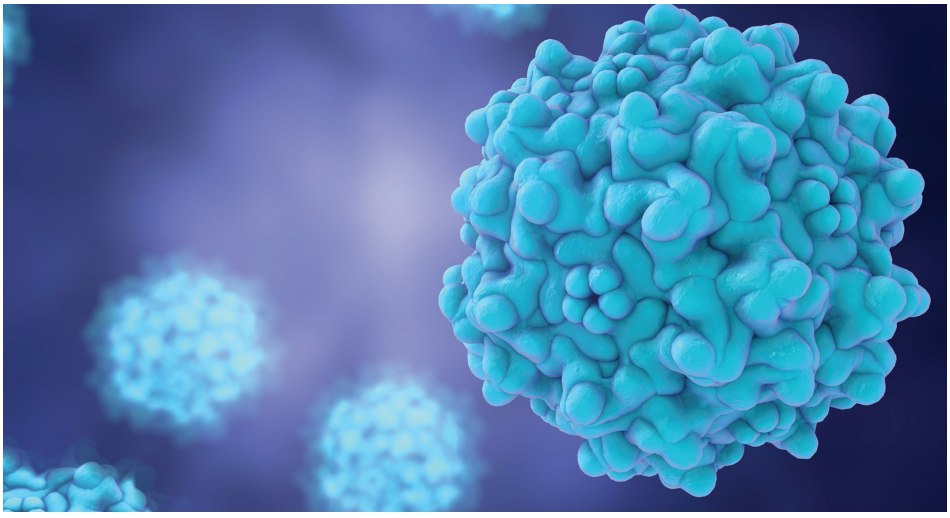
RARE DISEASES: OPPORTUNITIES AND CHALLENGES FOR TREATMENTS DELIVERED BY ADENO-ASSOCIATED VIRUSES

The word "rare" in the term rare disease can be confusing to the uninitiated. About 300 million people worldwide are living with one or more of over 6,000 rare diseases.[1] This is just under 4% of the world population. So it's not actually rare to have a rare disease.

It is rare, however, to have a rare disease with an approved drug to treat it. In the US, for example, there are treatment options for only 5% of rare diseases. As many such diseases have a genetic basis, the up-and-coming tools of gene therapy and gene editing are expected to revolutionize life for an increasing number of people with these conditions in the coming years. The technologies offer a way to fix genetic errors, either by editing genes or replacing them with a functional version. "Gene therapy is the most exciting technology to be added



Adeno-associated viruses are used in gene therapy.

Credit: Shutterstock

to our medical arsenal in my lifetime," says Nicole Paulk, an assistant professor of biochemistry and biophysics at the University of California, San Francisco. Gene therapy has the ability to cure, in a single step "some of these rare disorders at a genetic level," she adds.

Transporting replacement genes or gene-editing tools inside the body requires a vehicle with a built-in navigation system headed toward the cytoplasm of its target cells. The vehicle must also keep the genetic material—its passenger—safely encased. Upon arrival at the destination, the therapy disembarks and carries out its job. Because this is similar to how viruses work in nature, it's unsurprising that these delivery vehicles tend to be viruses (with therapeutic genes substituting for stripped-out viral genes).

The viruses most widely used for this purpose are adeno-associated viruses (AAVs).

An advantage of AAVs is that they don't make us sick. They "are not pathogens to us" and in fact are as harmless as the bacteria we host in our guts, says Paulk, whose lab develops AAV tools. The technology "has been used now in almost 300 clinical trials across the world for a variety of different diseases," she adds.

A RARE CHALLENGE

Even for common diseases, drug discovery is an arduous process, with fewer than 10% of drugs that enter Phase 1 clinical trials making it to market.[2] For rare diseases, the number of obstacles along a drug's path from bench to bedside is far greater. Developing a drug for a rare disease takes an average 15.1 years from first patent filing to market[3]—18% longer than the average for all new drugs.

