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SPOTLIGHT

EXPERT INSIGHT

A new era of *in vivo* gene therapy: the applicability of a differentiated HSV-1 based vector platform for redosable medicines

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Viral vector-based gene replacement approaches have traditionally focused on the use of adenoviruses, adeno-associated viruses, and lentiviruses for functional gene transmission. Innovation in payload delivery is critical for advancing the boundaries of genetic medicine. While underappreciated, herpes simplex virus type 1 (HSV-1) possesses a number of natural traits that make it an attractive alternative for gene therapy approaches, including episomal delivery, large payload capacity, a broad tissue tropism, and the ability to resist immune clearance via inhibition of innate and adaptive anti-viral immunity. Krystal Biotech has created a proprietary HSV-1-based gene delivery platform leveraging many of the natural properties innate to HSV-1, while engineering it to be replication-incompetent to reduce cytotoxicity. This platform has been validated clinically in dermatology, and its utility is being extended into programs across additional tissue types and organ systems, including initiation of a genetic pulmonary program in cystic fibrosis. This differentiated vector platform provides a broadly applicable, highly versatile gene delivery system for the development of direct and redosable genetically-coded medicines.

Cell & Gene Therapy Insights 2022; 8(5), 641–651

DOI: 10.18609/cgti.2022.096

The United States Food and Drug Administration (FDA) defines gene therapy as a means to modify or manipulate the expression of a gene or to alter the biological properties of living cells for therapeutic use [1]. An effective gene therapy approach requires efficient delivery of genetic material to target tissues or cells through the use of viral or non-viral vectors [2]. Central to the realization of gene therapy's potential is the advancement of vector technology to overcome the physical and immunological barriers to repeated gene delivery. While non-viral vectors may be an attractive future approach to gene therapy given their low cytotoxicity, reduced immunogenicity, and minimal risk of mutagenesis, the broad use of non-viral vectors has yet to be realized given the ongoing challenges related to gene transfer efficiency, gene expression duration, and safety [3]. In contrast, the use of viral vectors, which are the focus of this article, are the more commonly utilized tools for gene therapy given their evolutionarily-derived attributes of high efficiency gene transfer and specificity to target cells [4]. Viral vector-based gene replacement approaches have traditionally focused on the use of adenoviruses (Ad), adeno-associated viruses (AAV), and lentiviruses (LV) [5]. These viral vectors consist of a protein capsid and/or envelope that encapsulates the genetic payload, the transgene of interest, and regulatory elements that control stable or transient expression of the transgene as an episome or integrated into the host chromosome [2].

Adenovirus

Adenoviruses are non-enveloped viruses with double-stranded DNA that naturally cause infection of the upper respiratory tract. Their use as a vector in gene therapy was initially thought to be attractive given their payload capacity (4.5–6.5 kilobase (kb) transgene cassettes), high transduction efficiency (both in quiescent and dividing cells), epichromosomal persistence in the host cells, and broad tropism for various tissue targets [2, 6]. However, recent evidence suggest Ad vectors may have the potential to integrate into the host DNA through random (nonhomologous) recombination, thus raising safety concerns of their use in gene replacement approaches [7]. Further, the early generation Ad vectors proved to be highly immunogenic due to the innate immune response initiated by exposure of the host to the virus capsid protein resulting in severe cytokine storm [8–9]. This was evidenced in a 1999 clinical trial where a trial participant died from complications of Ad vector administration resulting in systemic inflammation and multiorgan failure [10–11]. While recent generation Ad vector-based vaccines and oncolytic therapies benefit from this intrinsic immunogenicity and cellular toxicity, these properties continue to limit their use as a treatment modality for genetic disease [2].

