Topical herpesvirus gene therapy enters final lap

The first herpesvirus replacement gene therapy for epidermolysis bullosa, a blistering skin condition, approaches the clinic.

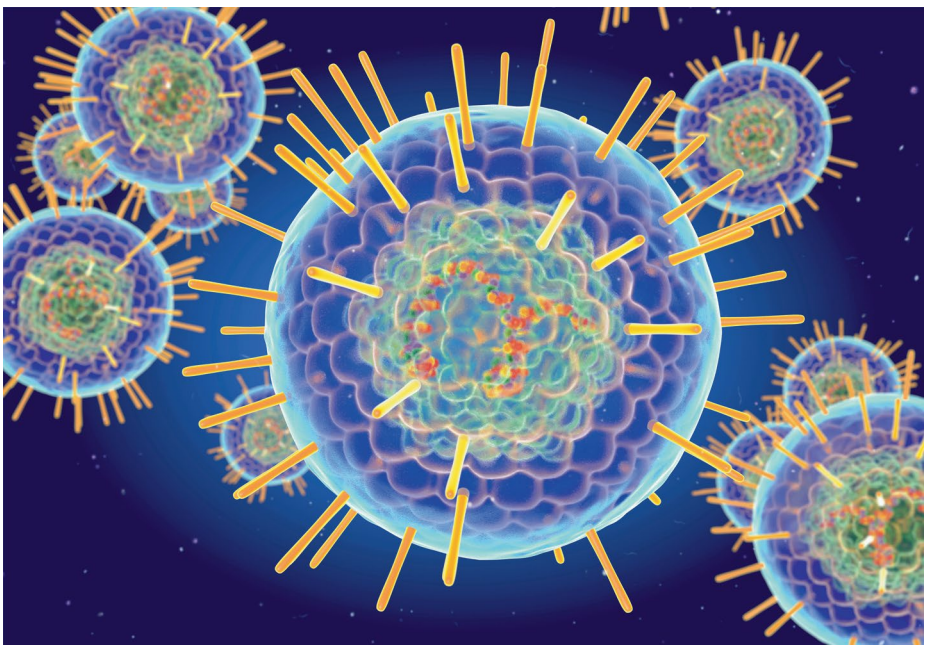
Late-stage clinical results have shown efficacy for a herpesvirus gene replacement therapy in the skin disorder epidermolysis bullosa, a devastating and extremely painful genetic disease that causes blisters and skin fragility. In March, phase 1 and 2 trial results were published; last November, a placebo-controlled phase 3 trial reached its primary endpoint. As *Nature Biotechnology* went to press, the company was gearing up to file an application for marketing with the US Food and Drug Administration (FDA).

In recessive dystrophic epidermolysis bullosa, a flawed *COL7A1* gene results in the loss of type VII collagen, an important structural protein of the skin. This defect causes the skin to separate below the lamina densa, a component of the basement membrane that lies between the dermis and the epidermis, resulting in widespread and disabling blistering and scarring of the skin. It also causes gastrointestinal and urogenital complications, anemia, growth retardation, eye problems and a high risk of developing squamous cell carcinoma. Patients have an extremely poor quality of life and a lowered life expectancy.

The treatment in question, B-VEC (Vyjuvek; beremagene geperpavec), is a recombinant herpes simplex virus type 1 (HSV-1) vector expressing the *COL7A1* gene, which encodes type VII collagen. B-VEC's progress against epidermolysis bullosa is in itself an important clinical milestone, given the historical dearth of approved therapies for the condition (Table 1). Beyond that, it is the first gene replacement therapy employing HSV-1, a vector that has received little attention from most gene therapy developers.

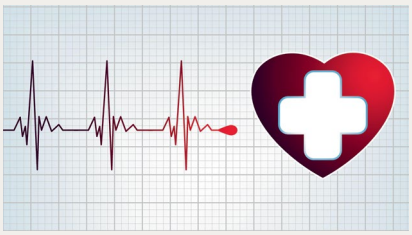
With a DNA packaging capacity ranging from 30 to 150 kilobases (kb), depending on their design, HSV-1-based vectors have an obvious advantage over the gene therapy vectors that dominate the field. Adeno-associated viruses (AAV) and lentiviruses have packaging capacities of only about 4.5 kb and 9 kb, respectively.

