REVIEW

Herpes simplex virus 1 (HSV-1) for cancer treatment

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Cancer remains a serious threat to human health, causing over 500 000 deaths each year in US alone, exceeded only by heart diseases. Many new technologies are being developed to fight cancer, among which are gene therapies and oncolytic virotherapies. Herpes simplex virus type 1 (HSV-1) is a neurotropic DNA virus with many favorable properties both as a delivery vector for cancer therapeutic genes and as a backbone for oncolytic viruses. Herpes simplex virus type 1 is highly infectious, so HSV-1 vectors are efficient vehicles for the delivery of exogenous genetic materials to cells. The inherent cytotoxicity of this virus, if harnessed and made to be selective by genetic manipulations, makes this virus a good candidate for developing viral oncolytic approach. Furthermore, its large genome size, ability to infect cells with a high degree of efficiency, and the presence of an inherent replication controlling mechanism, the thymidine kinase gene, add to its potential capabilities. This review briefly summarizes the biology of HSV-1, examines various strategies that have been used to genetically modify the virus, and discusses preclinical as well as clinical results of the HSV-1-derived vectors in cancer treatment.

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Introduction

There are two types of herpes simplex virus, type 1 and type 2 (HSV-1 and HSV-2), both belong to the *Herpesviridae* family, *Alphaherpesvirinae* subfamily. Herpes simplex virus type 2 is a common human pathogenic virus and is associated with sexually transmitted diseases. Herpes simplex virus type 1 is also a human pathogen, but is rarely associated with genital area infection. Acute HSV-1 infection generally involves gingivo stomatitis.¹ Chronic HSV-1 infection has also been described and is characterized by a skin keratitis.¹ Following primary infection, HSV-1 follows a retrograde course along sensory nerves to reach dorsal lumbar sacral ganglia. It may remain in a latent stage until occurrence of decreased immunity, at which point productive virus replication resumes. Subsequently, viral progenies travel along nerve pathways to previously involved sites in the skin or mucus membrane, causing a new round of infection. Small fluid-filled vesicles transiently develop. Duration of infection can be minimized with antiviral therapy such as acyclovir (ACV). Herpes simplex virus is capable of replicating in a wide variety of tissues, including lymphocytes. As a result of its intracellular locus of replication, it is able to escape

anti-HSV antibodies during the replication process. Herpes simplex virus type 1 genomic components controlling pathogenicity have been identified.

Herpes simplex virus type 1 has also been increasingly explored in cancer therapy. In this review, we will first describe the basic biology of HSV-1, followed by a discussion on strategies for HSV-1 vector engineering, and then focus on various applications of HSV-1 vectors in cancer therapies. We will review the most prevalent gene modified HSV-1 vectors in details and discuss properties of HSV-1, which enable its use as a unique gene delivery vehicle and/or oncolytic viral therapy.

An overview of herpes simplex virus type 1 biology

Virion structure

Herpes simplex virus type 1 is an enveloped double-stranded DNA (dsDNA) virus. Mature HSV-1 virion consists of four elements: (1) An electron-opaque core that contains the dsDNA genome wrapped as a toroid or spool. (2) An icosadeltahedral capsid surrounding the core. The capsid is composed of 162 capsomers arranged in a T=16 icosahedral symmetry. The capsid contains channels, which are controlled by tegument proteins, thus controlling the transport of DNA through the channel.² (3) An amorphous tegument surrounding the capsid. The tegument is largely unstructured and contains a matrix of proteins that play many important functions during HSV-1 infection. (4) An outer envelope exhibiting spikes on its surface. The envelope consists of a lipid bilayer with about 13 different viral glycoproteins embedded in it.

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Majority of the proteins of the mature virions are encoded by the viral genome, which consists of 152 kb of linear dsDNA arranged as the L (long) and the S (short) components that are covalently linked. Each component consists of unique sequences bracketed by inverted repeats (see Figure 1). The unique long segment (U_L) is flanked by ab and b'a' repeated sequences, and the unique short segment (U_S) is flanked by ac and c'a' repeated sequences. Homologous recombination between the terminal repeats results in the inversion of the L and S components of HSV-1 genome, yielding four linear isomers at equimolar concentrations (Figure 1). The isomers are designated as P (prototype), IL (inversion of the L component), IS (inversion of the S component), and ISL (inversion of both L and S components). Herpes simplex virus type 1 genome encodes approximately 90 unique transcription units (genes), approximately half of which are essential for viral replication in a permissive tissue culture environment. The rest are dispensable for growth in cells in culture. However, these so-called 'non-essential' genes are most probably not dispensable for replication in animal system. They often encode functions that are involved in virus–host interactions, for example, inducing immune evasion and host cell shut-off.

Cell entry

To initiate infection, the virus first attaches to cell surface receptors. Fusion of the viral envelope with the cell membrane rapidly follows the initial attachment. The de-enveloped tegument-capsid structure is then transported to the nuclear pores, where DNA is released into the nucleus. The initial attachment involves the interaction of viral envelope glycoproteins (such as gC and gB) with the glycosaminoglycan moieties of cell surface heparin sulfate.^{3–5} Subsequently, viral glycoprotein gD binds one of several cell surface receptors (such as nectin-1a and nectin-1b, 2a, 2d, HveA), resulting in commitment to virion–cell fusion.⁶ Many of the receptors are broadly expressed in a wide variety of human cell types and tissues. Thus, HSV-1 has a very wide host range. Fusion of viral envelope with cell membrane occurs after the binding of gD to its cognate cell surface receptor. Three other HSV-1 glycoproteins, gB, gH and gL, have been implicated in the fusion step.^{7–9} The transition from

attached to penetrated virus is very rapid and occurs within minutes.¹⁰ The capsid with associated tegument structures is then transported to the nuclear pore through the microtubular network.¹¹