Adeno-associated virus

Adeno-associated viruses are generally recognized as versatile vectors for gene therapy given their wide-ranging tropism profiles. Indeed, a significant majority of gene therapy development today is based on AAV vectors [2, 12]. AAVs lack the essential genes needed for replication, and they undergo circularization via inverted terminal repeat (ITR) recombination to form stable and persistent episomal configurations [2]. While there have been over 200 clinical trials based on AAVs worldwide, their limited transgene payload capacity (<5 kb transgene cassettes) and inherent immunogenicity are standing challenges in the field [2]. Regarding the latter, host adaptive immunity to the capsid results in reduced AAV efficacy [13–14]. Moreover, while early studies suggested AAVs do not integrate into the host genome, additional evidence has suggested AAVs (*e.g.,* AAV2) may cause insertional mutagenesis in humans [15–17]. While AAVs have been shown to be relatively safe in humans, resulting in the U.S. FDA approval of two AAV-based gene therapies (*i.e.,* Luxturna® (voretigene

DOI: 10.18609/cgti.2022.096

neparvovec-rzyl) and Zolgensma® (onasemnogene abeparvovec-xioi), additional genetic engineering of AAV vectors is needed to begin to address the issues associated with AAV integration, as well as pre-treatment immunity due to the presence of neutralizing antibodies against serotypes commonly found circulating in the population [18–19].

Lentivirus

Lentiviruses (LVs) are part of the retroviridae family of single-stranded RNA viruses [20]. LVs possess a payload capacity of up to 9 kb and integrate into the host genome in both dividing and non-dividing cells, with a preference for transcriptionally active sites, allowing for long-term transgene expression [21–22]. With over a dozen completed clinical trials, the use of LV vectors appears well-tolerated [2]. However, LV-based approaches to gene therapy are primarily limited to *ex vivo* delivery due to the potential for insertional mutagenesis and the associated risk of cancer development inherent to their use [2, 23]. Moreover, recent reports suggest LV vector integration can activate neighboring genes, promote chimeric gene fusions, and may cause aberrant splicing of cellular transcripts, raising additional concerns about the oncogenic impact of such integration [24–26]. The FDA has also recently placed a clinical hold on a LV-vector based gene therapy approach for the treatment of cerebral adrenoleukodystrophy, as one participant reportedly developed myelodysplastic syndrome likely associated with LV treatment, highlighting the potential risks associated with uncontrolled LV integration into the host genome [27]. However, groups are exploring the use of non-integrating lentiviruses in the preclinical setting with the intent to circumvent the risk associated with integrating LVs currently used clinically [28].

There remains an unmet need for a gene therapy platform that addresses a number of the challenges faced by Ad-, AAV-, and LV-based genetic medicines, such as vector integration into the host genome, pre- and post-treatment neutralizing immunity, and limited payload capacity.

HERPES SIMPLEX VIRUS AS THE BASIS FOR KRYSTAL BIOTECH'S GENE THERAPY PLATFORM

Herpes simplex virus type 1 (HSV-1) belongs to the human herpes virus (HHV) family of double-stranded DNA viruses. Of the known HHVs, HSV-1 is the best characterized given that it is highly prevalent in the human population, with estimates suggesting that more than two-thirds of those ≥12 years of age in the US have been exposed to the virus [29]. The HSV-1 virion is ~220 nm in diameter with a linear, double-stranded DNA genome that circularizes upon cellular infection. Importantly, the HSV-1 genome remains fully episomal and does not integrate into, or otherwise disrupt, the host genome [30–31].

Upon cellular infection, the cascade of HSV-1 gene expression that ultimately leads to replication, which is necessary for lytic disease and secondary neuronal infection/spread, is a tightly controlled temporal process. This begins with expression of the five immediate early (IE) genes (Table 1), which are a focus in vector development given their essential role in both replication and immune evasion. Subsequent expression of early and late genes, and consequent assembly of fully infectious virions, is entirely dependent upon the expression of the IE genes. In humans, HSV-1 efficiently resists immune clearance, partially explained by the innate immune-evasive properties of HSV tegument proteins and the observations that HSV-1 has evolved a number of genes devoted, at least in part, to inhibiting both innate and adaptive anti-viral immunity [32–33].

