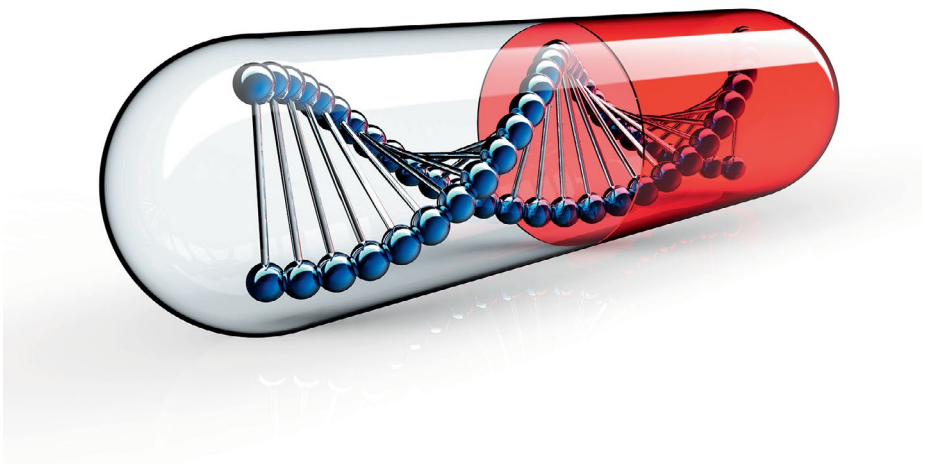
Why gene therapies must go virus-free

Gene therapies have made spectacular progress in delivering new cures for previously intractable disease, but they remain the world's most expensive treatments. Now companies are replacing the virus in gene therapies with new delivery technologies that promise not only to overcome the limitations of viral vectors but to slash production costs too.

By Cormac Sheridan



The world's most expensive drug is a genetic medicine. It's Hemgenix for hemophilia, and it costs $3.5 million per patient.

Moderna and Generation Bio teamed up in April to develop non-viral gene therapies for liver and immune-related conditions, in a deal that builds on Generation Bio's lipid nanoparticle (LNP) delivery platform. The collaboration is part of a growing trend to swap virus-driven gene therapeutics for innovative nucleic acid delivery platforms that escape the high costs and technical limitations of viral vectors. Engineered lipid or protein nanoparticles, DNA-based nanocarriers, and novel physicochemical methods point the way toward redosable genetic medicines (Table 1).

Virus-based vectors — particularly those based on adeno-associated virus (AAV) and lentiviruses — have dominated the first wave of gene therapies to gain regulatory approval. But their widespread deployment in dozens of clinical trials has exposed their limitations. "One of the issues is just the biomass needed to produce, for example, AAV at the scale and in the doses needed for large indications," says Akin Akinc, CEO of Aera Therapeutics, a company developing a protein-nanoparticle-based delivery system. AAV vectors are further limited by their 4.7-kilobase packaging capacity; poor tissue selectivity; risk of liver toxicity; and immunogenicity, which eliminates the possibility of redosing. Lentiviral vectors and, to a lesser extent, AAV vectors also elicit oncogenicity concerns arising from chromosomal integration and insertional mutagenesis. Moreover, the theoretical risk of replication-competent viruses emerging from recombination events during lentiviral production necessitates extensive testing in patients.

To provide a viable alternative, non-viral technologies need to satisfy certain well-defined requisites. They must accommodate a large payload and deliver it to specific organs, lack immunogenicity to allow redosing, and have a high safety margin and low production costs. Although most are still preclinical, the field is gaining momentum.

In recent months, Aera raised $193 million to progress a protein-nanoparticle system based on endogenous human proteins. These assemble spontaneously into capsid-like structures that can deliver a nucleic acid cargo. Intergalactic Therapeutics plans to start clinical trials next year of a gene therapy that employs in vivo electroporation to deliver covalently closed, circular DNA molecules to retinal cells. And ReCode Therapeutics has just entered the clinic with a nebulized LNP that delivers mRNA encoding a protein, DNAI, to the lungs of patients with primary ciliary dyskinesia arising from mutations in dynein axonemal intermediate chain 1 (*DNAI1*).

The global rollout of inexpensive LNP-based mRNA COVID-19 vaccines has demonstrated that it is feasible to manufacture and deliver billions of doses, including repeat doses, of at least one form of nucleic acid. There is, however, a substantial difference between delivering mRNA to antigen-presenting cells locally in muscle and delivering LNP-encapsulated DNA or other cargoes to the cell nucleus within target tissues, be they in the liver, muscle, lungs, heart or other organs, while avoiding triggering innate immune responses. The cell targeting needs to be more sophisticated while the DNA payload may also require modification to ensure it is efficiently localized to the nucleus upon cell entry. For example, Generation Bio's closed-end DNA (ceDNA) has a covalently closed structure to resist nuclease attack in the cytoplasm and two inverted terminal repeats flanking the transgenes and its regulatory elements to promote nuclear uptake.