Replication and progeny production

Transcription, replication and packaging of HSV-1 genome take place in the nuclei of the infected cells. During productive infection, more than 80 HSV-1 genes are expressed in a tightly regulated manner (reviewed in reference Roizman and Knipe¹²). Viral DNA is transcribed by host RNA polymerase II, but various viral factors are involved at all stages of infection to ensure that viral genes are expressed in a coordinately regulated and sequentially ordered manner. Upon entering nuclei, viral genome circularizes and transcription of the five immediate-early (IE) genes, ICP0, ICP4, ICP22, ICP27 and ICP47 commences immediately.¹³ A number of cellular transcription factors and a viral tegument protein that is transported to the nucleus along with the viral genome, VP16, stimulate the transcription of the IE genes. Meanwhile, host gene transcription, RNA splicing and transport, and protein synthesis are inhibited (a phenomenon known as host cell shut-off) to facilitate the transition from cellular to viral gene expression. Most of the IE gene products are involved in transcription regulation and stimulate expression of other HSV genes. Expression of the early (E) viral genes, which primarily encode enzymes involved in nucleotide metabolism and viral DNA replication, occurs before the onset of viral DNA synthesis and requires the presence of the IE gene products. Viral E gene products (including the viral DNA polymerase, single-stranded DNA-binding protein, origin-binding protein, DNA helicase-primase) localize into the nucleus and assemble onto the parental viral DNA, and start viral DNA synthesis in sub-nuclear structures called replication compartments. HSV-1 DNA replication involves origin-dependent initiation.¹⁴ There are three DNA replication origins in the viral genome, binding of these origin sequences by viral origin-binding protein (OBP) separates the DNA strands and initiate viral DNA synthesis. Viral DNA synthesis uses a rolling-circle mechanism, for the most part, producing 'head-to-tail' concatemeric molecules.¹⁵ Once viral DNA replication

Figure 1 Diagram of HSV-1 genome. U_L : unique long sequence. U_S : unique short sequence. a, b, c, a', b' and c' indicate the terminal repeat sequences. Arrows indicated the orientation of U_L and U_S .

has initiated, expression of late (L) genes commences, which mainly produces structural components of the virion.

Mature HSV-1 virions are formed in well-organized steps including capsid assembly, encapsidation of viral DNA in preformed capsids, and virion egress through Golgi apparatus.^{16,17} Initial stages of capsid assembly probably occur in the cytoplasm, where some capsid proteins become associated with one another.¹⁸ These protein complexes are then translocated into the nucleus where final assembly of capsids occurs. After capsid assembly, progeny DNA concatemers are cleaved into unit-length monomers, and the monomers are inserted into the preformed capsids.¹⁹ Two *cis*-acting elements on viral DNA, *pac1* and *pac2*, are required for precise cleavage and packaging of the HSV-1 genome.²⁰ After encapsidation of full-length viral genomic DNA, the nucleocapsids mature by budding through nuclear membrane and processing through Golgi stacks (a process known as egress). The tegument layer and the virion envelope are acquired during this process. The enveloped particles are then released through secretory vesicles. HSV-1 replicates efficiently: in fully permissive tissue culture cells, the entire replication cycle takes about 18–20 h.

Lytic infection vs latency

Herpes simplex virus type 1 is a neurotropic virus. After initial lytic replication in epithelia of the primary lesion, the viral progenies enter sensory neurons whose axon terminals innervate the affected area. The nucleocapsid and tegument are transported retrogradely along axons from the site of entry to the neuronal soma, where viral DNA and VP16 enter the nucleus. At this point, the virus may either initiate lytic replication or enter the latent state.²¹ Lytic replication, as described above, results in neuronal cell death and release of viral progenies. On the other hand, latently infected neuronal cells stay alive, and HSV-1 latency sometimes lasts the lifetime of the host.^{22,23} During latent infection of HSV-1, the viral genome persists as a stable episomal element without detectable expression of IE, E or L gene products. Only a set of non-translated RNA species, known as the latency-associated transcripts (LATs), are synthesized during latency.^{24–27} The LAT gene is located within the inverted repeat sequence that brackets the unique long segment, therefore, there are two copies of LAT gene in each HSV-1 genome. This gene encodes an 8.5 kb transcript that is present at low abundance (minor LAT). Splicing of this transcript produces a series of highly stable introns (major LATs) that accumulate to high levels within the nuclei of latently infected neurons. Expression of LATs is a hallmark of HSV-1 latency, although the LAT genes are not absolutely required for establishment or maintenance of latency.^{28–31} The LATs do not code for any protein and their role in HSV-1 latency remains unclear. However, a number of putative functions have been suggested (reviewed in Kent *et al.*³² and Bloom³³).

In a fraction of neurons harboring latent HSV-1, the virus is periodically reactivated. Cascade expression of the

viral IE, E and L genes resumes, resulting in the production of mature virions. Infectious virus particles are transported to the peripheral nerve terminals by anterograde axonal transport pathway, released, and infect cells at or near the site of initial infection.³⁴ Reactivation is not completely understood, but external stimuli, such as UV light, stress and fever, are known for decades to induce HSV-1 reactivation. Depending on several factors, including the host immune status, the reactivation may be asymptomatic or lead to a recurrent lesion, which may vary considerably in severity from punctuate lesions that are invisible to naked eyes to severe, debilitating lesions in immunocompromised individuals.

Interaction with host defense mechanisms

During infection of humans, HSV-1 may cause many diseases include primary and recurrent epithelial lesions as well as disseminated disease and encephalitis. Complications caused by HSV-1 are of particular concerns in immunocompromised individuals or in newborn children. Like many other viruses, HSV-1 encodes a number of functions that block host defense against infection. These functions include: (1) blocking *de novo* synthesis of cellular proteins that may adversely affect viral replication; (2) bypassing RNA-dependent protein kinase (PKR) pathway; (3) inhibiting apoptosis;³⁵ (4) inhibition of MHC class I peptide presentation on infected cells;^{36,37} and (5) blocking maturation of and antigen presentation by dendritic cells.

Blocking of host protein synthesis, known as host cell shutoff, is mediated by three mechanisms: degradation of host mRNA, selective degradation of cellular proteins, and inhibition of *de novo* host cell transcription and RNA processing. Degradation of host mRNA is mediated by a HSV-1 tegument protein encoded by the *UL41* gene.³⁸ The available evidence suggests that *UL41* protein is either itself a ribonuclease (RNase) or able to activate a cellular nuclease. This protein is abundantly present in mature HSV-1 virions, and is carried into cells during infection, causing RNA degradation before any viral gene expression. Herpes simplex virus type 1 also induces targeted degradation of a subset of cellular proteins, a process that is mediated by several viral proteins including ICP0, ICP22 and the *UL13* protein kinase.^{39,40} In addition, HSV-1 suppresses host gene transcription and RNA splicing during the initial stages of infection. The former is caused by direct modification of RNA polymerase II by viral ICP22 and *UL13* protein kinase,⁴¹ whereas the latter is mediated by ICP27.⁴²