While underappreciated as a gene delivery platform, HSV-1 addresses a number of challenges faced by other vector technologies currently utilized in gene therapy. HSV-1 is known to resist immune clearance and does not induce broadly neutralizing antibody

TABLE 1

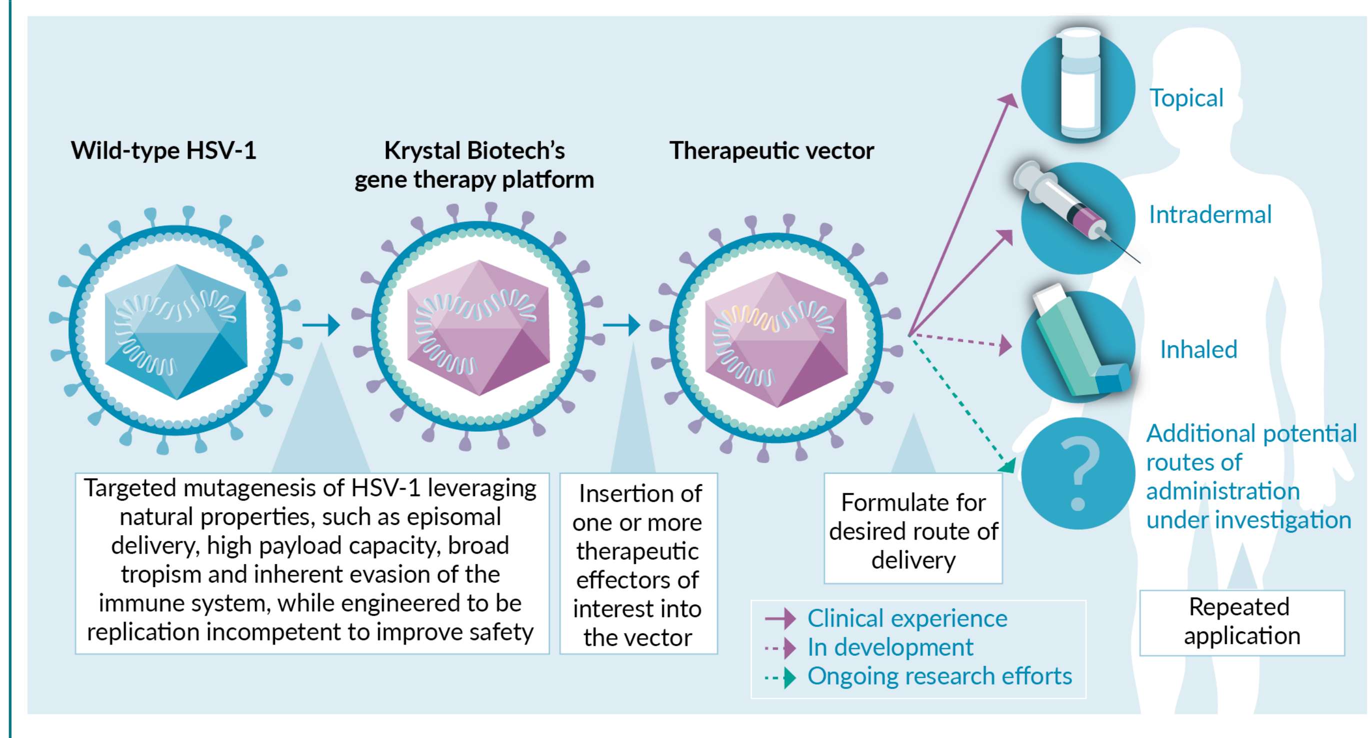
HSV-1 IE gene products and their functions

