Trends in Molecular Medicine

CellPress OPEN ACCESS

Review

Safety of non-replicative and oncolytic replication-selective HSV vectors

Alberto L. Epstein[1,*], and Samuel D. Rabkin[2,*]

Herpes simplex virus type 1 (HSV-1) is a DNA virus and human pathogen used to construct promising therapeutic vectors. HSV-1 vectors fall into two classes: replication-selective oncolytic vectors for cancer therapy and defective non-replicative vectors for gene therapy. Vectors from each class can accommodate ≥30 kb of inserts, have been approved clinically, and demonstrate a relatively benign safety profile. Despite oncolytic HSV (oHSV) replication in tumors and elicited immune responses, the virus is well tolerated in cancer patients. Current non-replicative vectors elicit only limited immune responses. Seropositivity and immune responses against HSV-1 do not eliminate either the vector or infected cells, and the vectors can therefore be re-administered. In this review we highlight vectors that have been translated to the clinic and host–virus immune interactions that impact on the safety and efficacy of HSVs.

Highlights

Preclinical and clinical studies indicate that immune responses against HSV-1 vectors, oncolytic or non-replicative, do not constitute a major impediment to their efficacy or safety.

Despite oHSV replication in tumors and their elicited immune responses, they have proved to be very safe and well-tolerated in cancer patients.

In contrast to early safety concerns, current non-replicative gene therapy vectors, which often express only the therapeutic transgene(s), are safe and elicit only limited immune responses.

Seropositivity and immune responses against HSV do not lead to the elimination of either HSV or infected cells, and the vectors can be re-administered several times.

Three HSV-1 vectors have already been approved, two for cancer therapy and one for gene therapy.

Other HSV-1 vectors are currently being clinically tested, and serious adverse events linked to the vector have not been observed.

Herpes simplex virus biology

HSV-1 (Box 1) is a neurotropic human pathogen with a worldwide infection prevalence of ~67% [1]. HSV-1 normally induces immunity that prevents reinfections with the same serotype, but not with HSV-2 [2]. HSV-1 infection can be asymptomatic, mild, or life-threatening. In most immunocompetent individuals, HSV-1 causes only mild and self-resolving diseases such as stomatitis, cold sores, or genital herpes [3]. In rare situations, HSV-1 infection is associated with diseases with high morbidity (herpes stromal keratitis, meningitis) and mortality (herpes simplex encephalitis) [3]. Why some individuals develop severe disease is not completely understood, but both innate and adaptive immune responses are fundamental to controlling HSV-1 and reducing pathogenesis [4]. Neonatal infection is more aggressive, leading to systemic dissemination with high morbidity if untreated [5].

HSV-1 has evolved numerous functions to evade, moderate, and subvert immune surveillance [2]. Reactivations are rapidly controlled by the immune system without significant inflammation or strong immune reactions [4,6] (Box 1). Engineering the virus to exploit and manipulate these counteracting functions is key to designing optimized HSV-1 vectors, for example to allow multiple vector administrations [7–10] in contrast to other vectors where re-administration has been impeded by their immunogenicity.

We review here current knowledge from >30 years of preclinical and clinical studies. We briefly summarize the host responses induced and counteracted by wild-type HSV-1, and the immune responses prompted by oHSV and defective, **non-replicative HSV-1 vectors** (see Glossary). HSV vectors have attributes that make them very appealing for gene and cancer therapy (Table 1), as demonstrated by the approved HSV vectors. Although difficulties working with or manufacturing HSV vectors are being overcome, the inaccurate view that they are inherently unsafe remains. Clinical studies have generally validated the safety profile observed in preclinical studies, and clinical adverse events (AEs) were less frequent than was originally postulated and also in comparison to other treatment modalities.

[1]EG 427 SAS, 29 Rue du Faubourg Saint Jacques, 75014 Paris, France
[2]Brain Tumor Research Center, Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA

*Correspondence:
alberto@eg427.com (A.L. Epstein) and
rabkin@mgh.harvard.edu (S.D. Rabkin).

Box 1. Molecular biology of wild-type HSV-1 infection

The HSV-1 DNA genome is contained in an icosahedral capsid, coated with tegument (a layer of at least 20 proteins that is important for gene expression and virion assembly), and a lipid envelope studded with 11 different virus-encoded glycoproteins [2]. The envelope glycoproteins function in viral entry, spread, and immune evasion. Glycoproteins gB, gD, gH, and gL are necessary for infection and interacting with nectin-1, the main receptor, to induce fusion of the virus and cellular membranes [91,92]. Following fusion, the tegument proteins and capsid dissociate, and the capsid is transported to the nuclear membrane where the genome enters the nucleus [93]. Late tegument proteins in incoming virus particles exert immediate effects even before viral gene expression, thus counteracting cellular antiviral defenses [2]. The HSV-1 life cycle can then follow a lytic or latent pathway. During lytic productive infection, a temporal cascade of gene expression occurs involving the sequential synthesis of immediate-early (IE, α), early (E, β), and late (L, γ) proteins [2]. IE infected cell proteins ICP0, ICP4, ICP22, and ICP27 regulate the expression of the E and L genes [2]. IE ICP47 is an inhibitor of human transporter associated with antigen processing (TAP) and blocks MHC I antigen presentation [85]. E proteins are required for replication of the virus genome, whereas L structural proteins are involved in virion morphogenesis and cell exit, as well as in modulating immune responses.

Transmission of HSV occurs through close contact and typically follows primary infection of epithelial cells in the oral (HSV-1) or genital (HSV-2) mucosa, followed by lifelong latency in sensory neurons, reactivation, and reinfection of peripheral mucosa. During latent infection, ICP0, an E3 ubiquitin ligase, is not efficiently expressed and cannot counteract genome silencing, and the transcription cascade is therefore not triggered: the virus genome remains in an epigenetically repressed state, with the exception of untranslated latency-associated transcripts (LATs) [94]. Latency in sensory neurons is a compromise where the silent HSV-1 genome and infected cells are preserved from immune attack, while the host CNS is protected from virus spread. Danger signals such as stress, UV light, or fever can stimulate reactivation from latency leading to resumption of the lytic gene expression cascade [2,94]. In regards to pathogenicity in humans, genetic polymorphisms/mutations in innate defenses are linked to a higher risk of encephalitis (e.g., *TLR3*, *UNC93B1*, *TRIF*, *IRF3*) [95]. Effective antiviral drugs such as acyclovir are available, in contrast to most other viruses [3].

Immune responses against HSV-1

HSV-1 establishes multiple intricate interactions with host defense systems, including intrinsic, innate, and adaptive immunity, with the goal of evading immune surveillance and maintaining life-long persistence [11,12]. Understanding these interactions is crucial for vector design to ensure safety and efficacy.

Table 1. Advantages of HSV vectors[a]

Vector attributes	Oncolytic	Non-replicative
Broad host range, surface receptors widely expressed	✓	✓
Non-integrating	✓	✓
Redirect cellular tropism, glycoprotein mutations/ligand expression	✓	✓
Very infectious	✓	✓
Cytotoxic at low multiplicity of infection	✓	✗
Induces inflammation	✓	✗
Deletion of essential genes for safety and to provide space for inserted sequences	✗	✓
Latency in neurons for life	?	✓
Genetic engineering enables rapid construction	✓	✓
Multiple administrations	✓	✓
Low immunogenicity	?	✓
Space for large sequence inserts	✓	✓
Antiviral drugs available	✓	✗

[a]Key, ✓, positive attributes; ✗, negative attributes; ?, uncertain.