One of the principal host defense mechanisms is mediated by PKR. RNA-dependent protein kinase is induced, via interferon α pathway, by double-stranded RNA (dsRNA) that forms during viral replication. Activated PKR causes the phosphorylation and inactivation of the translation factor eIF-2 α , resulting in a total shutdown of protein synthesis required for viral replication. Many viruses (for example, human adenovirus) have evolved strategies to inhibit PKR activity in order to allow viral replication. Herpes simplex virus type 1,

however, bypasses PKR-mediated host defense in a unique manner. Rather than inhibiting PKR activity, the viral ICP34.5 protein (also known as γ -34.5 or γ_1 -34.5 protein) directly binds cellular protein phosphatase 1 α and redirects it to dephosphorylate eIF-2 α , resurrecting the translational activity of eIF-2 α .^{43–45} Herpes simplex virus type 1 strains that lack ICP34.5 function are severely compromised in their ability to replicate in neurons. Such strains are unable to induce significant neurovirulence even when they were injected in high amounts into the central nervous system (CNS).^{43,46,47}

Interaction between HSV-1 and the host immune system is very complex; many factors may influence the outcome. Both nonspecific (mediated by macrophages and natural killer cells) and specific immune responses (mediated by B and T cells) have been implicated as important host defenses against HSV-1 infections.^{48–53} The relative value of each of these defense mechanisms varies significantly according to the genetic background of the host^{54,55} and the route of viral inoculation.⁵⁶ Herpes simplex virus type 1 has also evolved a number of strategies to interfere with dendritic cell maturation and antigen presentation mediated by dendritic cells (reviewed in Kobelt *et al.*⁵⁷ and Pollara *et al.*⁵⁸). Nevertheless, herpes simplex virus triggers strong cellular and humoral immunity. In general, cell-mediated immune responses are particularly important for viral clearance,^{56,59,60} as demonstrated by immunodepletion studies,^{52,61} adoptive transfer experiments^{53,62–64} and human clinical trials.⁶⁰ It is intriguing that recurrent HSV-1 lesions can occur in the presence of both humoral and cell-mediated immunity, although duration of recurrent lesions is typically shortened compared to the original infection.

Different ways of using herpes simplex virus type 1 for the treatment of human cancers

Broadly speaking, there are two types of HSV-1 vectors, both have been used in cancer treatment. (1) Replication-defective vectors, in which transgene expression cassettes are inserted in a viral genome with one or a few essential viral genes deleted. Such vectors can effectively express transgene products, but are unable to replicate, except in cells that complement the deleted viral functions *in trans*. A special group of replication-defective HSV vectors are amplicons.^{65–68} In this approach, an expression cassette for the therapeutic transgene(s) is placed in a plasmid ('amplicon' plasmid) that contains the viral packaging/cleavage signals and HSV-1 origin of replication. Defective HSV-like particles that contain concatemeric plasmid DNA is produced in eukaryotic cells that supply viral functions *in trans*. (2) Conditionally replicating vectors, in which deletion of some nonessential viral genes results in viruses that preferentially infect, replicate in, and lyse tumor cells. Conditionally replicating vectors can also be modified to carry therapeutic transgenes to augment antitumor effects. This review focuses on the recent progress in using replication-defective and con-

ditionally replicating HSV-1 vectors for the treatment of human cancers.

Replication-defective herpes simplex virus type 1 vector-mediated cancer gene therapy

A number of features make HSV-1 an attractive vector for cancer gene therapy. First, despite its neurotropism, HSV-1 possesses the ability to infect a wide range of host cells. Second, HSV-1 is highly infectious, able to transduce nondividing as well as dividing cells, and express transgene products with excellent efficiency. Third, as much as 30 kb of the HSV genome can be deleted and replaced by transgenes in replication-defective HSV-1 mutants, allowing for simultaneous delivery of multiple transgenes and use of heterologous promoters. Fourth, HSV-1 genome does not integrate into the cellular genome, eliminating the concern of insertional mutagenesis, as is the case for retrovirus and adeno-associated virus (AAV) vectors. Lastly, recombinant HSV-1 can be readily constructed, and purified stocks of virus containing 10¹⁰ infectious particles per milliliter can be prepared routinely without contamination of wild-type virus.

Generation of replication-defective herpes simplex virus type 1 vectors

Generation of replication-defective vectors can be accomplished by disruption of one or several essential IE genes.^{69,70} For example, vectors with ICP4 deletion are unable to replicate in non-complementing cells, and can be safely injected into the rat CNS.⁷⁰ However, since ICP4 negatively regulates other IE genes such as ICP22, ICP27 and ICP0, infection with an ICP4-null mutant results in overexpression of these other genes, whose products are toxic to the host cells.^{70,71} To prevent such toxicity, a series of recombinant HSV-1 vectors have been generated that harbor various combinations of IE gene deletions.^{72–74} A mutant with all five IE genes (ICP0, ICP4, ICP22, ICP27 and ICP47) deleted has been produced, and was shown to be entirely nontoxic to cells.⁷³ Other strategies to block replication and/or spread of HSV-1 include elimination of virion protein VP16^{75–77} and deletion of the gH gene.^{78,79} These strategies, combined with deletion of IE genes, produce nontoxic, nonreplicative HSV-1 mutants that serve as safe delivery vehicles for antineoplastic genes to such cancer types as malignant glioma where tumor cells and normal cells are highly intertwined.

Cancer gene therapy using replication-defective herpes simplex virus type 1 vectors

The neurotropic nature of HSV-1 makes it an attractive vector for treatment of cancers of the CNS, such as malignant gliomas. Malignant glioma is a common, fatal malignancy of the CNS with a median survival of 4–12 months following diagnosis.⁸⁰ An invasive tumor margin of malignant gliomas, along with sensitive local environment, makes it nearly impossible to completely remove

the tumor mass by surgery. Malignant gliomas are generally localized; distant metastasis occurs only under unusual circumstances.^{81,82} This trait enables direct inoculation of the tumor with recombinant vectors. In addition, transient high-level expression of transgenes may be desirable to eradicate tumor cells, obviating the need for long-term expression. These make HSV-1 vectors attractive for the gene therapy of malignant gliomas.

Cancer gene therapy mediated by HSV-1 vectors has largely focused on the delivery of suicide genes.⁸³ The most frequently used suicide gene is the native HSV-1 thymidine kinase (TK) gene, encoded by $UL23$. In normal replication cycle of HSV-1, TK is involved in synthesis of deoxyribonucleotides to facilitate viral DNA replication in cells with suboptimal precursor pools.^{84–86} In relation to suicide gene therapy, TK can convert nontoxic prodrugs such as ganciclovir (GCV) and ACV into cytotoxic metabolites that are incorporated into replicating DNA and cause premature termination to DNA synthesis. Thus, the TK suicide gene therapy is toxic to cells undergoing active DNA replication (such as cancer cells) but not towards neurons and quiescent glia. Earlier studies using replication-defective HSV-1 aimed at TK gene delivery have demonstrated antitumor activity *in vitro* and in animal models carrying human glioma.^{87,88} It should be pointed out that in these experiments, TK gene expression was driven by a HSV-1 IE promoter (such as ICP4 promoter) or an exogenous promoter (such as cytomegalovirus IE promoter), rather than its own promoter. This is necessary because in a replication-defective HSV-1 vector, many of the IE gene products are not available to activate transcription of TK gene, which is an early (E) class gene.