There are multiple reasons for this extended timeline. Knowledge gaps about the causes and natural clinical progression of many rare diseases, for example, make it hard to determine the best drug strategy for treatment. These same knowledge gaps also hinder the design of adequate research models, both in vitro and in vivo [see box], that recapitulate the disease. Furthermore, executing clinical trials for poorly understood diseases is challenging because defining clinical endpoints is complicated.

Enrolling enough patients for a rare disease clinical trial is another difficulty, because by definition a rare disease doesn't affect a large number of people. There is no globally agreed definition of a rare disease, as every country or region uses its own benchmark. In the US, for example, the US Food and Drug Administration (FDA) defines a rare disease as one that affects fewer than 200,000 people in the US. This equates to fewer than 6 people per 10,000. In the EU, any disease affecting fewer than 5 people in 10,000 is categorized as rare. And in China, there is no official definition.

A GROWING MARKET

In 1983, the Orphan Drug Act was passed to encourage the development of therapeutics for rare diseases. Incentives include market exclusivity extensions,

tax breaks, and the possibility of a fast-tracked FDA review process. The European Medicines Agency (EMA) adopted a similar scheme—the Orphan Medicinal Product designation—for EU countries in 1999. Regulators in both places are also more flexible about cohort size and the need for placebo groups in clinical trials for orphan drugs.

These incentives are working. "The Orphan Drugs Act has driven a lot of companies towards these spaces because it enables them to make developing these drugs financially viable," says Quentin Horgan, a pharmaceuticals analyst at London-based GlobalData. Between 1973 and 1983, only 10 drugs for rare diseases were approved in the US.[4] By the end of 2019, the FDA had approved drugs for more than 800 rare diseases.[5] The agency approved 22 treatments with orphan drug designation in 2019 alone.[6] That year, 44% of the novel small-molecule drug approvals were for orphan drugs, and one of the five approvals for novel biologics was also for an orphan product.

To raise awareness of rare diseases and their lack of treatment options, the nongovernmental organization EURORDIS (European Organisation for Rare Diseases) and its partners run an annual global awareness campaign called Rare Disease Day. The observance was first marked in 2008, on February 29—a rare day. The event has been held each year since on the last day of February.

MODEL ANIMAL CHALLENGES

Preclinical trials in animals are a vital part of all drug development. Their importance is magnified with rare diseases, when clinical trials are likely to run on very small patient populations.

Mice are the most commonly used animal model in studying genetic diseases in humans. To use mouse models against a genetic disease, their genome must be edited to replicate the human disease state. This requires that scientists know the error in the gene that causes the disease in humans, and that level of genetic detail simply isn't known for many rare diseases. "This means that when we make a new model, we don't know if it will compare well to the disease or not," says Shengfang Jin, formerly the vice president and head of discovery biology at Editas Medicine and currently chief scientific officer and co-founder of Oncology NewCo.

Making new mouse models is time consuming, but CRISPR-Cas9 has allowed for significant time savings.[18] " Conventional gene editing of mice involves generating the required mutation in their embryonic stem cells and then injecting them into a mouse embryo. This process takes around 9 months. With CRISPR-Cas9, the gene-editing tools are injected straight into the zygotes, translating into a timeline of around 3 months.

WuXi AppTec has developed an in vivo gene-editing platform using CRISPR-Cas9 for generating mouse models. The global provider of R&D and manufacturing services can support pharmaceutical and biotech companies looking to develop AAV-based gene therapeutics. WuXi AppTec's services include a scalable and flexible lab-scale AAV production platform suitable for use with all common serotypes and customized AAV vectors; quality assurance and quality control; and an in-house service for nonhuman primate studies for gene therapies.

For more information, watch a webinar here.

GENE REPLACEMENT THERAPIES

The rare disease biologic the FDA approved in 2019 was an AAV-based gene therapy for children under 2 years old with spinal muscular atrophy.[7] Zolgensma, developed by Novartis, was approved in the EU in mid-2020. Zolgensma delivers a functional copy of the SMN1 gene, which is defective in people with spinal muscular atrophy, through a single, one-time intravenous infusion.