Glossary

Human cytomegalovirus (HCMV)
IRS1: a protein kinase R (PKR) inhibitor that antagonizes host protein synthesis shutoff and can complement this activity that is lacking in γ34.5Δ oncolytic herpes simplex viruses (oHSVs).
ICP0: an immediate early (IE) protein and E3-ubiquitin ligase of HSV-1 that plays a key role in counteracting many of the cellular innate antiviral defenses.
ICP4: an IE HSV-1 protein that is the major transcriptional regulator for HSV, required for E and L transcription and repressor of IE gene expression and non-IE genes at IE times. As an essential protein, lack of its expression or mutation results in non-replicative viruses or vectors that can only be produced in cells expressing complementing amounts of this protein.
ICP6: also known as UL39, HSV-1 ICP6 is the large subunit of ribonucleotide reductase that is essential for virus growth in post-mitotic non-dividing cells and contributes to neuropathogenicity.
ICP34.5: also known as γ34.5, ICP34.5 is a late (L) HSV-1 protein and major neurovirulence factor that blocks host protein shutoff and autophagy; it is required for virus multiplication in neurons and other non-proliferative cells. Virus strains not expressing this protein can replicate in cancer cells but minimally in normal cells. For this reason the gene encoding this protein has been deleted in most oHSVs.
Lethal dose 50 (LD50): the dose of virus or other agent at which only 50% of treated animals survive.
Non-replicative HSV-1 vectors: these fall into two classes: (i) defective amplicons derived from plasmids lacking all HSV genes and that only contain a viral replication origin and packaging sequences, as well as exogenous sequences that are packaged as an ~150 kb genome in the presence of a helper HSV, and (ii) recombinant viruses that lack the expression of at least one essential IE gene.
Plaque-forming units (pfu): a measure of infectious virus based on the ability of a single virion to replicate and kill susceptible cells in a monolayer forming a plaque that can be counted.
PML nuclear bodies: also known as nuclear domains 10 (ND10), these are matrix-associated domains that recruit an astonishing variety of seemingly unrelated proteins, including several that

Intrinsic and innate immunity

Intrinsic and innate immunity, which are interconnected, are the first lines of defense that limit viral spread and regulate the ensuing adaptive response [13]. The intrinsic response is mediated by pre-existing restriction proteins that assemble into **PML nuclear bodies** driven by the **promyelocytic leukemia (PML)** protein and inhibit lytic infection without requiring interferon (IFN) expression [13]. This is counteracted by HSV-1 protein **ICP0**, allowing the infectious cycle to proceed [14]. Innate immunity is triggered by pathogen-associated molecular patterns (PAMPs) that induce IFN-stimulated gene (ISG) expression with antiviral and inflammatory activities [15]. Several cell types contribute to immune surveillance: natural killer (NK) cells, that are important for cytokine production and the recognition and killing of virus-infected cells [15], as well as plasmacytoid dendritic cells (pDCs) that are the major producers of IFN-α *in vivo* [16].

Intrinsic and innate cellular defenses are effective against HSV-1 during primary infection and reactivation from latency. However, HSV-1 expresses a large number of factors that counteract/modulate this. A note on nomenclature: HSV-1 proteins were historically named from the timing of their expression, either immediate-early (IE), early (E), or late (L) (also termed α, β, and γ, respectively; Box 1), or from their relative gel migration (e.g., infected cell polypeptide, ICP). Other HSV-1 genes/proteins are named from their locations in the genome, and UL and US proteins are encoded in the **unique long and short (UL and US) regions of the genome, respectively.**

HSV-1 factors modulating host defenses include the IE proteins ICP0 and ICP27, E protein UL42, and L proteins US3, US5, US11, UL21, UL31, UL36, UL41, UL46/VP11–12, UL48, UL49/VP22, and **ICP34.5** [4,17]. The IE and E viral functions eventually overcome cellular defenses, and the infection progresses, but with decreases and delays in virus production and spread, giving time to elicit adaptive immune responses that ultimately control the infection.

Adaptive immune responses to HSV-1

Antigen-specific adaptive immune responses take time to evolve and finally control virus spread [18]. Neutralizing antibodies against HSV-1 play a seemingly minor role compared to $CD8^+$ T cell-mediated immunity [19]. Conflicting results are often due to differences in the animal species, administration route, latency model, complex interactions between cell types, and HSV-1 versus HSV-2 [19,20].

Humoral responses. Antibody levels do not determine the outcome of HSV-1 infection in humans, and transfer of seropositive serum in mice had no appreciable effect on viral replication or establishment of latency [19]. However, B cell-deficient mice are more susceptible to herpetic encephalitis after ocular infection [21]. In human latency, B cells and HSV-specific antibodies are increased and respond to reactivation in the skin [22]. In acute infections, the combination of both antibodies and $CD4^+$ T cells protects neuronal tissue after immunization [23].

Cellular responses. $CD8^+$ T cells are recruited to HSV lesions early in infection and contribute greatly to immune control and cytolysis [19]. In recurrent genital HSV-2 patients, T cells expand after reactivation and then return to a steady state, resembling an acute recall response [24]. IFN-γ-mediated $CD8^+$ T effector functions contribute to protection against secondary infections, maintenance of latency, and limiting viral spread [19]. IFN-γ-producing $CD8^+$ T cells persist during latency in areas of T cell infiltration, but they do not clear the infection [19]. IFN-γ receptor depletion leads to impaired resolution of HSV infections [20] and significant mortality [25]. Depletion studies showed that both $CD4^+$ and $CD8^+$ T cells contribute to protection against HSV replication and shedding in the local mucosa [19]. Neuronal infection and reactivation are mainly controlled

contribute to innate antiviral cellular defenses and are targeted for proteolysis by ICP0.

Promyelocytic leukemia (PML): a key organizer of the so-called PML nuclear bodies. The gene encoding PML is rearranged in an oncogenic chromosomal translocation in some types of leukemia.

Unique long and short (UL and US) regions: the HSV-1 genome contains two unique regions, long (UL) and short (US), bordered by inverted repeat regions (IR and TR). Genes located in the UL are numbered UL1–UL56, and US genes are numbered US1–US12.

by noncytolytic $CD8^+$ T cells; however, in CD8-depleted or deficient mice, $CD4^+$ T cells are sufficient to clear virus from both local mucosal and neural sites [26]. Responses are further complicated by the modulation of MHC-I and -II expression [26].

HSV modulates adaptive immunity

HSV infects human immature dendritic cells (DCs) and blocks their maturation, impairs their activity, and triggers apoptosis [16]. HSV-1 IE ICP47 blocks the transporter associated with antigen presentation (TAP) [27], and L ICP34.5 inhibits autophagy processes involved in antigen presentation [28]. Infection of activated T cells is not productive but represses T cell receptor signal transduction and cytolytic activity [29]. HSV glycoproteins gE and gC bind to the Fc region of antibodies and complement, respectively, to inhibit antibody-dependent cell-mediated cytotoxicity [30].

The interaction between the host immune system and HSV, involving about a quarter of all HSV genes, is a long-term battle that often ends in a stalemate [12,18]. Alterations in HSV innate and adaptive immune evasion gene products can influence vector safety and efficacy and are important considerations in vector design [11].

HSV-1 vectors

There are two main types of non-integrating therapeutic vectors derived from HSV-1: (i) replication-selective oncolytic viruses for the treatment of cancer, named virotherapy [31] (Box 2), and (ii) defective non-replicative vectors for therapeutic transgene delivery to treat genetic and non-genetic diseases, named gene therapy [32] (Box 3). oHSVs, that are genetically engineered to replicate only in cancer cells (Box 2), have been evaluated in numerous patients [10]. They are potentially more pathogenic because of their replication competence, and thus provide insight into the most serious AEs that HSV vectors might elicit. Successful therapy with these two vector

Box 2. Oncolytic HSV-1 (oHSV)

oHSVs are genetically engineered to be replication-selective such that virus infection and/or replication is limited to cancer cells. They are a distinct class of cancer therapeutics – virotherapy – and have two unique mechanisms of action: (i) oncolytic/cytotoxic activity that directly kills cancer cells, while sparing normal cells and tissue, and amplifies *in situ*, and (ii) inducing tumor inflammation and antitumor immune responses [96]. Because cancer is often lethal and has a large commercial market, many more clinical trials have investigated the safety (Phase 1) and efficacy (Phase 2 and 3) of oHSV than of non-replicative HSV vectors. oHSV was the first genetically engineered oncolytic virus described and is one that has been most translated to the clinic [97]. oHSVs can be engineered for oncolytic activity and safety by deleting/mutating viral genes that are necessary for pathogenicity (γ34.5, UL56, UL39/ICP6), for replication in normal post-mitotic cells (UL39/ICP6, UL23/TK, UL2/UNG), or for inhibiting antiviral immune responses (γ34.5, Us3, ICP27, ICP47) and/or apoptosis (Us3, gJ, gG, ICP6, ICP22, LAT) [10,31,96,98]. These alterations enable oHSV to target dysregulated/defective cell physiology that is a common feature of most cancer cells, such as cell-cycle control, cell proliferation, apoptosis, and innate immunity [96]. oHSV can also be receptor-targeted to cancer-specific cell-surface molecules by deleting/mutating viral glycoprotein genes (e.g., gC, gD, and gB) and exchanging their binding domains with single-chain antibodies or ligands to rewire tropism [96]. Finally, oHSV can be transcriptionally targeted by driving the expression of IE ICP4 or L γ34.5 genes with cancer cell-specific transcriptional regulatory sequences (e.g., CAN-3110) or by repressing transcription using normal cell microRNA target sequences [96].