It has been well established that TK/GCV suicide gene therapy generates bystander cytotoxic effect, that is, tumor lysis greatly exceeds the degree of transduction.^{89,90} Several mechanisms may be responsible for the bystander effects, such as necrosis-induced inflammation and disruption of tumor vasculature.⁹¹ However, the most important may be passage of activated GCV (cytotoxic metabolites) from the HSV-TK transduced cell to its neighboring cells through gap junctions. This passage enables lysing of surrounding cancer cells that are not transduced. Gap junctions play a critical role in TK/GCV bystander effect.⁹² Gap junctions are intercellular channels formed by connexin molecules, one of which is connexin-43.^{93,94} Gliomas are often defective in connexin-43 expression and thus have defective gap junctions, limiting the passage of activated GCV. To maximize the bystander effect in such tumors, connexin-43 gene was incorporated into the HSV-1 vector.^{95,96} It was hypothesized that expression of connexin-43 could restore gap junctions and thereby facilitate dissemination of activated GCV through cell membrane. Studies in animal models demonstrated that connexin-43 by itself had an antitumor effect that was comparable with that of HSV-TK/GCV. Importantly, the combination of HSV-TK/GCV and connexin-43 proved to have synergistic antineoplastic effect.^{95,96}

Cancer therapeutic genes other than TK have also been delivered using replication-defective HSV-1 vectors for the treatment of malignant gliomas and other types of cancer. These include p53,⁹⁷ tissue inhibitor of metalloproteinases-2 (TIMP-2),⁹⁸ IL-2,^{99,100} IL-12,¹⁰¹ IFN- γ ,¹⁰² and granulocyte-macrophage colony-stimulating factor (GM-CSF).^{100,103} In general, these approaches have demonstrated promising antitumor potential following intratumoral injection in animal models. Furthermore, replication-defective HSV-1 vectors have been used extensively for simultaneously delivery of multiple therapeutic genes. Examples are co-expressing TK and TNF- α ,^{87,104} TK, connexin-43 and TNF- α ,¹⁰⁵ TK and IL-12,¹⁰⁶ and TK and I κ B α .¹⁰⁷ In these examples, significantly greater inhibition of tumor growth was achieved with vectors effecting multiple therapeutic modals compared to those that effect a single therapeutic modal.

However, more is not always better. A recent study tested a nonreplicating HSV-1 vector carrying two suicide genes, *Escherichia coli* cytosine deaminase (CD) and HSV-1 TK, followed by exposure with their reciprocal prodrugs (5-FC and GCV), in the 9L gliosarcoma model *in vitro* and *in vivo*.¹⁰⁸ This double suicide gene therapy was shown to be inferior to single gene treatments, suggesting that HSV-1 TK and CD are mutually counteractive in the prodrug-dependent killing of glioma cells. Although a conclusive explanation for this observation is not yet available, this result raises the possibility that single suicide gene systems employing HSV-TK or CD may be preferable over combinations of the two. Whether the same conclusion may be applied to suicide gene combination remains to be determined.

Conditionally replicating herpes simplex virus type 1 vectors as oncolytic agents

The earliest report of using viruses to treat cancer was published 100 years ago.¹⁰⁹ However, it is only recently that advances in molecular biology, virology and cancer biology has provided us with the tools necessary to develop novel oncolytic viruses for cancer therapy. The first tumor-selective oncolytic virus to demonstrate antitumor activity in a clinical setting was ONYX-015, a derivative of adenovirus type 5 with a deletion of the E1B-55K gene.^{110–113} Since then, numerous viruses have been explored as tumor-selective replicating vectors, including adenovirus, HSV, vaccinia virus (VV), reovirus, Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), measles virus, poliovirus and West Nile virus (reviewed in Chiocca,¹¹⁴ Kirn,¹¹⁵ Nemunaitis¹¹⁶ and Nemunaitis and Edelman¹¹⁷).

Efficient replication, cell lysis and spread of HSV-1, along with the virus' broad host range, make HSV-1 an attractive candidate for oncolytic viral agents. In permissive cells, the entire replication cycle of HSV-1 is usually completed within 20 h, releasing thousands of progeny virions upon cell lysis. This is in contrast to adenovirus, whose replication cycle typically lasts 48–72 h.¹¹⁸

Furthermore, it has been demonstrated that HSV-1 virions are capable of direct cell-to-cell spreading through cell junctions in addition to spreading through the extracellular space.^{119,120} This feature is extremely useful for an oncolytic virus, as it allows for efficient viral penetration within solid tumors with minimal systemic spread. Many of the limitations found with ONYX-015 in clinical trials, including insufficient antitumor potency as a single agent, inability to efficiently infect tumor metastases following intravenous (i.v.) delivery, insufficient virus spread within solid tumors, are, at least in part, due to slow replication of adenovirus. It is conceivable that these limitations can be relieved by viruses that are more potent in replication/spread. Other advantages of using HSV-1 vectors in oncolytic virotherapy include: (1) HSV-1 rarely produces life-threatening medical illness in immune-competent adults; (2) several anti-HSV-1 drugs (for example, ACV and fampiclovir) have been approved to treat HSV-1 infection.^{121,122} Taking advantage of the natural presence of TK, these drugs inhibit HSV-1 replication and provide a safety mechanism to shut off viral replication should systemic toxicity ensue; and (3) HSV-1 productively infects cells from many laboratory animals.¹² This makes HSV-1 easy to study in animal models, and preclinical results can be more readily translated into clinical trials. This is in contrast to, for example, oncolytic vectors derived from human adenovirus, for which a lack of good animal model has remained a major obstacle to preclinical evaluation.