To date, two other AAV-based gene therapies have gained approval. The first was Glybera, developed by uniQure for the treatment of lipoprotein lipase deficiency, a rare metabolic genetic disorder that disrupts the normal breakdown of fats in the body. Symptoms include recurrent abdominal pain episodes due to pancreatitis.

Luxturna was the second approved AVV-based drug to gain marketing approval—in the US in 2017 and the EU in 2018.[9] This gene therapy treats patients with rare retinal diseases, including Leber congenital amaurosis, that cause progressive tunnel vision. Developed by Spark Therapeutics, this biologic is injected into the retina to stop the progression of vision loss and even improve sight in some patients.

More AAV-based investigational treatments may be headed for market. BioMarin's BMN 270 and uniQure's AMT-061 are in Phase 3 clinical trials at the time of writing. They both aim to replace genes that provide instructions for making proteins essential for blood clotting. BMN 270 targets hemophilia A and AMT-061 hemophilia B.

It's important to note that AAV-based gene therapies don't all use the same AAV. Specific AAV serotypes can selectively deliver transgenes to different locations in the body. A drug developer selects the virus best suited for a particular disease's pathology. Zolgensma, for example, uses AAV9, whose targets include skeletal muscle.

The zebra is the official symbol of rare diseases in the United States.

Credit: Shutterstock

GENE-EDITING THERAPIES

AAV vectors are also being used as delivery vehicles for CRISPR-Cas9 gene-editing tools. These tools edit faulty genes rather than replace them wholesale, as is the case with the biologics discussed above. Emmanuelle Charpentier at the Max Planck Unit for the Science of Pathogens and Jennifer A. Doudna at the University of California, Berkeley, and Howard Hughes Medical Institute won the 2020 Nobel Prize in Chemistry for developing CRISPR-Cas9.[10] This tool was first described less than a decade ago and since then has been used prolifically in research laboratories.

CRISPR-Cas9 combines a protein, Cas9, that can neatly snip DNA with a customized piece of RNA. The piece of RNA, called CRISPR—short for clustered regularly interspaced short palindromic repeat—guides Cas9 to a particular spot on the genome. There, it can either turn a gene off or insert a new DNA sequence to repair the gene.

CRISPR-Cas9 is also starting to make it into humans. These investigational therapeutics fall into two broad categories, explains Jin. In the first category, cells are removed from a patient, edited, and then returned to the body. In the second category, editing is done in vivo; these therapeutics need targeted delivery vectors, such as AAV.

Another CRISPR pioneer, Feng Zhang at the Massachusetts Institute of Technology, says finding an effective delivery vector is a bottleneck for the development of CRISPR-Cas9 therapeutics. "The successes of AAV vectors in delivering gene therapies to ... patients propelled their adoption for in vivo delivery of CRISPR-based therapeutics," he and his collaborators wrote.[11]

The first CRISPR-Cas 9 treatment deployed in vivo in humans was the investigational treatment EDIT-101. Developed by Editas and Allergan, EDIT-101 deletes a mutation in the CEP290 gene that is responsible for Leber congenital amaurosis 10. Those with this rare eye disease can be blind from birth or develop blindness in the first decade of life. The hope is that EDIT-101 will restore their vision and halt the progression of blindness.

EDIT-101 received Orphan Medicinal Product designation from the EMA in 2017. In March 2020, it was injected directly into the retinas of its first participant in a Phase 1/2 clinical trial known as Brilliance.[12] "We are using AAV as a vehicle for [EDIT-101] to deliver the CRISPR-editing machinery," Jin says. The specific AAV used is AAV5.[13] In November 2020, Intellia Therapeutics announced in vivo dosing with an investigative CRISPR-Cas9 therapeutic, NTLA-2001.[14] The company is developing it as a single-dose treatment for transthyretin amyloidosis. This rare progressive systemic disease is caused by mutations in the TTR gene that cause the liver to produce a protein—transthyretin—in a misfolded form that builds up in the body.

