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Oncolytic HSV-1 Virotherapy: Clinical Experience and Opportunities for Progress

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Abstract

Oncolytic virotherapy with mutants derived from Herpes simplex virus (HSV) type 1 exhibit significant antitumor effects in preclinical models. Several mutants have now been tested in clinical trials for a variety of cancer types, and all have been found to be safe. While there have been hints of antitumor efficacy with prolonged survival in some cases compared with historical controls, dramatic responses have been elusive. We review the clinical experience published to date and discuss some of the biologic factors that may be limiting for virus infection and spread, as well as new strategies currently under development to enhance antitumor efficacy.

Keywords

Herpes simplex virus; oncolytic viruses; tumor microenvironment; angiogenesis; extracellular matrix; cytokines; immune response

Introduction

The field of oncolytic virotherapy encompasses any use of viruses with natural or engineered tumor-selective replication to intentionally infect and kill tumor cells. While the concept of using live viruses to infect and destroy tumors dates back over a century ago, the ease of creating novel genetically engineered viruses using molecular biology has accelerated the field of oncolytic virotherapy in the last two decades [1]. Here we describe the biology and clinical testing of oncolytic Herpes simplex virus type 1 (HSV-1)-derived viruses.

HSV-1 is an enveloped, double stranded DNA virus with a fully sequenced genome of about 152kb encoding approximately 80 genes. The large, well characterized genome permits the removal of genes known to not be essential for replication in cancer cells (estimated to be about 30kb), thus allowing the insertion of therapeutic transgenes within the viral backbone.

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These properties along with its ability to remain as an episome, which alleviates concerns of insertional mutagenesis, make HSV-1 a very desirable vector for therapeutic applications. Attenuated HSV-1 viruses deleted for certain genes important for viral replication in normal cells may retain their ability to infect and kill cancer cells. Such vectors have been exploited for their cancer cell-selective replication/lysis as oncolytic viruses in several preclinical and clinical studies. Initial studies focused on the use of HSV-derived vectors for brain tumors, but due to its broad host tissue tropism preclinical models and clinical trials have been expanded to include numerous different tumor types. In this review we focus on the HSV-1 vectors currently being tested in human patients, and discuss the various barriers faced by the oncolytic virus *in vivo*.

Early oncolytic viruses: dlsptk and hrR3

In the early part of the 20th century, wild-type HSV-1 was among the first viruses found to grow preferentially in animal tumors and slow their growth [2], but the subsequent isolation of attenuated mutants suitably safe for human use would require another 70 years. Single gene deletion oncolytic viruses such as dlsptk and hrR3 were among the first mutant oncolytic viruses investigated in preclinical studies for their anti-neoplastic properties. dlsptk is a mutant HSV-1 deleted for the viral HSV thymidine kinase (HSVTK) gene. Viral HSVTK is required for nucleotide metabolism essential for DNA replication in non-dividing cells, but the upregulated expression of mammalian TK compensates for this defect in cancer cells [3]. Martuza et al tested dlsptk for its therapeutic potential against human gliomas implanted into immunodeficient mice, and dlsptk induced significant tumor reduction and increased animal survival [4]. Because the viral TK gene is required for most antiherpetic drugs, its deletion rendered the virus insensitive to commonly used anti-herpetic drugs such as acyclovir and ganciclovir, making it less attractive for use in human patients. Nevertheless this represents the first study that highlighted the potential of using conditionally replication competent viruses as antineoplastic agents for therapy.

hrR3 is an oncolytic HSV-1 with an in-frame gene disruption of ICP6 by the insertion of the *E. coli* bacterial reporter gene, β-galactosidase [5]. The viral ICP6 gene encodes for the large subunit of ribonucleotide reductase (RR), which is essential for the *de novo* synthesis of deoxyribonucleotides, needed for viral DNA synthesis and replication. Similar to the HSVTK deletion mutants, viral RR deletion mutants replicate best in cells that compensate for the loss of ICP6 by expressing the mammalian counterpart of RR [3]. Increased expression of mammalian RR is found in most cycling cells and those with homozygous deletion of the p16 gene, indicating that RR deficient viruses are able to replicate selectively in cells that harbor such mutations [3]. hrR3 efficiently destroys cancer cells *in vitro* and *in vivo* [6, 7]. Due to an intact TK gene, hrR3 retains sensitivity to anti-herpetic agents, suggesting that hrR3-infected glioma cells would be sensitive to killing by such drugs. In fact, the combination of hrR3 with ganciclovir has been reported to be of therapeutic advantage in rats bearing intracranial tumors [8], though the use of anti-herpetic drugs in this context is generally thought to be counterproductive to the oncolytic effect of virus replication [9]. Additionally, the antitumor combination effects of hrR3 with radiation, anti-angiogenic agents, immune modulating agents, and chemotherapy drugs in animal models