Strategies for creating replication-conditional herpes simplex virus type 1 vectors

Many strategies have been used to create tumor-selective oncolytic viruses (reviewed in Nemunaitis and Edelman¹¹⁷ and Kirn¹²³). These strategies can be grouped into three general approaches. The first approach is to delete gene functions that are critical for efficient viral replication in normal cells but are dispensable in tumor cells, exemplified by replication-selective adenovirus mutant ONYX-015.^{110,124} Many replication-conditional HSV-1 vectors have included mutations and/or deletions in one or more of the genes encoding thymidine kinase, DNA polymerase, uracil DNA glycosylase, ribonucleotide reductase (RR), and ICP34.5.¹²⁵ The second approach is to limit the expression of a critical viral gene to tumor tissues through the use of tumor- and/or tissue-specific promoters, represented by the adenovirus mutant CV706.^{126,127} The third approach is to alter viral tropism through modification of surface proteins.^{128,129} Thus far, all of these approaches have been successfully used to engineer tumor-specific herpesviruses.

Single gene deletion

Herpes simplex virus type 1 mutants with TK gene deletion were created and tested for oncolytic virotherapy. Intraneoplastic administration of the TK-deleted strains induced tumor regression in several animal tumor models, with severe necrosis of the tumor tissue.^{130–134} However, in tumor models in severe combined immunodeficient (SCID) mice, these mutants were shown to spread into

normal tissues,¹³⁵ indicating a lack of tumor specificity. This, along with concerns that lack of TK would render these mutants insensitive to treatment with the anti-viral drug ACV, has led to the development of many other replication-conditional HSV-1 vectors.

A recombinant HSV-1 with the U_L39 gene deleted was examined (Table 1). The U_L39 gene encodes ICP6, the large subunit of HSV-1 RR. Activity of RR is required for efficient viral DNA replication.^{136,137} The U_L39-deleted virus, hrR3, is significantly attenuated for replication in normal cells but replicates efficiently in malignant cells.^{138–140} This virus has demonstrated selective replication in malignant tissues and has produced substantial antineoplastic effects and survival benefit in animal models of the brain,^{138,141,142} pancreas,¹⁴³ colon¹⁴⁴ and liver cancers.¹⁴⁵ Efficient replication of the viral vector is critical to its antineoplastic activity.^{49,50} The antitumor activity of hrR3 was enhanced by coadministration of cyclophosphamide (CPA).^{49,50} It was speculated that CPA, a strong immunosuppressive agent, may temporarily suppress systemic anti-viral immunity, allowing increased replication of the therapeutic virus and thus an enhanced antitumor effect.⁵⁰ Depletion of complement via cobra venom factor (CVF) treatment also facilitated *in vivo* viral propagation and improved antitumor activity of hrR3.¹⁴⁶

As mentioned above, ICP34.5 enables HSV-1 to replicate in neurons and has been described as a specific neurovirulence factor.^{43,46,47,147} There are two copies of ICP34.5 gene, because this gene is present in the inverted repeat region flanking the unique long segment. Several mutant strains of HSV-1 have been constructed in which both copies of the ICP34.5 gene were deleted (Table 1). These strains were replication-defective in neurons and other slow-growing cells, but replicate efficiently in rapidly dividing, transformed cells.^{148–151} In animal studies, the ICP34.5-deleted strains were shown to be nonvirulent when inoculated into the CNS of SCID mice, but replicated efficiently in xenograft tumors, induced tumor regression and significantly prolonged animal survival in treatment of glioma,^{152–155} mesothelioma,¹⁴⁹ melanoma,^{150,151} ovarian cancer,¹⁵⁶ and lung cancer¹⁵⁷ in animal models. One of the ICP34.5-deleted strains, HSV1716, has been tested in clinical trials involving patients with recurrent high-grade glioma. Following intratumoral inoculation of doses up to 10⁵ plaque forming units (PFU), there was no induction of encephalitis, no adverse clinical symptoms, and no reactivation of latent HSV.¹⁵⁸ Replication of the virus was demonstrated in human gliomas not only at the site of injection but also at distal tumor sites in some patients.¹⁵⁹ In a recently published study, HSV1716 was injected into cavity following surgical resection of glioma tumor mass. No clinical toxicity associated with the administration of this virus was observed. The treatment has demonstrated survival benefit in a subset of patients and a reduction of residual tumor size in at least one patient.¹⁶⁰ HSV1716, developed by Crusade Laboratories (Glasgow, UK), is in clinical trials for treatment of glioma, melanoma, head-and-neck cancer and mesothelioma.

Table 1 Summary of oncolytic HSV-1 strains

HSV-1 strain	Genetic alteration	Therapeutic transgenes	Development Stage	Clinical indications	Reference
dlspk	TK Gene deletion	None	Preclinical		130,135
hrR3	Disruption of UL39 (large subunit of ribonucleotide reductase)	None	Preclinical		138,143–145
HSV1716	759-bp deletion in both copies of ICP34.5	None	Human clinical trials	Glioma, melanoma, head-and-neck cancer	151,152,158–160,244
R3616	1000-bp deletion in both copies of ICP34.5	None	Preclinical		153,155,245
R4009	Premature stop codon in both copies of ICP34.5	None	Preclinical		153,155,245
G207	(1) 1000 bp deletion in both copies of ICP34.5; (2) Disruption of UL39	None	Human Clinical trials	Recurrent brain cancer (glioma astrocytoma, glioblastoma).	166,167,169,170,184
MGH-1	(1) 1000 bp deletion in both copies of ICP34.5; (2) Disruption of UL39	None	Preclinical		142,165
R7020 (NV1020)	(1) Deletion of one copy of ICP34.5; (2) TK under control of viral α 4 promoter, (3) Deletion of UL24,UL55,UL56	None	Human clinical trials	Liver, metastases derived from colorectal cancer	185,186,188,192,194
G47 Δ	(1) 1000 bp deletion in both copies of ICP34.5; (2) Disruption of UL39 gene; (3) Deletion of ICP47 gene.	None	Preclinical		195
Myb34.5	Deletion of both copies of endogenous ICP34.5 (2) Disruption of UL39 gene. (3) Insertion of an ICP34.5 gene under control of the B-myc promoter	None	Preclinical		196,198
DF3y34.5	(1) Deletion of both copies of endogenous ICP34.5 (2) Insertion of an ICP34.5 gene under control of the DF3/MUC1 Promoter	None	Preclinical		200,201
HF10	(1) Spontaneously generated clonal variant of HSV-1	None	Human clinical trial		246,247
NV1042	(1) HSV-1/HSV-2 intertypic recombinant (2) Contains only one copy of the ICP34.5 gene (3) Deletion of UL56 and ICP47	Murine IL-12	Preclinical		215,217–219
Onco Vex ^{GM-CSF}	(1) Deletion of both copies of ICP34.5 gene; (2) Deletion of ICP47.	GM-CSF	Human clinical trials	Breast cancer, head-and-neck cancer, Melanoma	222,223