The liver is another obstacle for any systemically delivered gene therapy not intended for that organ, because it scavenges from the circulation viral and non-viral vectors alike, along with other exogenous and endogenous macromolecules and nanoparticles. The key culprit is the serum protein apolipoprotein E (ApoE), says David Lockhart, president and CSO of ReCode. It promiscuously binds circulating LNPs or other delivery vehicles, and most of them end up in the liver, as it is rich in ApoE-binding low-density lipoprotein receptors. "If you don't avoid ApoE binding, the liver is a sink," he says.

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Table 1 | Selected virus-free gene therapy and gene editing development programs

Developer	Therapy	Description	Indication	Status
SQZ Biotechnologies	SQZ-eAPC-HPV	Autologous peripheral blood mononuclear cells modified with Cell Squeeze technology to express mRNAs encoding the human papillomavirus 16 (HPV16) antigens E6 and E7, the co-stimulatory signal CD86, and the membrane-bound cytokines interleukin (IL)-2 and IL-12	HPV16-positive solid tumors	Phase 1/2
ReCode Therapeutics	RCT1100	Nebulized selective organ targeting (SORT) LNP containing mRNA encoding dynein axonemal intermediate chain 1 (DNAI1)	Primary ciliary dyskinesia due to *DNAI1* mutations	Phase 1
Generation Bio	Not disclosed	Cell-targeting LNP containing double-stranded, linear, covalently closed-ended DNA construct encoding factor VIII	Hemophilia A	Preclinical
Intergalactic Therapeutics	IG-002	Covalently closed circular DNA encoding the full-length *ABCA4* gene, delivered subretinally by needle-based in vivo electroporation	ABCA4 retinopathies	Preclinical

Sources: ClinicalTrials.gov; company websites

To direct delivery to different organs, Recode adds a fifth, selective organ targeting (SORT) lipid to the classical four-lipid nanoparticle composition, an approach developed by company co-founder Daniel Siegwart of the University of Texas Southwestern Medical Center. "It's not one magic lipid – this is a class effect," Lockhart says. These lipids bind specific serum proteins whose receptors are strongly expressed by the target cells. Thus, adding a lipid that binds the adhesion protein vitronectin improves delivery to the lung, whereas one that binds β2-glycoprotein 1 boosts delivery to the spleen.

Generation Bio also adds a fifth, targeting ligand to its LNPs to avoid default trafficking to resident macrophages in the liver and spleen, but the mechanism differs from that of ReCode's. "It's a direct receptor engagement," says Matthew Stanton, CSO at Generation Bio. The company has not yet published details of its 'stealth' LNPs but claims it can avoid default trafficking to the liver and spleen, particularly to their resident macrophages. "That's what got Moderna so excited," he says.

Anjarium Biosciences of Schlieren, Switzerland, achieves cell-specific delivery with antibodies or antibody fragments, which it attaches to its proprietary 'hybridosome' nanoparticles. The latter are based on LNPs and exosomes. "We have a composite particle with elements of each," says CSO and co-founder Joël de Beer. Exosomes have proven to be difficult to load with genetic cargo, he says, but they can be readily modified externally. They are also immunologically silent, given their ubiquity in human circulation and tissues. "The issue with LNPs of course is biodistribution," he says. But they are efficient carriers of nucleic acids. The company's aim is to capture their combined benefits while avoiding their shortcomings.

Delivery vehicles based on other materials can avoid the issue of liver trafficking from the outset. Code Biotherapeutics is developing a DNA-based nanocarrier that has no inherent liver tropism. Its technology comprises a multivalent structure assembled by hybridizing multiple single strands into double-stranded monomers, which are then cross-linked to form a stable particle. Up to 18 copies of a therapeutic payload and another 18 of a targeting moiety can be attached externally. "We have pretty precise control over how we can build that," says Brian McVeigh, CEO and co-founder of Code.

Yet another option is to build carriers from phage proteins. Gensaic is using three capsid proteins from phage M13, a resident of the human microbiome, in protein-based nanoparticles. "These particles don't have a pre-existing tropism," says CEO Lavi Erisson. The protein-based system is readily amenable both to protein engineering methods, for the addition of targeting moieties, and to high-throughput screening methods, for the identification of particles with the desired attributes. The company's programs remain at an early stage, but efforts in Duchenne muscular dystrophy, cystic fibrosis and central nervous system disorders are underway.

Aera's approach also involves proteins that are present in the body. They are derived from retroelements, such as human endogenous retroviruses and retrotransposons, which constitute a sizeable percentage of the human genome. "By starting with human proteins, we're starting at a lower immunogenic risk," says CEO Akinc. Scientific founder Feng Zhang of the Broad Institute of MIT and Harvard has described one such particle, based on a protein called PEG10. Jason Shepherd of the University of Utah has supplied Aera with another protein nanoparticle, based on a protein called Arc, which is expressed in the brain. The company is actively seeking others. "I tend to think there's going to be better ones out there," says Akinc. For manufacturing, Aera is employing a cell-free process, unlike the cell-based process that Zhang and colleagues described in their original report. "We can precisely control what we're putting into the mixture," says Akinc. The company has not yet disclosed any development programs, but its initial focus will be on short interfering RNA (siRNA), mRNA and gene-editing cargoes. "We are interested in DNA delivery as well," he says.