A key question for B-VEC is how long the transgenes encoded by the HSV-1 vector can persist in the body. B-VEC is currently



Herpes simplex virus vectors are strong candidates for gene therapy. Credit: Science Photo Library / Alamy Stock Photo

FDA okays first cardiac myosin inhibitor



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The first therapy to improve heart function by targeting the pathology underlying hypertrophic cardiomyopathy (HCM) has claimed a green light from US regulators. In April, the US Food and Drug administration gave Bristol Myers Squibb's Camzyos (mavacamten) the go-ahead, delivering the first cardiac myosin inhibitor to patients. Obstructive HCM is a progressive disease that thickens the heart walls, making it more difficult for the heart to expand and fill with blood and eventually leading to atrial fibrillation, stroke and heart failure. In clinical trials in patients with severely symptomatic obstructive HCM, 16 weeks taking the orally active Camzyos improved the heart's function, measured by quality of life and cardiac biomarkers, and lowered the need for septal reduction therapy.

Approval was based on the phase 3 Explorer-HCM trial in 251 patients with New York Heart Association class II–III obstructive HCM, all of whom had left ventricular ejection fraction (LVEF)>55% or higher. Because the drug, an allosteric small moleule, depresses cardiac contractility by inhibiting cardiac-specific myosin, adverse reactions were a concern. But none of the subjects experienced left ventricular ejection fraction ≤30% nor congestive heart failure, syncope or sudden cardiac death in the Camzyos group. Two patients who transiently experienced LVEF ≤50% interrupted treatment and resumed it on a lower dose.

Bristol Myers Squibb acquired Camzyos when it bought MyoKardia for $13 billion cash in 2020. Camzyos is priced at $89,500 a year, and sales in obstructive HCM could reach $2 billion. The drug is available with a Risk Evaluation and Mitigation Strategy program and carries a boxed warning.

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Table 1 | Epidermolysis bullosa therapies in clinical development

Developer	Therapy	Description	Clinical stage
Amryt Pharma (Dublin, Ireland)	Filsuvez (oleogel-S10)	Birch bark extract containing triterpenes (betulin, betulinic acid, erythrodiol, lupeol and oleanolic acid), which reduce inflammation and promote keratinocyte differentiation and migration	Received a positive opinion from the European Medicines Agency, 22 April 2022; received a complete response letter from the FDA on 28 February 2022
Krystal Biotech	Beremagene geperpavec (B-VEC)	HSV-1 expressing COL7A1 and carrying deletions in the viral intermediate early gene *ICP4* and the *ICP22* gene, to prevent replication and reduce cytotoxicity, respectively.	FDA filing expected in 2Q22
Abeona Therapeutics	EB-101	Epidermal sheets prepared from autologous keratinocytes transduced ex vivo with an LZRSE retroviral vector expressing *COL7A1*	Phase 3
Castle Creek Biosciences	Dabocemagene autoficel (D-Fi; FCX-007)	Autologous fibroblasts transduced with a lentiviral vector expressing *COL7A1*	Phase 3
Holostem Terapie Avanzate (Modena, Italy)	Hologene 5	Autologous cultured epidermal grafts containing epidermal stem cells transduced with a γ-retroviral vector expressing the β3 chain of laminin 332	Phase 2/3[a]

[a]Hologene 5 is in development for junctional epidermolysis bullosa. Sources: ClinicalTrials.gov; PubMed; EMA

administered topically once a week — a far cry from the 'one-and-done' paradigm often touted as the advantage of gene therapies. What's more, the in vivo utility of HSV-1 vectors has yet to be demonstrated.

The first HSV-1-based gene replacement therapy entered clinical development more than a decade ago, when the US subsidiary of Stockholm-based Diamyd Medical tested a construct encoding human pre-proenkephalin in patients with cancer experiencing intractable pain. In 2015, the FDA approved the first oncolytic gene therapy, Amgen's T-VEC, or Imlygic (talimogene laherparepvec), against melanoma — an HSV-1 vector that encodes an immunostimulatory granulocyte macrophage colony-stimulating factor (GM-CSF) gene.

Despite these advances, the complexity of herpesvirus biology has hampered its exploitation as a gene-therapy vector. "The herpes system has always underperformed expectations," says Jude Samulski, of the University of North Carolina School of Medicine, one of the pioneers of AAV-based vector development. "It has such an elaborate relationship with the host that harnessing those activities has been a tour de force."