Multiple gene deletion

Herpes simplex virus type 1 strains carrying deletion of a single gene often retain residual replication, and toxicity, in normal cells.^{161–163} Therefore, additional deletions or alterations have been made to the HSV-1 mutant strains described above to further increase the safety margin of the vectors as well as to decrease the likelihood of reversion to wild type.¹⁶⁴ These strains are often referred to as the second-generation viruses to distinct them from the strains that contain deletions of a single viral gene. An important representative of the second generation oncolytic viruses is G207 (it should be noted that a nearly identical mutant, MGH-1, was constructed and tested

separately by another group¹⁶⁵). G207 contains deletions in both copies of ICP34.5 gene and a destructive insertion of *E. coli lacZ* gene in U_L39 gene which encodes ICP6.¹⁶⁶ It has been one of the most prominent herpes oncolytic vectors in both preclinical and clinical studies (Table 1). G207 has several favorable properties for treating human cancers: replication-competence in tumor cells, attenuated neurovirulence, temperature sensitivity, GCV hypersensitivity, and the presence of an easily detectable histochemical (*lacZ* gene product) marker.¹⁶⁶ The safety of G207 has been tested extensively in animal models from mice to non-human primates.^{167–170} No virus-related toxicities was observed at doses as high as 10⁹ PFU in monkeys,¹⁶⁷

a sharp contrast to wild-type HSV-1 strains, which caused severe toxicity and animal death. G207 has demonstrated potent antineoplastic activity in many different cancer models, both in immunodeficient and immunocompetent animals.^{125,171–177} The existence of pre-existing anti-HSV-1 immunity has no measurable effect on the oncolytic activity of G207, and multiple injections can be applied without being affected by immune resistance to the virus.^{178,179} It appears that G207 inhibits tumor growth by two mechanisms: direct oncolysis via virus replication¹⁶⁶ and induction of tumor-specific immunity via an increase in cytotoxic T-cell (CTL) activity.^{172,180–183} Based on the promising preclinical data, G207 is now evaluated in clinical trials. Phase I clinical trials in patients with malignant glial tumors confirmed the safety of G207 virotherapy. No virus-related toxicity or herpes encephalitis was observed at doses of up to 3×10^9 PFU injected directly into the tumor.¹⁸⁴ Radiographic and neuropathologic evidence suggested antitumor activity and long-term presence of viral DNA in some patients. Currently, MediGene AG (Frankfurt, Germany) is sponsoring further clinical development of G207 to assess its antitumor activity in patients with brain tumors.

Another HSV-1 mutant with multiple gene deletions, R7020, contains a 15-kb deletion across the joint region of the long and short segments of the HSV-1 genome (thus deletion of one copy of ICP34.5) in addition to a deletion in the endogenous TK gene (Table 1).¹⁸⁵ The long/short junction of R7020 contains a 5.2-kb fragment of HSV-2 DNA and an exogenous copy of the HSV-1 TK gene under the control of the powerful HSV-1 ICP4 promoter. Thus, R7020 remains sensitive to ACV and GCV. These genetic manipulations allow R7020 to replicate preferentially in neoplastic cells, resulting in a remarkable safety profile in extensive rodent and primate studies, as well as in limited human vaccine trials.^{185,186} Since one copy of the ICP34.5 gene is intact, R7020 replicated to much higher levels compared to the ICP34.5 double-deletion mutant R3616 both *in vitro* and in animal tumors.¹⁸⁷ Its antitumor effect was significantly better than that of R3616.^{188–193} R7020 is now evaluated in clinical trials under the name of NV1020 (MediGene AG, Frankfurt, Germany). In a phase I trial, single doses of NV1020 were administered by the hepatic artery to 12 patients with colon carcinoma liver metastasis.¹⁹⁴ Doses of up to 1×10^8 PFU were well-tolerated; all adverse events were reported as mild or moderate. Virological evidence suggested that NV1020 replicated in patient tumor tissues. Levels of the surrogate tumor marker carcinoembryonic antigen (CEA) declined in a subset of patients and there appeared to be anecdotal evidence of tumor regression or tumor stability on computerized tomography (CT) scan by day 28. Phase II clinical trials with NV1020 are ongoing.

Several other HSV-1 mutants with multiple gene deletions have been created and tested. G47 Δ is a derivative of G207 that contains an additional deletion in the ICP47 gene.¹⁹⁵ Because ICP47 is responsible for inhibiting the transporter associated with antigen presentation (TAP),^{36,37} its absence led to increased MHC

class I expression in infected cancer cells, promoting tumor antigen presentation and antitumor immunity. G47 Δ was significantly more efficacious *in vivo* than its parent G207 at inhibiting tumor growth in both immune-competent and immune-deficient animal models, while its animal safety profile was similar to that of G207.¹⁹⁵

Tumor-specific promoters

Several oncolytic HSV-1 strains have been created by utilizing tumor-specific promoters. Myb34.5¹⁹⁶ was constructed by deleting both endogenous copies of the ICP34.5 gene and re-inserting this gene into the ICP6 locus under control of the *B-myb* promoter. This virus is similar to hrR3 in its preferential replication in actively dividing cells by virtue of the disrupted ICP6 gene. In addition, its replication is further regulated by the *B-myb* promoter, which is active in E2F-deregulated cells and in cycling cells.¹⁹⁷ Compared to hrR3, Myb34.5 is more attenuated in normal cells both *in vitro* and *in vivo*. Its toxicity was greatly reduced in mice after i.v. administration. Cytotoxicity of Myb34.5 in tumor cells, however, was as robust as that of hrR3.¹⁹⁸ A similar virus, DF3 γ 34.5, in which expression of ICP34.5 is controlled by DF3/MUC1 promoter/enhancer sequence,¹⁹⁹ has shown preferential replication in tumors that express DF3/MUC1, restricted biodistribution, and reduced toxicity after systemic administration.^{200,201} Several other viruses have been constructed in which essential HSV-1 immediate-early genes (such as ICP4) are under the control of CEA promoter,²⁰⁰ albumin promoter,^{202,203} and calponin promoter.^{203,204} Replication of these viruses was shown to be restricted to cells that overexpress the respective proteins. These viruses are currently under preclinical examination.