HSV-1 IE genes	Function
Infected Cell Protein (ICP) 0	Activates viral promoters to support downstream viral replication, plays a role in evasion of the innate immune response via inhibition of multiple pathways, and promotes cell cycle arrest and apoptosis [34–36]
ICP4	Obligate transactivator of downstream viral gene expression and HSV-1's lytic cycle [37]
ICP22	Plays several roles in viral gene expression, cell cycle control, viral assembly, and nuclear egress [38]
ICP27	Regulates viral gene expression through multiple mechanisms including splicing regulation, processing, and mRNA export, and plays a role in evasion of the innate immune response [39]
ICP47	Not regulatory but instead prevents immune recognition and destruction of HSV-1 by binding to transport associated protein, which prevents antigen loading of MHC class I molecules [40]

responses [32, 41]. It has a genome size that easily accommodates large or multiple genes, and it can transduce both dividing and non-dividing cells without integrating into, or otherwise disrupting, host cell DNA [31]. In addition to its native sites of infection, the skin and mucosa, the ubiquity of HSV-1 entry receptors on myriad human cell types raises the possibility of efficient delivery to multiple tissues and organ systems upon targeted administration [42]. Krystal Biotech has combined many of the beneficial properties inherent to HSV-1 with a modification strategy of targeted IE gene deletions in ICP4 and ICP22 to render the vector replication-incompetent and less cytotoxic resulting in the development of an *in vivo*, non-invasive, and redosable vector platform suitable for localized gene delivery (Figure 1). Also, Krystal Biotech's products are manufactured using fully characterized virus and cell banks, and thus, do not suffer from the same inefficiencies inherent to multiple plasmid-based transfection methodologies utilized in the production of

FIGURE 1

An overview of Krystal Biotech's gene therapy platform approach.



DOI: 10.18609/cgti.2022.096

AAVs or LVs. Further, because of the targeted IE gene deletion in ICP4, the HSV-1 vector does not grow in non-complementing cells lacking exogenous ICP4. As an additional precaution, Krystal Biotech's vector engineering strategy was specifically designed to maintain sensitivity to commonly prescribed antiviral medications (*e.g.*, acyclovir and valacyclovir) targeting herpes viruses, to address the extremely unlikely event that herpetic lesions or other viral-associated disease manifestations were to appear in a patient exposed to the modified virus.

CLINICAL VALIDATION OF KRYSTAL BIOTECH'S GENE THERAPY PLATFORM IN RARE SKIN DISEASE

Dystrophic epidermolysis bullosa (DEB) is a serious, ultra-rare genetic blistering disease caused by mutations in the *COL7A1* gene, encoding type VII collagen (COL7) [43–44]. Pathogenic mutations to *COL7A1* result in absent or dysfunctional anchoring fibrils and loss of adhesion of the epidermis to the dermis [45–46]. DEB is characterized by skin fragility that leads to widespread, painful, and lifelong recurrent blistering [45–47]. Patients with DEB are at increased risk for serious complications, including aggressive squamous cell carcinoma [44, 48–53]. A significant unmet need exists for therapies to molecularly correct the underlying cause of DEB.

Beremagene geperpavec (B-VEC) is an investigational topical, redosable gene therapy based on Krystal Biotech's vector platform that is designed to restore functional COL7 via delivery of full-length *COL7A1* genes [41, 54]. Preclinical data demonstrated that B-VEC efficiently restored COL7 expression in recessive DEB (RDEB) primary skin cells, as well as in a diseased animal model, demonstrating its capability for therapeutic gene delivery [41]. In an open-label, placebo-controlled Phase 1/2 clinical study (GEM-1; NCT03536143), repeated application of B-VEC resulted in full-length COL7 protein expression and anchoring fibril formation in nine patients with RDEB. Notably, B-VEC was well tolerated, and wounds treated with the vector demonstrated improvement in closure compared to placebo at 3 months [41]. A Phase 3, double-blind, placebo-controlled, intra-patient-randomized study (GEM-3; NCT04491604) evaluating the efficacy and safety of B-VEC in patients with DEB has been completed [54].