Even so, over the years, developers have pursued several HSV-1-based gene delivery technologies. Among these, amplicon vectors are, in theory, the safest as they do not carry any viral genes. The basic format comprises a plasmid origin of replication and an antibiotic resistance gene, to enable production in bacteria. This is combined with viral sequences to ensure the transgene construct is packaged into infectious particles. These additional 'helper' viral genes have created contamination problems during production, however. "The more stringent you become in terms of eliminating helper virus, the lower the titer becomes," says Joseph Glorioso, of the University of Pittsburgh, a pioneer of HSV-based vector development.

Persistence is a particular problem for herpesvirus in dividing cells, as amplicon vectors, which exist as extrachromosomal episomes, are lost during cell division. Glorioso is collaborating with Neochromosome and Replay Therapeutics to develop modern high-payload amplicon HSV platforms. But the technology is still less advanced than approaches involving more traditional virus-based vectors. "Amplicon vectors are more difficult. You can't scale them as a product," says Suma Krishnan, co-founder and president, research and development, of Krystal Biotech.

Krystal's B-VEC sidesteps that problem using a recombinant HSV-1 virus. Krystal produces its HSV-1 vector by infecting cells derived from a master cell bank with virus seed stock that carries two copies of the 9-kb *COL7A1* gene, as well as deletions in the viral intermediate-early gene *ICP4*, to eliminate viral replication, and *ICP22*, to reduce cytotoxicity. "But that's about it," says Glorioso "This virus makes products that are highly cytotoxic, like ICP0, and so the chances of getting long-term expression without killing the cells that the virus infects — it's almost impossible, I would say." More modern HSV-1 vectors that do not encode ICP0, a ubiquitin ligase centrally involved in evading the host immune response, are available. "I think this can be improved on quite a bit," says Glorioso.

Insulator elements — sequences ordinarily involved in epigenetic regulation — are another innovation that can improve vector performance by boosting transgene expression. "These insulators will keep transgenes active for many, many months, and they'll even work with promoters that are tissue specific," says Glorioso. At the same time, he credits the team at Krystal for selecting a condition appropriate to its vector technology. "It's a novel application; that's what makes it clever," he says. "They've done a great job in moving a problem forward."

Imlygic — B-VEC's one notable precedent — is also based on a recombinant HSV-1 vector, albeit one that has a radically different design and purpose. As well as expressing GM-CSF, Imlygic contains deletions in ICP34.5 (the neurovirulence gene) and ICP47 (an immune escape gene) and an additional thymidine kinase gene (conferring sensitivity to the antiviral acyclovir). These changes were made to promote replication of the vector in cancerous cells. However, Imlygic was a commercial flop. It was licensed for direct injection into cutaneous, subcutaneous or nodal lesions in patients with recurrent melanoma who have already undergone surgery, with the aim of stimulating the immune system to attack tumor cells. It was overshadowed by the more compelling clinical outcomes obtained with antibody-based immune checkpoint inhibitors, and its efficacy was compromised by host antiviral innate immune responses to HSV and limited intratumoral spread of the vector due to the extracellular matrix, fibrosis and necrosis.

B-VEC is, like Imlygic, administered locally. It is directly applied in a gel suspension to the painful wounds and lesions that erupt on the skin of patients with epidermolysis bullosa. It infects both keratinocytes and fibroblasts, which produce and export functional type VII collagen proteins. By forming large structures called 'anchoring fibrils', the newly formed collagen promotes adhesion of the dermal and epidermal layers of the skin. In the phase 3 trial, Krystal Biotech disclosed in a press release that 67% of B-VEC-treated wounds were deemed to be healed at six months, compared with 22% of those treated with placebo. The healing effect on an individual wound is expected to last for ~90 days, says Krishnan, as type VII collagen has a half-life of about 30 days. Not all wounds are treated at any one time. "Continuous weekly dosing will improve wound burden in these patients," she says. B-VEC's relatively benign safety profile and ease of administration has enabled the company to obtain permission from the FDA to treat patients at home during an ongoing open-label extension study. "We are the first to get that," says Krishnan.