HSV can be a lethal pathogen in humans, and it is therefore essential to err on the side of safety in designing these vectors. Given its large genome size, oHSV can be 'armed' with therapeutic transgenes or sequences for gene therapy. The only oncolytic virus approved in the USA is talimogene laherparepvec (T-Vec, Imlygic®), a GM-CSF 'armed' oHSV, for the treatment of advanced melanoma [10]. G47Δ (Delytact®) was recently approved in Japan for the treatment of GBM [9]. There have been an additional 22 different oHSVs in clinical trials, currently or previously, for a range of solid tumors: breast, colorectal, gallbladder, GBM, head and neck, liver, melanoma, neuroblastoma, prostate, soft tissue sarcoma, pancreatic, and metastatic (clinicaltrials.gov) [58,90]. oHSV treatment induces immune and inflammatory responses in the tumor microenvironment (TME), which can be both beneficial and detrimental to therapy [11,98]. Induction of an immunologically 'hot' TME, so-called *in situ* vaccination [98], is important for clinical success [9,59,99,100].

Box 3. Non-replicative gene therapy vectors.

There are two classes of non-replicative defective HSV1 vectors, (i) amplicon and (ii) recombinant [32]. Defective amplicon vectors are derived from plasmids containing no HSV genes and only *cis*-acting sequences for DNA replication and packaging (~2 kb), as well as therapeutic genes or sequences [101]. With helper HSV, the amplicon plasmid is amplified and packaged as an ~150 kb genome into infectious viral particles [78]. First-generation amplicon vectors contained contaminating helper virus which could be immunogenic [75]. The development of systems that generate helper-free amplicons resulted in no or only faint inflammation [76]. Non-replicative recombinant HSV-1 vectors are deficient in at least one essential IE function and are produced in complementing cells expressing the missing function(s). They also lack some non-essential genes to create space for the insertion of exogenous sequences such as transgenes. First-generation non-replicative HSV-1 vectors could not multiply because of the absence of IE ICP4. However, they displayed cytotoxicity due to the expression of other IE functions, mainly ICP0 and ICP27 [102]. These early studies introduced the notion that non-replicative HSV-1 vectors were toxic and immunogenic, an outdated view that has persisted. Further engineering resulted in vectors deleted or functionally deficient in the expression of all the IE genes [79,80]. They are fully non-toxic in infected cells but preserve robust and durable transgene expression when they are driven by adequate regulatory sequences and are protected from epigenetic silencing [79,80].

types and their interactions with host defense mechanisms are diametrically opposite. oHSVs should kill infected cancer cells in an immunogenic fashion and be highly inflammatory, whereas non-replicative HSV-1 vectors should not alter infected cells and be non-immunogenic (Figure 1). Both vector types must be safe and non-pathogenic in patients.

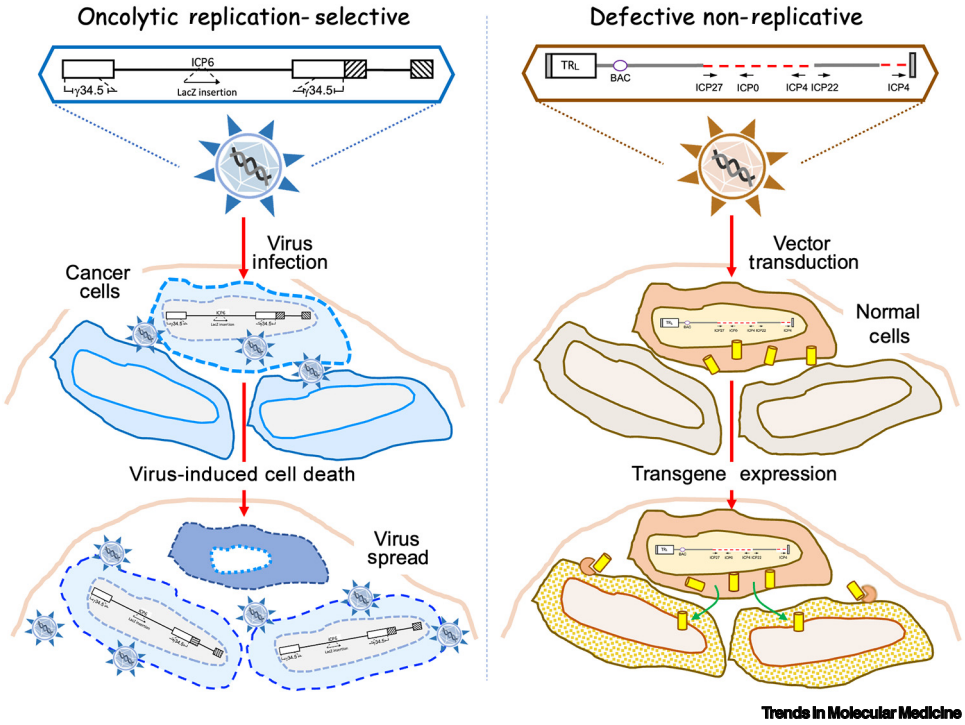

Figure 1. Herpes simplex virus type 1 (HSV-1) vector types. (Left) Replication-selective oncolytic HSV (oHSV) for cancer therapy. The virus can replicate in and kill target cancer cells, generating more oHSV that can spread in the tumor, thus repeating the cycle. (Right) Non-replicative HSV vectors for gene therapy. Vector transduction/infection of target normal cells is minimally intrusive except for the consequences of transgene expression. Transgene products, but not the virus, can spread to other cells, act on receptors or be taken up by cells, or function in the cells where they are expressed, depending on the product. Both vector types are structurally identical and packaged in the same virus particles unless genetically altered, and only differ in their genome. Abbreviations: BAC, bacterial artificial chromosome; ICP, infected cell protein; TR, terminal repeat.

Oncolytic HSV-1

oHSV preclinical safety studies

US FDA investigational new drug (IND) approval to initiate a clinical trial requires in-depth safety studies in appropriate preclinical animal models that are sensitive to HSV pathogenesis, such as BALB/c or A/J mice, immunodeficient mice, and sometimes *Aotus nancymae* New World primates [33,34]. However, pathogenicity can vary greatly between different inbred mouse strains and with different 'wild-type' HSV-1 strains [35]. Typically oHSV is administered via routes related to the therapeutic target; for example (i) intracerebral (IC) for brain tumors or central nervous system (CNS) disorders, (ii) intravenous (IV) for systemic delivery to metastatic disease, (iii) intracerebroventricular (ICV) for leptomeningeal or disseminated CNS disease, (iv) intraperitoneal (IP) for ascites and abdominal cavity tumors, and (v) intrahepatic artery delivery for metastatic tumors and liver disease [36]. HSV-sensitive models and oHSV dose escalation should reveal potential toxicity that can be avoided or managed. Toxicity is often measured by determining the number of animals that fail to survive oHSV injection, morbidity, and histopathology of tissue from challenged animals [37].

Intracerebral injection. The most common route of oHSV administration is intratumoral, and treatment of glioblastoma (GBM) and other brain tumors therefore involves IC injection [38,39]. The brain is the most sensitive organ to HSV pathogenicity, often leading to death or severe sequelae [3], and is therefore a common target for identifying toxicity. All oHSVs in clinical trials for GBM have a deletion (Δ) of the γ34.5 gene, the major contributor to viral neurovirulence [40]. oHSV G207 (Figure 2A; γ34.5Δ, **ICP6**-negative, LacZ$^+$ [41]), was the first oHSV developed for GBM treatment and to enter clinical trials in the USA [42]. IC injection of G207 (10^7 **plaque-forming units, pfu**) produced no apparent symptoms or evidence of disease, whereas 50% of mice receiving 10 000-fold less wild-type HSV-1 succumbed within 1 week [41,43]. G207 superinfection of mice surviving HSV-1 at the same location caused no disease or evidence of HSV-1 reactivation [43]. G47Δ (Figure 2A; γ34.5Δ, ICP6-negative, ICP47Δ, LacZ$^+$; Teserpaturev/Delytact®), a third-generation oHSV derived from G207 by deletion of ICP47, which restores MHC I presentation and increases virus replication in tumor cells, was as safe as G207 in mice [44]. Chimeric oHSV C134 (Figure 2A) that expresses **human cytomegalovirus (HCMV) IRS1** to overcome γ34.5Δ-mediated decreases in virus replication, had a **lethal dose 50 (LD50)** of >10^7 pfu after IC injection, showing that HCMV IRS1 does not increase toxicity [33].

Other routes of administration. ICV injection of G207 (10^7 pfu) resulted in no disease symptoms in any mice compared to 50% morbidity with 1000-fold less HSV-1 [43]. In a separate study of ICV G207 (10^7 pfu), three of 10 mice needed to be euthanized due to rapid weight loss, which could be blocked by low-dose ICV G207 (10^4 pfu) or poly I:C [45]. This is a rationale for administering an initial low or priming dose of oHSV followed by high-dose injections, as with talimogene laherparepvec (T-Vec; Figure 2A) in patients [46,47]. Concerns about ventricular toxicity in mice led to the exclusion of patients with GBM tumors <1 cm from the ventricles. Other routes of G207 delivery that were found to be non-toxic include IV, intrahepatic artery [48], and intraprostatic [49]. IV delivery is the preference of oncologists and the pharmaceutical industry, but is not very efficient for oHSV because of neutralizing antibodies and innate factors [48]. Oral or intra-esophageal administration of G47Δ produced only a transient small decrease in toxicity scores [50], and intra-sciatic nerve injection produced no neuronal ultrastructural abnormalities [51]. *In vitro*, G207 infection of human hematopoietic stem cells had no effect on CD34$^+$ stem cells [52]. Repeated doses of T-Vec were well tolerated in BALB/c mice, rats after intrahepatic artery injection, and dogs [53]. However, injection of subcutaneous tumors in nude (T cell-deficient) and severe combined immunodeficiency (SCID; B and T cell-deficient) mice resulted in lethal systemic infections in 20% and 100% of mice, respectively [53], indicating a role for T and B cells in limiting toxicity.