NTLA-2001 is being given intravenously; Intellia says it is using a proprietary lipid nanoparticle–AAV hybrid system to deliver the gene-editing tools to the liver.

FUTURE CHALLENGES FOR AAV VECTORS

The reason for using hydrid approaches is to overcome some of the inherent issues with AAV as a delivery system for both gene delivery and editing. One challenge is the size of the AAV vector. The viral particles are only about 25 nm in diameter, which limits the amount of cargo they can carry.[11] The most common Cas9 for gene editing is derived from the bacterium Streptococcus pyogenes. It contains over 1,300 amino acids and is too large to be packaged in AAVs.

Approaches being explored to circumvent the size limitation include using tools that compress the genetic payload into a smaller bundle, engineering AAVs with more flexible shells that can accommodate more material, and employing hybrid vectors. "We can split the genome in half and put half the genome in one AAV virus and half of the genome in another AAV virus," Paulk says. "Then you can engineer those to both enter the same cell and then have the genomes recombine there."

Pre-existing immunity is another hurdle for AAV. People with prior exposure to wildtype AAV can generate antibodies that destroy the therapeutic before it can get to work. This results in low efficiency of gene expression; for this reason, participants are tested for prior immunity before they are enrolled in trials.

Immunogenicity is also suspected as triggering immune-mediated toxicities observed in a few AAV clinical trials using high doses. AAV capsid proteins and genome components can activate the innate immune system and lead to liver inflammation.[15] This appears much more likely when high doses of AAV-based therapies are used.

In August 2020, for example, a Phase 2 trial for an AAV-based gene therapy for the rare neuromuscular disease, X-linked myotubular myopathy, was paused after two participants died.[16] Both reportedly had received high doses of the investigational treatment, AT132. The drug is designed to fix a mutation in the MTM1 gene that inhibits normal development of skeletal muscles. After treatment, both patients (who each had pre-existing liver dysfunction) displayed worsening liver dysfunction followed by sepsis. In December, the FDA granted the AT132's developer, Audentes Therapeutics, permission to restart the trial at a lower dose.[17] Additional efforts to further improve the safety profile of AAV-based gene therapies are expected to continue as more start navigating the taxing path from bench to bedside.

REFERENCES

1. Stéphanie Nguengang Wakap et al., "Estimating Cumulative Point Prevalence of Rare Diseases: Analysis of the Orphanet Database," *Eur. J. Hum. Genet.* 28 (Feb. 2020): 165–73, https://doi.org/10.1038/s41431-019-0508-0.
2. Helen Dowden and Jamie Munro, "Trends in Clinical Success Rates and Therapeutic Focus," *Nat. Rev. Drug Discovery* 18 (May 18, 2019): 495–96, https://doi.org/10.1038/d41573-019-00074-z.
3. Suz Redfearn, "Tufts: Facing Many Challenges, Orphan Drugs Take 18% Longer

to Develop," CenterWatch, May 14, 2018, https://www.centerwatch.com/articles/12603-tufts-facing-many-challenges-orphan-drugs-take-18-longer-to-develop.