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Other types of nanoparticles – polymer-based, for instance – do not traffic to the liver by default so developers do not have to deal with that problem. "Typically they're designed or discovered on a per-tissue basis," says Sean Kevlahan, CEO and co-founder of Nanite. The rules that determine why a particular particle traffics to a particular tissue are not fully understood, however, and the company is addressing this issue. "If you don't understand why, then you can't design them effectively," he says. To explore the vast chemical space of polymer nanoparticles, the company has developed rapid synthesis and computational methods. It recently secured funding from the Cystic Fibrosis Foundation to identify polymer-based nanoparticles equipped to penetrate the thick mucus that

clogs the airways of patients and deliver an mRNA payload.

For working ex vivo or for tissues that are readily accessible, scientists may choose to apply physicochemical tools. Apart from one from SQZ Biotech, which is now struggling to stay afloat, most tools remain in preclinical development, although they are edging closer to the clinic. Intergalactic is employing a proprietary needle-like electrode to deliver its C^3 double-stranded DNA molecules to target cells. These are assembled in vitro from libraries of transgenes and regulatory elements and then combined into the final product in engineered bacterial cells, which ensures ease of manufacture and low cost of goods. The final DNA product is fully human and does not contain any viral or bacterial sequences. In the company's lead program, aimed at ABCA4 retinopathy, a solution containing the DNA is administered by subretinal injection and the same area is then exposed to a low-energy electric field. The method is broadly applicable. "Any tissue that can be biopsied is a potential candidate for local [gene] delivery," says José Lora, Intergalactic's CSO.

Maynooth, Ireland-based Avectas is developing a novel method of ex vivo gene transfer, in which it transiently exposes cells to a solubilizing spray, which contains ethanol and other constituents. This temporarily permeabilizes the cell membrane, allowing macromolecules, such as nucleic acids or gene editing components, to cross into the cytoplasm. Lab tests on T-cells suggest it is on a par with electroporation in terms of gene transfer efficiency, but the transfected cells exhibit less perturbation of gene expression. "Ultimately, that results in a cell that has the potential to be more highly functional," says Justin McCue, chief technology officer at Avectas.

Ultimately, gene therapy's claims of 'one and done' may need revising as transgene expression wanes over time, and if the therapy is virus-based, potential immunogenicity means patients will be unable to receive a second dose. Non-viral gene delivery particles that can reliably demonstrate a lack of immunogenicity could eliminate that issue. What's more, they could also change the safety equation during clinical development.

"It's not about getting to the maximum-tolerated dose and seeing if you're efficacious," says Stanton. Instead, developers can stack multiple "well-tolerated doses" over time, he says, which allows patient-specific dose titration. And if those nanoparticles are also able to evade the liver and reach their target organ, the whole experience of gene therapy will become similar to taking a biologic drug. It will take time and effort to deliver this vision — but it is now starting to take shape.

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News in brief

First herpesvirus gene therapy

The US Food and Drug Administration has approved the first treatment for the rare blistering skin disorder dystrophic epidermolysis bullosa. The therapy, Vyjuvek (beremagene geperpavec), developed by Krystal Biotech, is also the first to use the herpes simplex virus type 1 (HSV-1) as a gene therapy vector.

Patients with dystrophic epidermolysis bullosa experience painful and persistent skin wounds and rarely survive beyond age 30. The disabling skin wounds arise from mutations in the collagen gene *COL7A1*, defects in which cause loss of dermal and epidermal cohesion and lead to blistering, scarring and high rates of skin cancer. Vyjuvek replaces the mutated gene with two normal copies of the collagen type VII α1 chain (*COL7A1*) in keratinocytes and fibroblasts. This enables collagen type VII to form fibrils that anchor the epidermis and dermis together, leading to wound healing.

In trials, 67% of Vyjuvek-treated wounds and 22% of placebo-treated wounds closed completely. Its modified HSV-1 vector has greater DNA packaging capacity than the adeno-associated viruses commonly used in gene therapies. But Vyjuvek, a topical treatment, is not a 'one-and-done' gene therapy: repeat dosing was needed during the trials until the wounds remained closed for approximately 100 days. Redosing is possible because the HSV-1 vector is less immunogenic than adeno-associated virus and lentiviral vectors. The estimated annual cost of Vyjuvek is $631,000 per patient.

Krystal has two other HSV-1-based gene replacement therapies in clinical trials: KB407, encoding the human CFTR channel for cystic fibrosis, and KB301, encoding type III collagen to improve the cosmetics of aging skin.