> "The wider gene therapy field is "fundamentally shifting," Samulski says, from gene replacement toward gene editing, as base editing, prime editing and epigenetic editing technologies start to mature."

Krystal Biotech has yet to reveal its pricing expectations, but its manufacturing process is simpler than that of AAV vectors as it does not entail the co-transfection of multiple plasmids into a production cell line. "The cost of goods is going to be much more favorable for us than for AAV," says Krishnan. "We can be competitive from a cost perspective." The master cell bank has been engineered to provide the proteins that are absent from the HSV-1 vector, to enable viral replication and packaging to proceed. "It's very reproducible. It's the same mother cell that produces daughter cells," says Krishnan. If B-VEC is to be administered on a weekly basis, the cost per dose will probably need to be more akin to that of a vaccine than a one-off gene therapy treatment.

There is more to come too: Krystal is about to start a trial of an HSV-1-based gene therapy in cystic fibrosis. A cosmetics-focused subsidiary, Jeune Aesthetics, has already reported phase 1 data for KB301, an HSV-1 vector encoding type III collagen, which is in development for improving the appearance of aging skin. Whether these developments herald the arrival of a whole new approach to gene therapy is an open question, however.

In the meantime, Krystal Biotech has been active in the courts as well as the clinic. PeriphaGen acquired the Diamyd pain program and the underlying technology in 2012 and, some years later, entered a relationship with Krystal Biotech. The partnership soured, however, and resulted in litigation over alleged misappropriation of trade secrets and a financial settlement, comprising an initial $25 million payment to PeriphaGen and up to $75 million more linked to the approval and commercial performance of B-VEC. The recent settlement removed any legal uncertainty about the program.

B-VEC for epidermolysis bullosa is a clever topical application of the HSV-1 platform. It remains unclear whether HSV-1-based vectors will develop into a more mainstream gene therapy modality or remain a niche technology limited to topical settings. "I don't see it as a vector being used more routinely," says Kyriacos Mitrophanous, CSO at Oxford, UK-based gene therapy manufacturer Oxford Biomedica.

For the moment, AAV and lentiviral vectors will continue to dominate the field and continue to attract most of the research attention and investment, despite shortcomings like limited payloads, immunogenicity that prevents redosing, and safety concerns.

"There is interest in alternative vector systems," says Brian Mullan, chief technology officer at gene therapy manufacturer Yposkesi, of Corbeil-Essonnes, France, citing the example of Ring Therapeutics, which is building a gene therapy platform based on a family of human commensal viruses called anelloviruses.

The wider gene therapy field is "fundamentally shifting," Samulski says, from gene replacement toward gene editing, as base editing, prime editing and epigenetic editing technologies start to mature. In that context, he says, the large payload capacity of HSV-1 vectors will become less significant. "I would say the cargo is the most important thing that is shifting," he says. ❐

Cormac Sheridan
Dublin, Ireland

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AI's plastic recycling

Credit: Art Directors & TRIP / Alamy Stock Photo

Researchers have used machine learning to create a new enzyme variant that degrades plastic trash even at low temperature. Since the discovery in 2016 of enzymes that can depolymerize polyethylene terephthalate (PET) plastic, biochemical engineers have been trying to industrialize the process by creating more efficient enzymes to break down the plastic. Natural PET hydrolyzing enzymes (PETases), originally isolated from bacterium *Ideonella sakaiensis*, require partially processed plastic and take a long time to digest plastic to completion at ambient temperatures. Alternative chemical methods are energy intensive, require un-ecological processing and create low-quality subunits that can only be downcycled into less valuable products. Now scientists at the University of Texas have applied AI to create a set of PETases with improved activities. After training their algorithm on over 19,000 protein structures from the Protein Data Bank, they could predict which amino acids were not optimal in different local environments. By engineering amino acid substitutions, the scientists created PETases with 3- to 29-fold greater activities, depending on the temperature, than natural or previously known PETases. The engineered enzymes, dubbed FAST-PETases (short for functional, active, stable and tolerant), depolymerize PET within a day at 50 °C, work on over 50 different untreated PET consumer products, and produce subunits that can reconstitute PET, closing the recycling circle. Roughly 35 million tons per year of PET plastic waste from discarded plastic bottles and mixed fiber clothing end up in landfills. PET, however, is only one of the five most commonly used plastics and is already the most recycled of the lot.

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