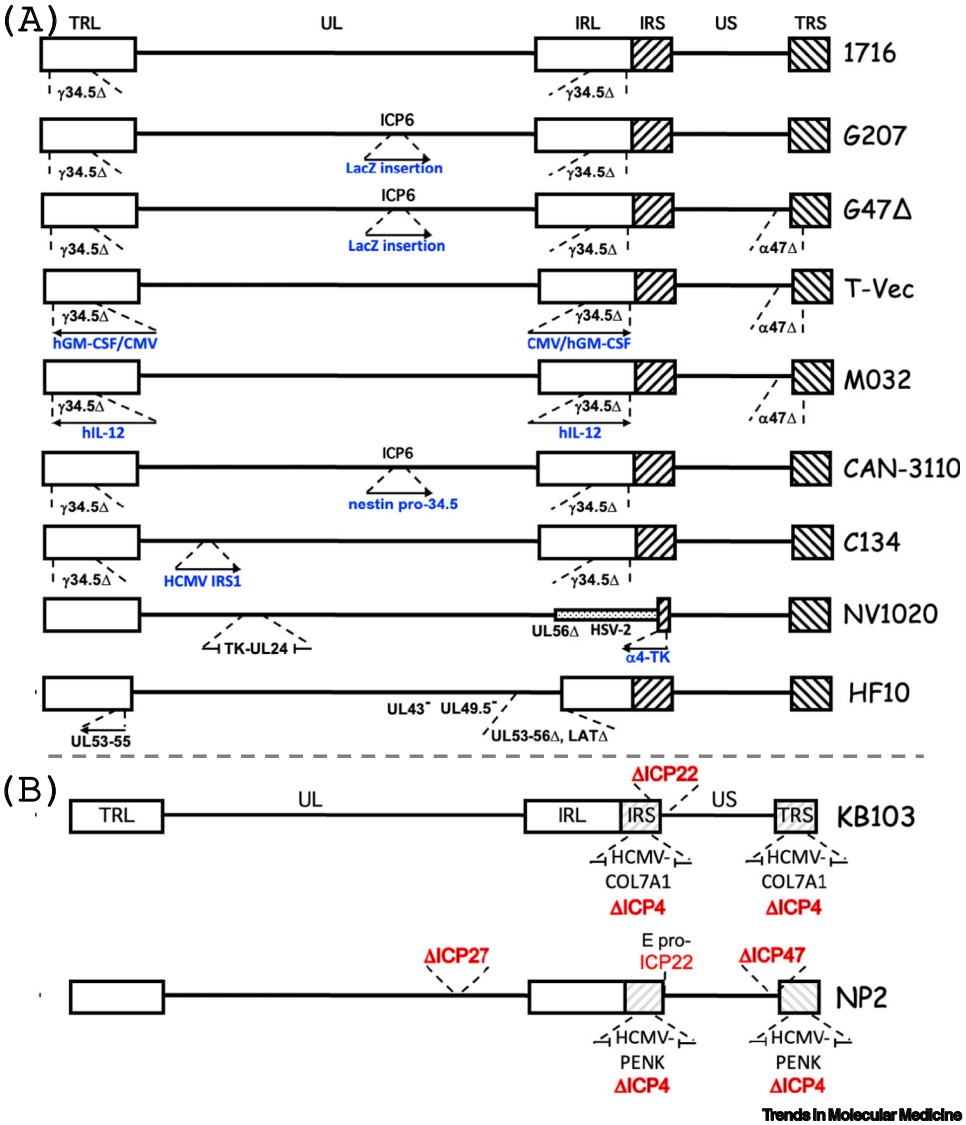

Figure 2. Genetic structure of oncolytic herpes simplex viruses (oHSVs) in clinical trials, completed or current, and non-replicative herpes simplex virus type 1 (HSV-1) vectors NP2 and KB103 (B-VEC). The HSV genome consists of unique long (UL) and unique short (US) regions bracketed by terminal repeat long (TRL) and internal repeat long (IRL), and internal repeat short (IRS) and terminal repeat short (TRS). (A) For oHSV, transgene inserts are in blue font, and deletions are indicated by dashed lines and virus genes Δ. oHSV names are indicated on the right and genomic alterations are indicated: 1716 [60], G207 [41], G47Δ [44], T-Vec [46], M032 [57], CAN-3110 [65], C134 [103], NV1020 [70], and HF10 [72]. (B) For recombinant HSV vectors, immediate-early (IE) genes are indicated in red, deletions by Δ, and their location is indicated on the genome. COL7A1 and PENK driven by the human cytomegalovirus (HCMV) IE promoter are inserted into the deleted ICP4 region. IE ICP22 is downregulated by expression from an early (E) promoter.

Non-human primates. The New World owl monkey (*Aotus nancymae*) is exquisitely sensitive to HSV infection, and the clinical symptoms and histopathology are similar to those in human neonates [54]. After IC HSV-1 (10^3 pfu), encephalitis with classic histopathologic features rapidly develops [55]. By contrast, IC injection of G207 (10^9 pfu) resulted in no AEs except a seizure that resolved in a few days, a million-fold difference in neuropathogenicity [55]. All monkeys receiving

IC G207 (3×10^7 pfu) developed anti-HSV serum antibodies, and no virus was shed [56]. This is similar to what has been seen in patients [39,46]. After intraprostatic injection of G207 (10^7 pfu), none of the monkeys displayed any disease symptoms, virus shedding, virus spread to other organs, or significant histological changes [49]. M032 (Figure 2A) was evaluated in *Aotus* because human IL-12 is not active in mice and was found to be safe except for one high-dose animal [57].

oHSV clinical safety studies

Twenty-five different oHSVs have progressed to clinical trials for a range of malignancies, 16 expressing therapeutic transgenes, and have provided important safety data from patients. Many of the clinical trials involve combinations with other therapies which can contribute to toxicity and/or mask oHSV effects [58]. We focus here on a representative oHSV cohort with different genetic alterations and published clinical results. Although safety is a key consideration in early Phase 1 trials, the overall goal is to demonstrate antitumor efficacy at a safe dose. Importantly, this has been accomplished with two oHSVs that have been approved for clinical use (T-Vec and G47Δ) [9,47].

G207. Virus (1×10^6 to 3×10^9 pfu) stereotactically injected into GBM tumors was found to be safe and potentially efficacious [42] (NCT00028158; https://clinicaltrials.gov/). Two of four resections were positive for G207 DNA, whereas in five autopsies there was no viral histopathology and HSV was not detected [42]. G207 shifted tumor-associated immune cell subtypes, and post-treatment expression of CXCL10 and IDO1 correlated with survival [59]. A recent clinical trial that combined G207 with 5 Gy radiation in pediatric high-grade gliomas resulted in a median overall survival (mOS) of 12.2 versus 5.6 months for historical controls [38]. There were no serious AEs attributable to G207 or virus shedding, and seroconversion only occurred in three of five patients receiving a high dose, indicating dose-dependency [38].

HSV1716. HSV1716 (Figure 2A; Sephrevir®) was the first oHSV in clinical trials in Europe [60]. Because of animal testing regulations in the UK regarding non-human primates, much lower doses ($\leq 10^5$ pfu) were inoculated. In three GBM trials, no clinical toxicity was attributable to the virus [61]. HSV1716 has been evaluated in several extracranial tumors, including in pediatric patients [62]. No dose-limiting toxicities were seen after IV administration in nine young seronegative patients, whereas all patients with data seroconverted, and four had HSV-1 DNA in their blood on day 4, possibly representing virus replication [63].

M032. MO32 (Figure 2A) was evaluated in a canine clinical trial in 21 pet dogs with glioma because human IL-12 is active in dogs. There were no AEs attributable to M032, although modulation of the tumor microenvironment (TME) and some clinical benefits were observed [64].