Vector re-targeting

Vector re-targeting has been explored rather extensively as a unique strategy to increase tumor specificity of oncolytic adenoviral vectors.²⁰⁵ Although the complexities of HSV-1 entry are not fully unraveled, several studies have demonstrated the feasibility of restricting HSV-1 infection to predefined subpopulations of cells (reviewed in Burton *et al.*¹³). For example, an HSV-1 mutant was engineered in which the heparan sulfate binding domain of gB was abrogated, and N-terminal truncated gC was fused to full-length erythropoietin (EPO).²⁰⁶ This virus strain showed greatly reduced glycosaminoglycan binding, as well as a new acquisition of specific binding to the EPO receptor. Although this virus is noninfectious and is not useful as the backbone of oncolytic viral vectors, it has demonstrated feasibility of HSV vector re-targeting.²⁰⁶ Recently, more effective re-targeted HSV vectors have been reported (for examples, see Argnani *et al.*²⁰⁷ and Zhou *et al.*²⁰⁸). A number of cell surface proteins were shown to be overexpressed in cancer cells, such as epidermal growth factor receptor (EGFR),²⁰⁹ folate receptor,²¹⁰ CD44.²¹¹ It is reasonable to assume that engineered HSV-1 vectors that specifically target these cell surface proteins will provide additional tumor selectivity for oncolytic viruses.

Armed oncolytic virus

Because infection and lysis of 100% of the tumor cells is hard to achieve *in vivo* using oncolytic viruses alone, oncolytic viruses are often 'armed' with genes that can augment their cytolytic capacities. Meanwhile, successful cancer gene therapy requires high levels of transgene expression in a high percentage of tumor cells *in vivo*. Oncolytic viruses that specifically replicate in tumor tissues and amplify template copy number provide an ideal platform for achieving this goal. Oncolytic HSV-1 vectors have been used successfully to deliver immunoregulatory molecules, prodrug converting enzymes and angiogenesis inhibitors.²¹²

Synergism of antitumor effect was observed when oncolytic HSV-1 mutants were engineered to express cytokines. An elegant experiment done by Andreansky *et al.*²¹³ clearly demonstrated that cytokine expression may be an important adjunct to oncolytic HSV-1 therapy. IL-4 (an immunostimulatory cytokine) and IL-10 (an immunosuppressive cytokine) were separately inserted in a HSV-1 vector with both copies of ICP34.5 gene deleted. In a glioma model in immunocompetent mice, inoculation of these vectors produced dramatically opposite physiologic responses. The IL-4-expressing vector significantly prolonged survival of tumor bearers compared to the parental virus, whereas tumor-bearing mice that received the IL-10-expressing vector had a median survival that was identical to that of saline-treated controls. Numerous studies have shown that oncolytic HSV-1 vectors that express IL-12 have significantly greater antitumor efficacy compared to their counterparts, which do not carry an IL-12 gene.^{101,214–218} This additional antitumor effect appeared to be mediated by both induction of tumor-specific cytotoxic T-lymphocytes^{216,219} and inhibition of tumor angiogenesis.²¹⁸ Expression of IL-2 from an amplicon vector also improved the overall antitumor effect of an oncolytic HSV-1 vector in a combination study.^{220,221} Overall toxicity of the oncolytic HSV-1 vectors was not altered by insertion and expression of the cytokine genes.

A genetically engineered strain of HSV-1, which expresses human immunostimulatory molecule GM-CSF, has been created, preclinically tested and is now evaluated in clinical trials under the name of OncoVex^{GM-CSF} (BioVex Ltd, Abingdon, UK). Four modifications were incorporated into this virus (Table 1).²²² First, a fresh clinical isolate of HSV-1 (JS1 strain) was used as the backbone for this virus instead of a laboratory strain which has been passaged numerous times in tissue cultures and may have partially lost their lytic capability in human tumor cells. Second, both copies of the ICP34.5 gene were deleted. Third, the viral ICP47 gene was deleted. Finally, human GM-CSF gene was inserted in this vector. It was reasoned that combination of GM-CSF with oncolytic therapy may be particularly effective as the necrotic cell death accompanying virus replication should serve to effectively release tumor antigens to induce a GM-CSF-enhanced immune response, resulting in systemic antitumor effects. OncoVex^{GM-CSF} was tested *in vitro* in human tumor cell lines and *in vivo* in mouse tumor

models, both demonstrating promising antitumor effects.²²² *In vivo*, both injected and non-injected tumors showed significant shrinkage or clearance and mice were protected against re-challenge with the same tumor cells. In phase I clinical trials involving patients with solid tumors including breast cancer, head and neck cancer, and melanoma, OncoVex^{GM-CSF} was shown to be well tolerated and substantial biological effects were observed.²²³ Phase II studies with OncoVex^{GM-CSF} are now underway.

Others have identified use of oncolytic HSV-1 mutants to express prodrug-converting enzymes. For example, HSV1yCD was engineered such that the viral RR gene was disrupted by sequences encoding yeast cytosine deaminase (yCD), which metabolizes the prodrug 5-fluorocytosine (5-FC) to 5-fluorouracil (5-FU). The combination of HSV-1 replication and intratumoral conversion of 5-FC to 5-FU was more effective than either modality alone in treatment of mice with diffuse liver metastases, and significantly prolonged animal survival.²²⁴ Similar results were obtained from rRp450, a replication-conditional HSV-1 mutant which does not express RR but carries the rat cytochrome P450 transgene. Cytochrome P450 activates prodrugs such as CPA to generate highly toxic metabolite. It was shown that rRp450-mediated oncolysis was enhanced by administration of CPA, both in tissue culture and in animal tumor models.^{145,225–227} Cyclophosphamide is an immunosuppressive agent. As discussed above, immunosuppression by CPA facilitates HSV-1 replication *in vivo*, thus enhancing the oncolytic effect of replication-competent HSV-1 vectors. However, enhancement of rRp450 antitumor effect appeared to be independent of the immunosuppressive property of CPA, as it was observed in immunodeficient mice as well.^{225,228}

In contrast to yCD/5-FC and p450/CPA, TK/GCV combination did not augment the antitumor efficacy of the oncolytic HSV-1 strains. Both HSV1yCD and rRp450 retain endogenous TK gene. When treated with GCV, antineoplastic efficacy of both viruses was reduced.^{145,224,227} These results were explained by the finding that activation of 5-FC and CPA only minimally affected HSV-1 replication, whereas activation of GCV markedly attenuated HSV-1 replication.^{224,227} Thus, combination of suicide gene therapy with oncolytic viral vectors must be evaluated carefully and on a case-by-case basis. Antiviral effect of GCV against HSV-1 strains that carry the TK gene may be used as a 'turn-off' safety valve if viral-related toxicity develops.