4. Koichi Mikami, "Orphans in the Market: The History of Orphan Drug Policy," *Soc. History of Med.* 32, no. 3 (Aug. 2019): 609–30, https://doi.org/10.1093/shm/hkx098.
5. US Food and Drug Administration, "Rare Disease Day 2020: FDA Continues Important Work on Treatments for Rare Diseases," Feb. 21, 2020, https://www.fda.gov/news-events/fda-voices/rare-disease-day-2020-fda-continues-important-work-treatments-rare-diseases.
6. US Food and Drug Administration, New Drug Therapy Approvals 2019, Jan. 6, 2020, https://www.fda.gov/drugs/new-drugs-fda-cders-new-molecular-entities-and-new-therapeutic-biological-products/new-drug-therapy-approvals-2019.
7. Novartis, "AveXis Receives FDA Approval for Zolgensma, the First and Only Gene Therapy for Pediatric Patients with Spinal Muscular Atrophy," press release, May 24, 2019, https://www.novartis.com/news/media-releases/avexis-receives-fda-approval-zolgensma-first-and-only-gene-therapy-pediatric-patients-spinal-muscular-atrophy-sma.
8. uniQure, "uniQure Announces It Will Not Seek Marketing Authorization Renewal for Glybera in Europe," press release, April 20, 2017, https://www.globenewswire.com/news-release/2017/04/20/962549/0/en/uniQure-Announces-It-Will-Not-Seek-Marketing-Authorization-Renewal-for-Glybera-in-Europe.html.
9. US Food and Drug Administration, Approved Product Information for Spark Therapeutics' Luxturna, July 26, 2018, https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/luxturna.
10. The Royal Swedish Academy of Sciences, "The Nobel Prize in Chemistry 2020," press release, Oct. 7, 2020, https://www.nobelprize.org/prizes/chemistry/2020/press-release/.
11. Dan Wang, Feng Zhang, and Guangping Gao, "CRISPR-Based Therapeutic Genome Editing: Strategies and In Vivo Delivery by AAV Vectors," *Cell* 181, no. 1 (April 2, 2020): 136–50, https://doi.org/10.1016/j.cell.2020.03.023.
12. Heidi Ledford, "CRISPR Treatment Inserted Directly into the Body for First Time," *Nature* 579, no. 7798 (March 12, 2020): 185, https://doi.org/10.1038/d41586-020-00655-8.
13. News brief, "First CRISPR Therapy Dosed," *Nat. Biotechnol.* 38 (April 2020): 382, https://doi.org/10.1038/s41587-020-0493-4.
14. Intellia Therapeutics, "Intellia Therapeutics Doses First Patient in Landmark CRISPR/Cas9 Clinical Trial of NTLA-2001 for the Treatment of Transthyretin Amyloidosis," press release, Nov. 9, 2020, https://www.globenewswire.com/news-release/2020/11/09/2122648/0/en/Intellia-Therapeutics-Doses-First-Patient-in-Landmark-CRISPR-Cas9-Clinical-Trial-of-NTLA-2001-for-the-Treatment-of-Transthyretin-Amyloidosis.html.
15. Helena Costa Verdera, Klaudia Kuranda, and Federico Mingozzi, "AAV Vector Immunogenicity in Humans: A Long Journey to Successful Gene Transfer," *Cell*

28, no. 3 (March 4, 2020): 723–46, https://www.cell.com/molecular-therapy-family/molecular-therapy/fulltext/S1525-0016(20)30003-4.
16. News brief, "High-Dose AAV Gene Therapy Deaths," *Nat. Biotechnol.* 38 (Aug. 2020): 910, https://doi.org/10.1038/s41587-020-0642-9.
17. Audentes Therapeutics, "Audentes Therapeutics Announces FDA Lifts Hold on ASPIRO Clinical Trial of AT132 for Treatment of X-Linked Myotubular Myopathy (XLMTM)," press release, Dec. 24, 2020, https://www.audentestx.com/press_release/audentes-therapeutics-announces-fda-lifts-hold-on-aspiro-clinical-trial-of-at132-for-treatment-of-x-linked-myotubular-myopathy-xlmtm/.
18. Silvia Mirillo-Cuesta et al., "The Value of Mouse Models of Rare Diseases: A Spanish Experience," *Front. Genet.* (Oct. 14, 2020), https://doi.org/10.3389/fgene.2020.583932.

AMERICAN CHEMICAL SOCIETY
1155 Sixteenth Street, NW,
Washington, DC 20036