical trials to evaluate new treatments for people. The value of animal studies continues, even after a new drug shows promise or is approved.

A recent study on a gene therapy given to monkeys and pigs, similar to one that has already had spectacular results in children, may warn of possible dangers of escalating doses – or not.

The findings, published in *Human Gene Therapy*, come just weeks after the first FDA approval of a gene therapy, for Luxturna. It treats a form of hereditary blindness.

Gene therapy was a long time in coming. The first clinical trial was in 1990. The field stalled in 1999, following the death of 18-year-old Jesse Gelsinger four days after treatment for a urea cycle disorder.

At the helm of that heartbreaking clinical trial in 1999 was James Wilson, MD, Ph.D., director of the gene therapy program at the University of Pennsylvania and scientific founder of REGENXBIO, one of several companies developing adeno-associated virus (AAV) viral vectors for gene therapies – a very different virus from the one that killed Jesse. He's also main author of the recent report on acute liver toxicity in monkeys who received gene therapy for spinal muscular atrophy (SMA) similar to the approach already used in nine children.

When Jim Wilson sounds an alarm, people listen. But what does it mean for gene therapy?

Viral Vectors Vary

Engineered viruses deliver working copies of genes to cells where they can have an effect. Viruses naturally home to certain types of cells (dividing or not) in certain types of tissues or organs (blood, liver, nervous, muscle).

Even within viral types, like AAV, are variants within variants, like the same model of iPhone from a dozen people but each with a distinctive set of apps. AAV doesn't insert its cargo into human chromosomes. The virally-delivered "transgene" remains in tiny circles outside the chromosomes, lost as cells divide. (Mature muscle and nerve cells don't).

Viral vectors vary in the healing genes they carry, but also in surrounding control DNA sequences and whether the genetic material is single-stranded or doubles back on itself. The types of "capsid" proteins forming the viral exteriors, like panes of a greenhouse, form the viral

face to the immune system.

A Timeline to Toxicity

Unlike treating blindness, tackling conditions like SMA or Duchenne muscular dystrophy (DMD) requires systemic delivery of viral vectors – to motor neurons for SMA and to skeletal muscle for DMD. That means higher doses.

I waited a few weeks to post about the monkey study to sort out the chronology, which is overlapping and a bit confusing.

In November 2017, FDA placed a partial clinical hold on a gene therapy trial for DMD at Solid Biosciences LLC, questioning whether all of the viruses to treat a patient with a high dose can come from one batch. This info went out in a news release January 25. That was a week or so after Dr. Wilson resigned from Solid's scientific advisory board, citing "emerging concerns about the possible risks of high systemic dosing of AAV," according to the SEC filing.

Solid's gene therapy trial, at the University of Florida, is called IGNITE DMD. (Several trials with different sponsors are underway). IGNITE's treatment, SGT-001, delivers a shortened version of the gene, a "microdystrophin," in AAV9. The ClinicalTrials.gov entry lists three dosage groups, but not the exact numbers.

The dystrophin gene that's missing or shortened in boys who have DMD is one of extremes: it's gargantuan, yet it's percentage of muscle proteins minuscule, it's impact profound. Without dystrophin, the complexes of glycoproteins that enable skeletal muscle cells to withstand the force of contraction topple. A boy rapidly loses the ability to move.

On January 30, Dr. Wilson's retreat from the Solid board made sense,

with online publication of the *Human Gene Therapy* paper. I'll return to that soon.

On February 14, the first IGNite participant, a teen, received 50 trillion viral genomes per kilogram of his body weight. A few days later he was hospitalized after laboratory tests revealed low platelets and red blood cells, as well as an innate immune response (complement activation). But his clotting and liver were okay, and he "responded well to medical treatment," according to a news release from the company.

Yet on March 14, FDA placed IGNITE DMD on full clinical hold, due to the "suspected unexpected serious adverse reaction" that Solid had reported.

Meanwhile, back in the business sector, Bloomberg News reported, oddly, that as of February 2018, Dr. Wilson was no longer a co-founder of REGENXBIO nor the Special Advisor, which he had become on April 7, 2017, after being Chief Scientific Advisor from September 2014. A company rep told me that REGENXBIO doesn't use the vector (AAVhu68) that sickened the monkey.

Monkeys and Piglets Sound an Alarm

Dr. Wilson's team administered AAVhu68 (which they isolated from a person) loaded with copies of the human SMN1 gene that lies behind spinal muscular atrophy to 3 rhesus macaque monkeys and 3 mini-piglets. The animals received 200 trillion viral particles per kilogram of body weight – the same high dose given to 9 of the 12 kids in the SMA clinical trial.

In all animals, lower motor neurons readily took up the viruses. That's good. But one monkey became very sick on day 4.