G47Δ. In a pivotal G47Δ Phase 2 clinical trial in residual or recurrent GBM, 19 patients were treated with up to six intratumoral injections (10^9 pfu each) over ~5 months[i] [9]. All patients experienced virus-related symptoms, mostly low-grade AEs (fever, vomiting, nausea, and lymphocyte/white blood cell/neutrophil count decrease), and transient grade 3 or lymphocyte decreases occurred in five patients [9]. There was a significant increase in the 1 year survival primary endpoint – 84.2% in treated patients versus 15% in historical controls [9] – leading to G47Δ approval for recurrent GBM in Japan [9]. There was a large increase in tumor-infiltrating $CD8^+$ and $CD4^+$ T cells, but not T regulatory cells, that persisted for months after treatment [9]. In the earlier Phase 1 (dose-escalation)/2 clinical trial using two intratumoral injections of G47Δ, 1 year survival was 38.5%, possibly due to fewer injections. All seronegative patients seroconverted by 1 week after treatment [39]. A similar AE profile was seen, except for a coincidental increased frequency of seizures in three of three patients in the Phase 1 high-dose cohort and none in seven same-

dose patients in Phase 2, and small single intratumoral hemorrhages in three of 13 patients, likely due to biopsy [39]. It is difficult to know whether seizures were due to the virus or physical trauma from multiple injections or to tumor progression.

CAN-3110. CAN-3110 (Figure 2A; rQNestin34.5v2 [65]). In a dose-escalation (1×10^6 to 1×10^{10} pfu) Phase 1 trial of CAN-3110 in recurrent GBM/high-grade glioma (HGG) patients (NCT03152318), HSV-1 but not HSV-2 seropositivity significantly correlated with better survival (mOS = 14.2 versus 7.8 months), which may be due to enhanced antitumor and antiviral immunity, whereas virus persistence correlated with seronegativity [66]. There were no obvious dose effects on survival and no dose-limiting toxicities, although five serious AEs, including two prolonged seizures, were possibly related to the virus [66].

Talimogene laherparepvec (T-Vec). T-Vec (Figure 2A; Imlygic®) is the only oncolytic virus so far approved in the USA. In the pivotal Phase 3 clinical trial (OPTiM; NCT00769704), advanced melanoma patients were randomized to intralesional T-Vec or subcutaneous GM-CSF [67]. Owing to low-grade 'flu-like' symptoms in seronegative patients in the Phase 1 trial [46], the first dose was low (10^6 pfu/ml, depending on lesion size up to 4 ml in total) to seroconvert patients and was followed by 10^8 pfu/ml once every 2 weeks [67]. The durable response rate (DRR) was 19.0% versus 1.4% for GM-CSF, and mOS was 23.3 versus 18.9 months [67], leading to FDA approval in 2015 for advanced melanoma and subsequently by the European Medicines Agency [47]. The main AEs, highest in the first cycle, were fatigue (50%), chills (49%), pyrexia (43%), nausea (36%), 'flu-like' illness (31%), and injection site pain (28%), of which <2% were grade 3–4 [67]. Biodistribution and shedding following treatment were minimal, and T-Vec DNA was only detected in 11% of swabs of herpetic lesions [68]. T-Vec is currently in 16 active clinical trials for a variety of cancers, many with drug combinations (clinicaltrials.gov). Overall, no dose-limiting toxicities have been identified so far [47]. The safety profile in 'real-world' use is comparable to the OPTiM trial [69].

NV1020. NV1020 (Figure 2A) is derived from R7020, an HSV-1/2 intertypic recombinant developed as an HSV-2 vaccine [70]. NV1020 was administered via hepatic artery followed by conventional chemotherapy in a Phase 1/2 dose-escalation clinical trial for metastatic colorectal carcinoma to the liver [71] (NCT00149396). A transient febrile reaction was observed after each virus infusion, but there were no virus-related grade 3/4 toxicities and no shedding [71].

HF10. HF10 (Figure 2A; canerpaturev/C-REV/TBI-1401) is a spontaneously arising mutant oHSV that has been used in clinical trials for refractory solid tumors [72]. In a Phase 1/2 trial in patients with superficial tumors or melanoma (NCT02428036), it caused low levels of HF10-related AEs, mainly chills, fatigue, and pyrexia.

In summary, the clinical trial results have elucidated important safety features of oHSV immunovirotherapy: (i) a maximum tolerated dose has not been reached in any of the trials; (ii) AEs, mostly not serious, could be ascribed to oHSV, and the majority were the expected 'flu-like' symptoms [46,67,71], but seizures were observed in some GBM patients that could have been due to oHSV or tumor progression; (iii) there is limited if any shedding of oHSV; (iv) even in the brain, seroconversion was common among seronegative patients, indicative of an immune response to oHSV; (v) oHSV is probably replicating in the tumor but not in normal tissue; and (vi) the immune responses arising from oHSV infection do not seem to be associated with toxicity. However, the inflammation and immunity induced by oHSV play a large role in the efficacy seen in patients and in mouse models [9,66,73].

Clinician's corner

HSV-1, a neurotropic human pathogen, is a large enveloped virus with a 152 kb double-stranded DNA genome containing ~82 genes. Antiviral drugs are available, but a vaccine is not.

HSV-1 has evolved a complex and balanced interaction with humans through numerous strategies to evade host defenses. After lytic infection of epithelial cells, the virus typically enters a latent state in sensory neurons from which it can reactivate, even in the face of adaptive immune responses which limit virus pathology but do not clear the infection. However, the virus can cause significant and lethal disease, especially in the central nervous system.

HSV-1 vectors, both attenuated oncolytic and non-replicative gene therapy vectors, have so far been found to be safe and effective in clinical trials. Both oncolytic and non-replicative HSV vectors have been approved in the USA: T-Vec for the treatment of advanced melanoma and B-VEC for dystrophic epidermolysis bullosa, respectively.

Non-replicative recombinant HSV-1 vectors can deliver long and multiple transgenes (~30 kb), including genes that cannot be delivered by most other vectors such as the cDNAs of dystrophin, von Willebrand factor, or type 1 neurofibromatosis (NF1), as well as genes involved in ophthalmic diseases such as Stargardt disease and Usher syndrome. Defective amplicon vectors can carry up to 150 kb of exogenous sequence or multiples of shorter sequences, and non-replicative HSV-1 vectors are emerging as appealing and powerful vector systems.

Non-replicative HSV vectors used in gene therapy elicit no or a very low level of virus immunity that is too weak to impact on therapeutic outcomes. They can be re-administered several times without inducing rejection of the virus or of the infected cells.

For oncolytic HSV, where immune responses are larger and a key component of efficacy, the induction of antiviral and antitumor immunity does not seem to have any serious adverse safety effects, but existing HSV seropositivity may have a

positive impact on efficacy. The adverse events profile in patients is typical of natural virus infections.

Non-replicative gene therapy vectors

There are two general classes of non-replicative defective HSV: recombinant (virus genome lacking essential genes) and amplicon (helper virus-dependent plasmid-based) [32] (Box 3). We will describe the immune responses elicited by these vectors and then the main features of their clinical trials. The immune responses elicited by non-replicative vectors, that are unable to spread and induce disease, and express no or only a few viral proteins, are expected to be mild and mostly limited to non-specific intrinsic and innate cellular responses. However, this remains understudied and has been examined mostly in rodent brains [74]. A first-generation HSV-1 amplicon vector expressing LacZ that was produced using an IE **ICP4**-deleted (Δ) mutant helper HSV was very inflammatory after IC injection, leading to activated microglial infiltration followed by activated lymphocytes and macrophages which persisted [75]. A delayed inflammatory response was also seen at secondary sites projecting to the injection site [75]. This was probably due to helper virus contamination of amplicon stocks [76] leading to toxicity arising from IE proteins other than ICP4. IC injection of recombinant ICP4Δ HSV-1 vector (similar to the helper virus above) activated immune responses that were dramatically lower than with a replicating vector [77]. Modifications to the amplicon plasmid (bacterial DNA deletion and insulator-like insertions) and diminished helper virus greatly improved safety and transgene expression [78]. Inactivation of all IE genes fully eliminated recombinant HSV vector cytotoxicity in cultured cells [79] as well as neurotoxicity and lymphocyte infiltration in the brain [80].

HSV-1 seropositivity did not eliminate inoculated virus or infected cells, induce significant immune state modifications, or significantly affect transgene expression [81–83], likely due to the disappearance of incoming viral structural proteins and the lack of viral gene expression. The real concerns are immune reactions against the therapeutic transgene, an issue that is common to all gene therapy vectors and which depends on therapeutic protein immunogenicity, particularly for proteins not normally expressed by the host.