Autosomal recessive congenital ichthyosis (ARCI) is a life-long, severe genetic skin disease resulting from germline mutations in the *TGM1* gene encoding tranglutaminase-1, a protein essential for proper formation of the skin barrier [55]. Patients with *TGM1*-associated ARCI are typically born in a collodion membrane and develop plate-like scales on the skin following the shedding of the collodion membrane, [56]. Disease complications include pronounced dehydration, increased risk of infection, and a significantly decreased quality of life [56].

KB105 is a second investigational, topical, redosable gene therapy candidate developed using the modified HSV-1 vector platform via insertion of the functional form of *TGM1*. Preclinical data demonstrated that KB105 efficiently transduced ARCI patient keratinocytes *ex vivo* and barrier-impaired mouse skin *in vivo*, resulting in human *TGM1* expression [57]. A Phase 1 exploratory, open-label, placebo-controlled, intra-patient study (NCT04047732) evaluated three adult patients with a genetic diagnosis of *TGM1*-deficient ARCI to understand the safety and preliminary efficacy (molecular correction, phenotypic improvement) of KB105 [56]. Repeat dosing was well-tolerated with no drug-related adverse events or immune response to HSV-1 or TGM1. Treatment with KB105 restored functional TGM1 protein, which was correctly localized in the epidermis. Areas treated with KB105 showed reduced reversion to the ichthyotic scaling phenotype; however, phenotypic evaluation was limited by small treatment areas and these observations need to be confirmed in larger

studies. The Phase 2 portion of this study is ongoing.

DERMATOLOGY BEYOND RARE SKIN DISEASE

Given clinical substantiation of the underlying vector technology, there existed a unique opportunity to differentiate Krystal Biotech's platform beyond the traditional confines of the therapeutic setting.

Dermal collagen represents >90% of human skin and is composed primarily of type I collagen (COL1) and type III collagen (COL3) fibrils which provide strength to the skin and are critical for the maintenance of skin tissue architecture. COL3 appears early during collagen fibrillogenesis, and its subsequent replacement by COL1 is a critical step for collagen fibril maturation and extracellular matrix reorganization [58]. Due to the essential role collagen plays in the process of skin biorejuvenation, and the diminution of dermal collagen being the primary contributor to the aged phenotype, direct and indirect collagen stimulation/supplementation/replacement has been the focus of aesthetic product development for much of the last four decades. However, directed supplementation of functional full-length human COL3, produced by and secreted from the patient's own dermal cells, has not been explored clinically to treat superficial skin depressions.

Jeune Aesthetics, Inc., a wholly-owned subsidiary of Krystal Biotech, is evaluating KB301, an investigational aesthetic product based on Krystal Biotech's differentiated HSV-1 platform, encoding the *COL3A1* gene. A Phase 1 study with two cohorts evaluating the safety, tolerability, and preliminary efficacy in adults (PEARL-1; NCT04540900) has been conducted. In cohort 1, repeated intradermal injection of three different doses of KB301 were evaluated in seven healthy subjects, demonstrating tolerability with no clinically significant immunogenicity findings [59]. Cohort 2 was a randomized, double-blind, placebo-controlled assessment of the safety and preliminary efficacy of KB301 for the improvement of fine lines and skin texture in the lower and upper cheek, and for the improvement in skin thickness on the knee [60]. Twenty-seven adult patients were enrolled with the treatment side randomized 2:1 to receive KB301 or placebo as multiple micro depot injections. Low dose KB301 was evaluated in the knee. Low dose or high dose KB301 was evaluated in the lower cheek and low dose KB301 was evaluated in the upper cheek. Repeat administration of KB301 was well-tolerated with minimal injection site reactions, all resolving within 3–5 days. Treatment with KB301 also demonstrated clinical benefit versus placebo, including improved Subject Satisfaction Scores across all three treatment areas. Safety and efficacy of KB301 will be further evaluated in a Phase 2 study.