The animal was listless, it's body weight up 15% and the liver ballooning. The monkey rapidly went into shock, with difficulty breathing and dilated pupils. Upon autopsy 95% of the liver was degenerating, with what little structure remained clogged with clots. Liver enzyme levels were through the roof, tiny clots everywhere, and a spike in cytokines indicated inflammation. Even the fat was blood-tinged, and a host of organs affected—spleen, thymus, lymph nodes, digestive organs, heart, and lungs, but the central nervous system and kidneys spared. The other two monkeys didn't get sick but had elevated liver enzymes. Still, the human gene made it into the targeted neurons.

Liver cells had taken up 1000 times more viral vectors than the neurons, but that wasn't surprising. Blood vessels in the liver are fenestrated with large openings, allowing the viruses to easily disperse within the spongy bulk of the organ. The problem: delivering enough viruses to reach nerve or muscle overwhelms the liver, like putting down a ton of grass seed to grow a lawn.

To Dr. Wilson, the scenario of a rapid viral assault on the liver in a gene therapy patient must have evoked memories of Jesse Gelsinger.

The three little pigs had a different problem – they staggered about, losing proprioception, the sense of where the body is in space. Their livers were okay; like the monkeys the viruses more readily entered liver cells than neurons, but not enough to harm them. However, the fatty sheaths on peripheral neurons were frayed.

Although half a dozen animals isn't a huge sample, the study was potentially alarming in two ways: individuals vary in response to a viral vector, and a small increase in the number viruses delivered can translate into a rapid and possibly life-threatening reaction.

Perspective

One news report labeled the findings a "toxic disaster," but Terence Flotte and Hildegard Buning wrote an editorial to accompany the report in *Human Gene Therapy* that put the potentially inflammatory findings into perspective. They ticked off a number of limitations of the study:

- Only six animals, all given the same dose.
- Toxicity could have been due to contaminants.
- The vector was not identical to the one used in the SMA clinical trial.
- The monkeys and pigs received human SMN1, not their own versions.
- It wasn't possible to tell whether the liver toxicity (monkeys) or neuron damage (pigs) was due to the high viral dose or to the delivered gene.

Another study coming out in *Molecular Therapy* reports liver damage resulting from transfer of a different AAV9-based vector, capsid, and gene – so the response may be common beyond a threshold of viral dose. And that's important to know as trials try higher doses in search of faster or greater effects.

Drs. Flotte and Buning point out that only certain tissues require high doses – crossing the blood-brain barrier to reach neurons, and hitting enough muscle cells to improve mobility. Yet higher doses of any drug can be dangerous. Even too much acetaminophen can damage the liver.

The effect on the monkey's liver was too fast to have been an immune response. In contrast, elevated liver enzymes seen among four of a dozen young children in the SMA gene therapy trial were likely due to T cells responding to the capsid, and resolved with steroids.

Don't ignore the findings, but don't overact, caution Drs. Flotte and Buning. The monkey/piglet findings might inspire redesign of trials:

trying different doses, altering vector prep to minimize the number of empty capsids, altering timepoints, engineering the genes, comparing single-stranded AAV to "self-complementary" double-stranded preparations, finding new biomarkers, using the immunosuppressant rapamycin to temper liver uptake of AAV, and expanding natural history studies that provide the backdrop of how a disease progresses.

Looking Forward

The Human Gene Therapy editorial concludes, "the one and only guiding principle in critical moments such as this should be the welfare of patients with the diseases being treated with these therapies."

I agree. Halting trials might mean that kids with SMA will have to wait longer for the gene therapy that turned around the life of three-year-old Evelyn Villarreal. She had gene therapy at 8 weeks old, and can now run and talk. Her older sister died of the disease at 15 months.

I hope that the apparent glitch from the highest doses of one subtype of AAV are just a brief hiccup in the momentum that has been propelling gene therapy forward since blind kids became able to see, and children with SMA began to walk.

Three boys treated in September 2017 with 100 trillion AAV8 vectors per kilogram of weight for X-linked myotubular myopathy (MTM) by early January showed improvements in breathing and mobility, and like the SMA kids, can raise their heads, roll over, and sit. The clinical trials for MTM followed studies in baby monkeys and the pioneering efforts of Alison and Paul Frase, who lost a young son to the disease and developed a natural dog model. (I'll tell their story another time.)

My thoughts? Liver damage in one monkey blasted with viruses is a mere hiccup in the promising trajectory of gene therapy development.

But the report is essential. It's normal for biomedical research to proceed in fits and starts, for clinical trials to advance even as experiments on non-human animals continue, with an occasional circling back to optimize the way forward.

More information: Christian Hinderer et al. Severe Toxicity in Nonhuman Primates and Piglets Following High-Dose Intravenous Administration of an Adeno-Associated Virus Vector Expressing Human SMN, *Human Gene Therapy* (2018). DOI: 10.1089/hum.2018.015