Gene therapy for cancer-related pain

A first clinical trial exploited the ability of HSV-1 vectors to express transgenes after establishing a latent infection in the dorsal root ganglia (DRG). A dose-escalation Phase 1 clinical trial of NP2 (NCT00804076), a non-replicative HSV-1 recombinant vector expressing human preproenkephalin ((PENK) Figure 2B), was conducted in terminal cancer patients with intractable focal pain [81]. NP2 was injected into the dermatomes, leading to virus being taken up by nerve terminals and transported to the DRG, where the vector institutes a persistent, quasi-latent state with transgene expression. No treatment-related serious AEs were reported and no subject seroconverted [81]. Pain relief was reported for the middle and high doses [81]. Because NP2 establishes a silent latent infection in the DRG, no expression of toxic functions is expected to take place. This clinical trial showed that intradermal delivery of NP2 is safe and potentially efficacious, the primary endpoints. A Phase 2 clinical trial (NCT01291901) was unfortunately discontinued for financial reasons.

Gene therapy for severe skin diseases

B-VEC (beremagene geperpavec, Vyjuvek™; KB103) (Figure 2B) is a non-replicative HSV-1 vector expressing the *COL7A1* (~9 kb) gene that is used to treat recessive dystrophic epidermolysis bullosa (RDEB), a shattering skin disease resulting from collagen VII (C7) mutations that impair anchoring fibrils [82,84]. Repeated doses of B-VEC were topically administered to freshly renewed skin to treat the symptoms but not cure the disease. Unlike the NP2 study, this trial exploits the ability of B-VEC to strongly express transgenes in the superficial layers of the skin and be re-administered as many times as required. Latency was not tested and is irrelevant, as is the fact that B-VEC can express some toxic functions such as ICP0 and ICP27 because skin

cells are constantly being renewed. B-VEC expresses IE ICP47, which inhibits antigen presentation to reduce immune recognition [85], thus facilitating multiple administrations [82].

A randomized, placebo-controlled, Phase 1/2 clinical trial (NCT03536143), in which matched wounds from RDEB patients received topical B-VEC or placebo repeatedly over 12 weeks, met primary and secondary objectives [82]. No grade 2 or above B-VEC-related AEs, vector shedding, or skin immune reactions were noted [82]. This was the first non-replicative HSV clinical trial targeting non-neuronal cells. A Phase 3, double-blind, intra-patient randomized, placebo-controlled clinical trial of weekly applications of B-Vec gel in RDEB patients (NCT04491604) was completed with similar results for safety and lack of significant immune responses [84]. The primary endpoint was complete wound healing at 6 months, and this was achieved in 67% of wounds and was significantly better than placebo (22%). Six of eight seronegative patients seroconverted, and 13 of 18 developed antibodies to C7 without significant immunologic reaction, and there was no association between HSV-1 serostatus or C7 seroconversion [84]. Based on this, B-VEC was the first topical gene therapy approved in the USA, in 2023 [86]. The ease of topical application has advantages over invasive procedures, but more data will be necessary to confirm long-term efficacy [87].

Following a similar approach, KB105, a non-replicative HSV-1 vector encoding human transglutaminase I (TGM1), was developed to treat autosomal recessive congenital ichthyosis (ARCI) [83]. Preclinical studies demonstrated that repeated topical administration induced TGM1 protein in the target epidermal layer only at the dose site, without fibrosis, necrosis, or acute inflammation [83].

To summarize, these clinical trials and preclinical studies clearly indicate the absence of severe AEs and confirm the safety of the vectors, although with varying levels of efficacy. Importantly, in no case did vector administration or transgene expression result in immune reactions that might prevent treatment efficacy.

Concluding remarks

The major conclusion stemming from these studies is that both non-replicative and oncolytic HSV-1 vectors are safe and potentially efficacious. HSV-1 vectors possess numerous salient features that make them very appealing for gene therapy (Table 1). The evidence from preclinical and clinical studies indicates that immune responses against HSV-1 vectors, either oncolytic or non-replicative, do not constitute a major impediment to efficacy or safety, and multiple dosing is likely better than a higher single dose [8,9,39], even in HSV-seropositive patients. In the case of non-replicative HSV vectors, they elicit no or very low levels of immunity that are too weak to impact on therapeutic outcomes.

What is next? Studies with non-replicative HSV-1 vectors demonstrated that they can be used for stable transgene expression from latently infected DRG neurons or for strong but transient expression in non-neuronal cells in the skin. These vectors have not yet been used in more mainstream gene therapy applications in non-neuronal cells, such as in diabetes or muscular dystrophies. The next challenge is to demonstrate that these vectors can produce safe and stable transgene expression in non-neuronal cells by establishing a latent-like infection. This will require both an absence of toxic functions and prevention of long-term epigenetic silencing of the vector genome (see Outstanding questions). Although this goal seems ambitious, the results suggest that we are not far from reaching it [79,80,88]. Obtaining such results in preclinical models of non-neurologic or CNS disease would represent important progress toward uncovering the therapeutic potential of non-replicative HSV-1 vectors [89].

Outstanding questions

How do non-replicative HSV-1 vectors perform in tissues other than neurons or renewable skin cells which are naturally infected by HSV-1 and for which data are available?

What happens in other tissues (liver, pancreas, muscle, kidney, etc.) regarding epigenetic silencing, transgene expression, and immune responses, for example?

How can we exploit the large size of the HSV-1 genome to deliver very long transgenes and/or regulatory sequences that provide physiological control?

How can we regulate the level and timing of transgene expression from HSV vectors?

How can we overcome amplicon vector manufacturing difficulties to enable the delivery of sequences >100 kb in length?

How can we improve the delivery/targeting of HSV vectors, especially systemically, whether they are oncolytic for tumors or non-replicative for specific cell types or organs?

Why has oHSV been so 'safe' in patients with cancer? Is it related to the limited and localized infections typically seen with HSV-1, or are we making oHSVs too attenuated for optimal efficacy and safety?

What aspects of oHSV biology have the greatest impact on antitumor efficacy? What is the balance between oncolytic/cytotoxic activity, inflammation, and innate and adaptive immune responses?

How representative and/or prognostic of the targeted human disease are the preclinical models used to evaluate vector activity and safety?

For oHSV, where the immune responses are larger and a key component of efficacy, immunity does not seem to have a serious negative impact on safety and possibly has a positive impact on efficacy. In contrast to early safety concerns about using HSV as a vector, the overall safety and tolerability in patients are much better than expected. The spectrum of AEs attributable to the virus has been relatively modest, as might be predicted for a viral infection, and less than those seen with most cancer therapies [90]. A maximum tolerated dose of oHSV has not been reported in any of the clinical trials so far. Whether HSV vectors exhibit a typical dose–response curve or whether a maximum tolerated dose is more efficacious than a lower dose remains unclear. For HSV-1, a human pathogen, the ability to genetically eliminate pathogenicity, owing to our understanding of virus–host interactions, is an important advance that has enabled the use of HSV as a successful and safe viral vector for gene and cancer therapy [58].

Acknowledgments

S.D.R. was supported in part by a grant from the National Institutes of Health (R01 CA160762) and the Thomas A. Pappas chair in Neurosciences.

Declaration of interests

A.L.E. is cofounder, chief scientific officer, and shareholder of EG 427 SAS. He is a coinventor on patents related to the use of replication-incompetent HSV-based vectors owned by the University of Versailles Saint Quentin (France) and EG 427. S.D.R. is a coinventor on patents relating to oncolytic HSVs that are owned and managed by Georgetown University and Massachusetts General Hospital, and which have received royalties from Amgen and ActiVec Inc. He is on the Scientific Advisory Board of EG 427 SAS, has received honoraria and equity, and has acted as a consultant and received honoraria from Replimune and Cellinta.