Combination with other therapies

Combination of oncolytic HSV-1 strains with chemotherapy or radiotherapy generally displays enhancement in antitumor activity.^{175,188,229–232} A variety of chemotherapy agents (mitomycin C, cisplatin, methotrexate and doxorubicin) were shown to augment the antitumor effects of oncolytic virus HSV1716.^{231,233} Ionizing radiation was shown to enhance anti-cancer activity of hrR3 without significantly altering viral replication.²³⁴ Similarly, combination of ionizing radiation and the

ICP34.5-deleted HSV-1 strain R3616 induced a synergistic antitumor response, resulting in better tumor regression and better survival rates.^{235,236} At least one report, however, indicated that ionizing radiation had no effect on antitumor activity of G207.²³⁷ It is possible that different genetic background of these mutants may cause these viruses to respond differently to chemo- and radiotherapy. More research is necessary to clearly define the interaction between radiation and oncolytic activity of viral vectors.

Limitations of herpes simplex virus type 1 vectors

Although extensive studies using HSV-1 vectors for cancer treatment have demonstrated remarkable safety and generated encouraging antitumor efficacy data, several limitations do exist for the HSV-1-derived vectors and may limit their usefulness. For example, multigene deleted HSV-1 vectors are in general more difficult to produce than the wild-type virus, resulting in lower yield. Complementing cell lines with multiple viral genes integrated must be generated and used to produce the mutant viruses, which adds another level of complexity. Long-term stability (over 6-month period) of HSV-1-derived vectors, whether in aqueous solution or in lyophilized form, has not been adequately addressed. Induction of antiviral or antitransgene product immune response may reduce the effectiveness of HSV-1-derived vectors, especially when multiple injections of the vectors are necessary. Although it has been shown that the existence of pre-existing anti-HSV-1 immunity has no measurable effect on the oncolytic activity of G207, and multiple injections can be applied without being affected by immune resistance to the virus,^{178,179} the impact of pre-existing immunity on HSV-1 cancer gene therapy and virotherapy still needs to be carefully evaluated by further studies. More research in laboratory and in clinics would be required to address all these issues.

Herpes simplex virus type 1 is a human pathogen with broad cell type tropism and great replication capacity, so naturally, safety of HSV-1-based therapies is an important concern. In this review, we outline a wide variety of strategies that aim to minimize toxicity of HSV-1 vectors and prevent undesired damage to normal tissues. In spite of the impressive safety records with these agents in animal studies and in human clinical trials, therapies based on HSV-1 vectors (especially when replication-conditional viruses are used for virotherapies) still raise bio-safety and risk management issues. This is particularly important considering the potential of homologous recombination of recombinant viral genome with the latent wild-type HSV-1 present in a substantial human population and with viral DNA sequence in complementing cell lines. These are discussed in the following section.

Safety considerations

The properties that make HSV-1 a useful tool for cancer therapy also make it potentially dangerous to the treated

patients and their contacts. For example, efficient replication of HSV-1 in a wide spectrum of human cells raises the possibility of serious damage to untargeted, normal tissues. This is particularly important when replication-competent viruses are employed in systemic administration. An increasing population with immunodeficiency also adds to the possibility of an epidemic spread of the virus. Because many recombinant HSV-1 vectors carry biologically active transgenes, effects of the transgene products must be considered in addition to the pathogenicity of the viral vectors. Extensive clinical studies are necessary to address these safety concerns.

A significant risk lies with the possibility of recombination. Homologous recombination could occur when a replication defective or a conditionally replicating HSV-1 vector infects a cell which already harbors a wild-type latent virus, homologous recombination between the incoming vector and the resident wild-type virus might occur and could result in the generation of a fully virulent virus that carries a biologically active gene. Although studies using animal models did not show a significant risk of wild-type virus re-activation,^{168,238} this possibility has not been clearly ruled out in human patients. This poses a significant risk not only to the individual who receives the treatment, but also to anyone who comes in contact with the person. To prevent this risk, it is essential to insert transgenes in regions of the viral genome that are essential for virus replication. This way, incorporation of the transgene(s) must result in inactivation of the recipient genome by disrupting the essential genes at the insertion loci. Recombination could also result from propagation of defective viruses in complementing cell lines, leading to the production of replication-competent viruses that could contaminate vector stocks. The way to avoid such recombination is by carefully designing the system so that there is no homology or minimal homology between the vector and the complementing sequence in the cell line.²³⁹

Should unwanted replications occur, methods for treating or controlling HSV-1 replication do exist (for a detailed review, see Villarreal²⁴⁰). As mentioned earlier, viral TK may be used as a safety valve when combined with prodrugs such as GCV, fampiclovir, penciclovir, ACV, and foscarnet. Inhibition of HSV-1 replication has been demonstrated using this approach both *in vitro* and *in vivo*.^{164,224,227}

Conclusions and future direction

Herpes simplex virus-based vectors for cancer treatment exist in three forms: amplicon, replication-defective vectors and replication-conditional viruses. This review focuses on the recent progress using replication-defective vectors and replication-conditional viruses. While strategies using replication-defective HSV-1 to deliver therapeutic transgenes have yielded encouraging results, the fastest growing area in HSV-1 vector-mediated cancer treatment lies with replication-conditional viruses. Not only do they provide a means to selectively target and kill

cancer cells in their own right but they also are attractive vectors for tumor-specific delivery of multiple foreign antitumor gene products, making it possible to combine oncolysis of the viral vectors with antitumor effects of multiple transgenes to achieve synergistic tumoricidal effects. There is still much to be learned and to be improved, but currently available data have certainly indicated a promising perspective for HSV-1 and other virus-based therapies. Twenty years ago, cancer therapy with monoclonal antibodies faced many challenges ranging from safety to efficacy to manufacturing issues, not unlike the challenges that gene therapy and virotherapy are facing today. To date, eight monoclonal antibodies have been approved by FDA for treating human cancers,²⁴¹ and a lot more are currently under clinical investigation. With active research in cancer biology, virology, immunology and the related field, we have reason to anticipate new therapies based on viral vectors in the future. As regards to HSV-mediated therapies, important issues currently under investigation to improve the efficacy and safety of include: evaluation and selection of the therapeutic transgenes, improvement of vector delivery efficiency and specificity. Further understanding of the biology of this virus will improve our ability to manipulate it to our advantage. Two approaches aimed at vector optimization deserve our attention: First, using fresh clinical isolates of HSV-1, rather than the laboratory strains which may have accumulate undesired genetic alterations, as the backbone for genetic engineering.²²² Care must be exerted as these strains are conceivably more virulent, and thus posing a greater risk, than many of the lab strains. Alternatively, it is possible to isolate viral mutants with enhanced anti-tumor efficacy by serial passage of a laboratory strain in cancer cells under carefully designed conditions.^{242,243}

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