Following clinical proof-of-concept in skin to address both monogenic disease and aesthetic protein supplementation, Krystal Biotech's platform is now being investigated more broadly, including for its ability to deliver non-traditional effectors for genetic medicine. Given skin being the initial focus as a target tissue, the first departure from traditional gene replacement was an attempt to treat chronic, complex skin indications with vectors encoding synthetic constructs. Preliminary efforts in this regard focused on recombinant HSV-1 vectors to deliver and locally express therapeutic antibodies. A library of such vectors was engineered, including candidates encoding single-chain antibodies targeting TNFα, IL4-Rα, and IL-17, and preliminary *in vitro* and *in vivo* efficacy was demonstrated, including in multiple murine atopic dermatitis models [61]. The use of Krystal Biotech's vector platform, which enables effector expression at the site of application without systemic vector exposure and the ability to re-dose over time, could be particularly attractive for these alternative payloads. Discovery-phase exploration of vectors designed to deliver RNAi and gene editing machinery are under investigation.

 DOI: 10.18609/cgti.2022.096

ADDITIONAL PLATFORM APPLICATIONS OUTSIDE OF THE SKIN

Cystic fibrosis (CF), a disease characterized by chronic pulmonary infections, increased airway secretions, and eventually respiratory failure, is the most common inherited genetic disorder in the United States [62]. Targeted delivery of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene to address the diseased phenotype, employing both viral and non-viral gene therapy approaches, have been explored extensively but have suffered from some combination of limited capacity to encode a large effector like *CFTR*, toxicity to the administered epithelium, robust immune system activation upon single or repeated exposure, and/or inefficient gene transfer to the apical (air-exposed) membrane of polarized airway cells.

Krystal Biotech is investigating the use of its gene delivery platform for the treatment of CF with KB407, an investigational therapeutic encoding two copies of full-length human *CFTR* [63]. Preclinical pharmacology of KB407 indicated that the vector capably transduces relevant primary CF patient airway epithelial cells in 2D culture, efficiently produces functional human CFTR protein, molecularly corrects multiple CFTR defects without significant toxicity in a clinically relevant 3D organotypic system, and effectively directs localized expression of human CFTR in mice and non-human primate lung epithelium. Krystal Biotech believes that direct supplementation or replacement of full-length human CFTR upon nebulization of KB407 presents a unique opportunity for safe, non-invasive, and mutation-agnostic molecular correction of CF. A Phase 1 study is planned to commence in 2022.

CONCLUSIONS

Much progress has been made to advance gene therapy over the years; however, it has also shed light on limitations of commonly used *ex vivo* and *in vivo* viral vectors. Krystal Biotech recognized the attributes of HSV-1 as the basis for a gene delivery system, established a differentiated vector platform through targeted modification to render the virus replication incompetent and less cytotoxic, and has clinically validated it in multiple dermatologic conditions, including a Phase 3 study of B-VEC in DEB. During the course of developing B-VEC from concept to clinic, there was a growing recognition of the potential applicability of the underlying vector technology in other skin conditions as well as additional organ systems. Evidence to date suggest this proprietary platform holds promise in the development of broadly applicable, redosable gene therapies that can be delivered via minimal- to non- invasive routes of administration.

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DOI: 10.18609/cgti.2022.096

AUTHORSHIP & CONFLICT OF INTEREST

Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *The authors thank Whitney Ijem, Stacie Oliver, and Serene Forte for their insights and contributions to early iterations of this manuscript. They also thank Anastasia McManus for her editorial review.*

Disclosure and potential conflicts of interest: *TP, DAP, SK are employees/receive a salary from Krystal Biotech, Inc. TP is an inventor on a number of patents/patent applications assigned to Krystal Biotech, Inc. TP, DAP and SK received stock options as a portion of their compensation from Krystal Biotech, Inc. The authors have no other conflicts of interest.*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

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Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Apr 27 2022;* ***Revised manuscript received:*** *Jun 02 2022;* ***Publication date:*** *Jun 28 2022.*