Resources

[i]https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000003240

References

1. James, C. *et al.* (2020) Herpes simplex virus: global infection prevalence and incidence estimates, 2016. *Bull. World Health Organ.* 98, 315–329
2. Knipe, D. *et al.* (2021) Herpes simplex viruses: mechanisms of lytic and latent infection. In *Fields Virology* (7th edn) (Howley, P.M. *et al.*, eds), pp. 235–296, Wolters Kluwer
3. Whitley, R. and Baines, J. (2018) Clinical management of herpes simplex virus infections: past, present, and future. *F1000Res* 7, 1726
4. Zhu, H. and Zheng, C. (2020) The race between host antiviral innate immunity and the immune evasion strategies of herpes simplex virus 1. *Microbiol. Mol. Biol. Rev.* 84, e00099
5. Pinninti, S.G. and Kimberlin, D.W. (2018) Neonatal herpes simplex virus infections. *Semin. Perinatol.* 42, 168–175
6. Danastas, K. *et al.* (2020) Herpes simplex virus type 1 interactions with the interferon system. *Int. J. Mol. Sci.* 21, 5150
7. Goss, J.R. *et al.* (2002) Herpes simplex-mediated gene transfer of nerve growth factor protects against peripheral neuropathy in streptozotocin-induced diabetes in the mouse. *Diabetes* 51, 2227–2232
8. Chahlavi, A. *et al.* (1999) Effect of prior exposure to herpes simplex virus 1 on viral vector-mediated tumor therapy in immunocompetent mice. *Gene Ther.* 6, 1751–1758
9. Todo, T. *et al.* (2022) Intratumoral oncolytic herpes virus G47Δ for residual or recurrent glioblastoma: a phase 2 trial. *Nat. Med.* 1630–1639
10. Bommareddy, P.K. *et al.* (2018) Oncolytic herpes simplex viruses as a paradigm for the treatment of cancer. *Ann. Rev. Cancer Biol.* 2, 155–173
11. Saha, D. *et al.* (2016) Oncolytic herpes simplex virus interactions with the host immune system. *Curr. Opin. Virol.* 21, 26–34
12. Verzosa, A.L. *et al.* (2021) Herpes simplex virus 1 infection of neuronal and non-neuronal cells elicits specific innate immune responses and immune evasion mechanisms. *Front. Immunol.* 12, 644664
13. Alandijany, T. (2019) Host intrinsic and innate intracellular immunity during herpes simplex virus type 1 (HSV-1) infection. *Front. Microbiol.* 10, 2611
14. Rodríguez, M.C. *et al.* (2020) The HSV-1 ubiquitin ligase ICP0: modifying the cellular proteome to promote infection. *Virus Res.* 285, 198015
15. Tognarelli, E.I. *et al.* (2019) Herpes simplex virus evasion of early host antiviral responses. *Front. Cell. Infect. Microbiol.* 9, 127
16. Ma, F. *et al.* (2021) Herpes simplex virus interference with immunity: focus on dendritic cells. *Virulence* 12, 2583–2607
17. Krawczyk, E. *et al.* (2023) HSV replication: triggering and repressing STING functionality. *Viruses* 15, 226
18. Truong, N.R. *et al.* (2019) Mechanisms of immune control of mucosal HSV infection: a guide to rational vaccine design. *Front. Immunol.* 10, 373
19. St Leger, A.J. *et al.* (2021) Local immune control of latent herpes simplex virus type 1 in ganglia of mice and man. *Front. Immunol.* 12, 723809
20. Lee, A.G. *et al.* (2020) T cell response kinetics determines neuroinfection outcomes during murine HSV infection. *JCI Insight* 5, e134258
21. Deshpande, S.P. *et al.* (2000) Pathogenesis of herpes simplex virus-induced ocular immunoinflammatory lesions in B-cell-deficient mice. *J. Virol.* 74, 3517–3524
22. Ford, E.S. *et al.* (2021) B cells, antibody-secreting cells, and virus-specific antibodies respond to herpes simplex virus 2 reactivation in skin. *J. Clin. Invest.* 131, e142088
23. Iijima, N. and Iwasaki, A. (2016) Access of protective antiviral antibody to neuronal tissues requires CD4 T-cell help. *Nature* 533, 552–556
24. Davé, V. *et al.* (2023) Recurrent infection transiently expands human tissue T cells while maintaining long-term homeostasis. *J. Exp. Med.* 220, e20210692

25. Cantin, E. *et al.* (1999) Gamma interferon (IFN-gamma) receptor null-mutant mice are more susceptible to herpes simplex virus type 1 infection than IFN-gamma ligand null-mutant mice. *J. Virol.* 73, 5196–5200
26. Boeren, M. *et al.* (2023) T cell immunity in HSV-1- and VZV-infected neural ganglia. *Trends Microbiol.* 31, 51–61
27. Oldham, M.L. *et al.* (2016) A mechanism of viral immune evasion revealed by cryo-EM analysis of the TAP transporter. *Nature* 529, 537–540
28. Ripa, I. *et al.* (2022) Interplay between autophagy and herpes simplex virus type 1: ICP34.5, one of the main actors. *Int. J. Mol. Sci.* 23, 13643
29. Sloan, D.D. and Jerome, K.R. (2007) Herpes simplex virus remodels T-cell receptor signaling, resulting in p38-dependent selective synthesis of interleukin-10. *J. Virol.* 81, 12504–12514
30. Lubinski, J.M. *et al.* (2002) Herpes simplex virus type 1 evades the effects of antibody and complement in vivo. *J. Virol.* 76, 9232–9241
31. Aldrak, N. *et al.* (2021) Oncolytic herpes simplex virus-based therapies for cancer. *Cells* 10, 1541
32. Artusi, S. *et al.* (2018) Herpes simplex virus vectors for gene transfer to the central nervous system. *Diseases* 6, 74
33. Cassady, K.A. *et al.* (2017) Pre-clinical assessment of C134, a chimeric oncolytic herpes simplex virus, in mice and non-human primates. *Mol. Ther. Oncolytics* 5, 1–10
34. Kollias, C.M. *et al.* (2015) Animal models of herpes simplex virus immunity and pathogenesis. *J. Neurovirol.* 21, 8–23
35. Dix, R.D. *et al.* (1983) Comparative neurovirulence of herpes simplex virus type 1 strains after peripheral or intracerebral inoculation of BALB/c mice. *Infect. Immun.* 40, 103–112
36. Li, L. *et al.* (2020) Delivery and biosafety of oncolytic virotherapy. *Front. Oncol.* 10, 475
37. Chiocca, E.A. *et al.* (2020) Preclinical toxicology of rQNestin34.5v.2: an oncolytic herpes virus with transcriptional regulation of the ICP34.5 neurovirulence gene. *Mol. Ther. Methods Clin. Dev.* 17, 871–893
38. Friedman, G.K. *et al.* (2021) Oncolytic HSV-1 G207 immunovirotherapy for pediatric high-grade gliomas. *N. Engl. J. Med.* 384, 1613–1622
39. Todo, T. *et al.* (2022) A phase I/II study of triple-mutated oncolytic herpes virus G47Δ in patients with progressive glioblastoma. *Nat. Commun.* 13, 4119
40. Kardani, K. *et al.* (2023) Oncolytic herpes simplex viruses for the treatment of glioma and targeting glioblastoma stem-like cells. *Front. Cell. Infect. Microbiol.* 13, 1206111
41. Mineta, T. *et al.* (1995) Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. *Nat. Med.* 1, 938–943
42. Markert, J.M. *et al.* (2000) Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther.* 7, 867–874
43. Sundaresan, P. *et al.* (2000) Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation in mice. *J. Virol.* 74, 3832–3841
44. Todo, T. *et al.* (2001) Oncolytic herpes simplex virus vector with enhanced MHC class I presentation and tumor cell killing. *Proc. Natl. Acad. Sci. U. S. A.* 98, 6396–6401
45. Kang, K.D. *et al.* (2022) Safety and efficacy of intraventricular immunovirotherapy with oncolytic HSV-1 for CNS cancers. *Clin. Cancer Res.* 28, 5419–5430
46. Raman, S.S. *et al.* (2019) Talimogene laherparepvec: review of its mechanism of action and clinical efficacy and safety. *Immunotherapy* 11, 705–723
47. Kaufman, H.L. *et al.* (2022) Talimogene laherparepvec: moving from first-in-class to best-in-class. *Front. Mol. Biosci.* 9, 834841
48. Tang, G. *et al.* (2023) The dilemma of HSV-1 oncolytic virus delivery: the method choice and hurdles. *Int. J. Mol. Sci.* 24, 3681
49. Varghese, S. *et al.* (2001) Preclinical safety evaluation of G207, a replication-competent herpes simplex virus type 1, inoculated intraprostatically in mice and nonhuman primates. *Hum. Gene Ther.* 12, 999–1010
50. Yajima, S. *et al.* (2021) Efficacy and safety of a third-generation oncolytic herpes virus G47Δ in models of human esophageal carcinoma. *Mol. Ther. Oncolytics* 23, 402–411
51. Antoszczyk, S. *et al.* (2014) Treatment of orthotopic malignant peripheral nerve sheath tumors with oncolytic herpes simplex virus. *Neuro-Oncology* 16, 1057–1066
52. Wu, A. *et al.* (2001) Biological purging of breast cancer cells using an attenuated replication-competent herpes simplex virus in human hematopoietic stem cell transplantation. *Cancer Res.* 61, 3009–3015
53. European Medicines Agency (2016) *Imlygic: talimogene laherparepvec (Assessment Report EMA/734400/2015)*, EMA Published online 5 February, 2016. https://www.ema.europa.eu/en/documents/overview/imlygic-epar-summary-public_en.pdf
54. Katzin, D.S. *et al.* (1967) Experimental herpes simplex infection in the Owl Monkey. *Proc. Soc. Exp. Biol. Med.* 125, 391–398
55. Hunter, W.D. *et al.* (1999) Attenuated, replication-competent herpes simplex virus type 1 mutant G207: safety evaluation of intracerebral injection in nonhuman primates. *J. Virol.* 73, 6319–6326
56. Todo, T. *et al.* (2000) Viral shedding and biodistribution of G207, a multimutated, conditionally replicating herpes simplex virus type 1, after intracerebral inoculation in *Aotus*. *Mol. Ther.* 2, 588–595
57. Roth, J.C. *et al.* (2014) Evaluation of the safety and biodistribution of M032, an attenuated herpes simplex virus type 1 expressing hIL-12, after intracerebral administration to aotus nonhuman primates. *Hum. Gene Ther. Clin. Dev.* 25, 16–27
58. Scanlan, H. *et al.* (2022) Herpes simplex virus 1 as an oncolytic viral therapy for refractory cancers. *Front. Oncol.* 12, 940019
59. Miller, K.E. *et al.* (2022) Immune activity and response differences of oncolytic viral therapy in recurrent glioblastoma: gene expression analyses of a phase IB study. *Clin. Cancer Res.* 28, 498–506
60. Rampling, R. *et al.* (2000) Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther.* 7, 859–866
61. Harrow, S. *et al.* (2004) HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. *Gene Ther.* 11, 1648–1658
62. Streby, K.A. *et al.* (2017) Intratumoral Injection of HSV1716, an oncolytic herpes virus, is safe and shows evidence of immune response and viral replication in young cancer patients. *Clin. Cancer Res.* 23, 3566–3574
63. Streby, K.A. *et al.* (2019) First-in-human intravenous Seprehvir in young cancer patients: a phase 1 clinical trial. *Mol. Ther.* 27, 1930–1938
64. Omar, N.B. *et al.* (2021) Safety and interim survival data after intracranial administration of M032, a genetically engineered oncolytic HSV-1 expressing IL-12, in pet dogs with sporadic gliomas. *Neurosurg. Focus.* 50, E5
65. Kambara, H. *et al.* (2005) An oncolytic HSV-1 mutant expressing ICP34.5 under control of a nestin promoter increases survival of animals even when symptomatic from a brain tumor. *Cancer Res.* 65, 2832–2839
66. Ling, A.L. *et al.* (2023) Clinical trial links oncolytic immunoactivation to survival in glioblastoma. *Nature* 623, 157–166
67. Andtbacka, R.H.I. *et al.* (2019) Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III-IV melanoma. *J. Immunother. Cancer* 7, 145
68. Andtbacka, R.H.I. *et al.* (2019) Biodistribution, shedding, and transmissibility of the oncolytic virus talimogene laherparepvec in patients with melanoma. *EBioMedicine* 47, 89–97
69. Ressler, J.M. *et al.* (2021) Real-life use of talimogene laherparepvec (T-VEC) in melanoma patients in centers in Austria, Switzerland and Germany. *J. Immunother. Cancer* 9, e001701
70. Kelly, K.J. *et al.* (2008) Herpes simplex virus NV1020 as a novel and promising therapy for hepatic malignancy. *Expert Opin. Investig. Drugs* 17, 1105–1113
71. Geevarghese, S.K. *et al.* (2010) Phase I/II study of oncolytic herpes simplex virus NV1020 in patients with extensively pretreated refractory colorectal cancer metastatic to the liver. *Hum. Gene Ther.* 21, 1119–1128
72. Eissa, I.R. *et al.* (2017) Genomic signature of the natural oncolytic herpes simplex virus HF10 and its therapeutic role in preclinical and clinical trials. *Front. Oncol.* 7, 149

73. Glorioso, J.C. *et al.* (2021) Oncolytic HSV vectors and anti-tumor immunity. *Curr. Issues Mol. Biol.* 41, 381–468
74. Ryan, D.A. and Federoff, H.J. (2009) Immune responses to herpesviral vectors. *Hum. Gene Ther.* 20, 434–441
75. Wood, M.J. *et al.* (1994) Inflammatory effects of gene transfer into the CNS with defective HSV-1 vectors. *Gene Ther.* 1, 283–291
76. Olschowka, J.A. *et al.* (2003) Helper-free HSV-1 amplicons elicit a markedly less robust innate immune response in the CNS. *Mol. Ther.* 7, 218–227
77. Zeier, Z. *et al.* (2009) A limited innate immune response is induced by a replication-defective herpes simplex virus vector following delivery to the murine central nervous system. *J. Neurovirol.* 15, 411–424
78. Soukupová, M. *et al.* (2021) Improvement of HSV-1 based amplicon vectors for a safe and long-lasting gene therapy in non-replicating cells. *Mol. Ther. Methods Clin. Dev.* 21, 399–412
79. Miyagawa, Y. *et al.* (2015) Herpes simplex viral-vector design for efficient transduction of nonneuronal cells without cytotoxicity. *Proc. Natl. Acad. Sci. U. S. A.* 112, E1632–E1641
80. Verlengia, G. *et al.* (2017) Engineered HSV vector achieves safe long-term transgene expression in the central nervous system. *Sci. Rep.* 7, 1507
81. Fink, D.J. *et al.* (2011) Gene therapy for pain: results of a phase I clinical trial. *Ann. Neurol.* 70, 207–212
82. Gurevich, I. *et al.* (2022) In vivo topical gene therapy for recessive dystrophic epidermolysis bullosa: a phase 1 and 2 trial. *Nat. Med.* 28, 780–788
83. Freedman, J.C. *et al.* (2021) Preclinical evaluation of a modified herpes simplex virus type 1 vector encoding human TGM1 for the treatment of autosomal recessive congenital ichthyosis. *J. Invest. Dermatol.* 141, 874–882
84. Guide, S.V. *et al.* (2022) Trial of beremagene geperpavec (B-VEC) for dystrophic epidermolysis bullosa. *N. Engl. J. Med.* 387, 2211–2219
85. Bauer, D. and Tampe, R. (2002) Herpes viral proteins blocking the transporter associated with antigen processing TAP – from genes to function and structure. *Curr. Top. Microbiol. Immunol.* 269, 87–99
86. Dhillon, S. (2023) Beremagene geperpavec: first approval. *Drugs* 83, 1131–1135
87. De Rosa, L. and De Luca, M. (2022) The joint battle to tackle epidermolysis bullosa through gene therapy. *Trends Mol. Med.* 28, 533–535
88. Han, F. *et al.* (2018) Cellular antisilencing elements Support transgene expression from herpes simplex virus vectors in the absence of immediate early gene expression. *J. Virol.* 92, e00536
89. Epstein, A.L. (2022) HSV-1's contribution as a vector for gene therapy. *Nat. Biotechnol.* 40, 1316
90. Koch, M.S. *et al.* (2020) HSV-1 oncolytic viruses from bench to bedside: an overview of current clinical trials. *Cancers (Basel)* 12, 3514
91. Madavaraju, K. *et al.* (2020) Herpes simplex virus cell entry mechanisms: an update. *Front. Cell. Infect. Microbiol.* 10, 617578
92. Jambunathan, N. *et al.* (2021) Two sides to every story: herpes simplex type-1 viral glycoproteins gB, gD, gH/gL, gK, and cellular receptors function as key players in membrane fusion. *Viruses* 13, 1849
93. Smith, G.A. (2021) Navigating the cytoplasm: delivery of the alphaherpesvirus genome to the nucleus. *Curr. Issues Mol. Biol.* 41, 171–220
94. Cohen, J.I. (2020) Herpesvirus latency. *J. Clin. Invest.* 130, 3361–3369
95. Skouboe, M.K. *et al.* (2023) Inborn errors of immunity predisposing to herpes simplex virus infections of the central nervous system. *Pathogens* 12, 310
96. Peters, C. and Rabkin, S.D. (2015) Designing herpes viruses as oncolytics. *Mol. Ther. Oncolytics* 2, 15010
97. Zhang, S. and Rabkin, S.D. (2021) The discovery and development of oncolytic viruses: are they the future of cancer immunotherapy? *Expert Opin. Drug Discov.* 16, 391–410
98. Jahan, N. *et al.* (2021) In situ cancer vaccination and immunovirotherapy using oncolytic HSV. *Viruses* 13, 1740
99. Ribas, A. *et al.* (2017) Oncolytic virotherapy promotes intratumoral T cell infiltration and improves Anti-PD-1 immunotherapy. *Cell* 170, 1109–1119
100. Ramelyte, E. *et al.* (2021) Oncolytic virotherapy-mediated anti-tumor response: a single-cell perspective. *Cancer Cell* 39, 394–406
101. Fraefel, C. and Epstein, A.L. (2020) Preparation of herpes simplex virus type 1 (HSV-1)-based amplicon vectors. *Methods Mol. Biol.* 2060, 91–109
102. Krisky, D.M. *et al.* (1998) Deletion of multiple immediate-early genes from herpes simplex virus reduces cytotoxicity and permits long-term gene expression in neurons. *Gene Ther.* 5, 1593–1603
103. Shah, A.C. *et al.* (2007) Enhanced antiglioma activity of chimeric HCMV/HSV-1 oncolytic viruses. *Gene Ther.* 14, 1045–1054