underscores the significance of using oncolytic HSV-1-derived viruses as therapeutic agents [10, 11] [12].

Based on the promising preclinical efficacy research, the safety of delivering hrR3 via intracerebral and intracorneal inoculation in mice was investigated [13]. While inoculation of wild-type HSV-1 revealed a lethal dose in 50% of animals (LD_{50}) of 10 plaque forming units (pfu), the LD_{50} for hrR3 was more than 500,000 pfu in 4 week old mice (mice survived the highest dose tested), suggesting that hrR3 is relatively safe for *in vivo* use. The LD_{50} was lowered by two orders of magnitude in 1 day old mice, attributed in that study to the rapid cell division occurring in the brain during the first week of post-natal life in the mouse. In older mice, no hrR3 virus replication was detected in brains compared with an increase of >4 logs of wild-type KOS virus [13]. The safety and bio-distribution of hrR3 was also tested in nude rats and Balb/c mice [14, 15]. The same dose was administered into the portal-venous circulation, and virus was detected by PCR in the liver and spleen but not in the brain, lung, or colon [15].

rRp450

rRp450 is an oncolytic HSV-1 that contains a deletion of ICP6, which should result in a similar phenotype to the mutation of ICP6 in hrR3. Inserted into the ICP6 locus is the rat cytochrome P450 2B1 (*CYP2B1)* gene, which encodes for the enzyme that activates oxazophosphorine prodrugs (e.g., cyclophosphamide and ifosfamide) into their anticancer metabolites (e.g., phosphoramide mustard) [16]. Treatment of rats bearing intracranial gliomas and mice with hepatic and sarcoma xenografts resulted in significant anti-tumor efficacy [17, 18]. The replication of rRp450 has been shown to be attenuated in human and mouse hepatocytes by >4 logs compared with wild type KOS [19], and indeed the vector appears to be safe when given by intracranial, intraperitoneal, and intravenous routes alone and in combination with cyclophosphamide [18]. rRp450 has also now even been shown to exhibit an antitumor effect when given intravenously, which can be enhanced by combination with anti-VEGF antibodies [20]. Based on these safety and efficacy studies, a clinical trial to test rRp450 in patients with liver metastases and primary liver cancer is underway at Massachusetts General Hospital (www.clinicaltrial.gov NCT01071941).

ICP34.5-Deficient Oncolytic HSVs

Interferon-induced, double-stranded RNA-activated protein kinase (PKR) is one of the principal cellular defenses against viral infection and is maintained at low levels in normal cells. PKR is activated upon viral infection and leads to phosphorylation of eIF-2α, resulting in the shutoff of host protein synthesis. Viruses have evolved to counteract such cellular antiviral defense reactions. For example, the HSV gene R_L-1 encodes for ICP34.5, which can reverse the effects of activated PKR. Thus, attenuation of both copies of this gene leads to reduction of neurovirulence associated with HSV-1 *in vitro* and *in vivo* [21, 22]. Despite the reduced replication in normal cells, HSV-1 virions deleted for ICP34.5 efficiently replicate in and are cytotoxic to a majority of glioma cell lines and primary tumor-derived cells [22, 23]. Treatment of mice bearing experimental brain tumors with HSV1716 resulted in a statistically significant increase in survival [24, 25]. HSV1716 was the first mutated

HSV-1 to be tested for efficacy by direct inoculation into the brains of patients with malignant brain tumors. There was no evidence of encephalitis or viral shedding or reactivation of endogenous latent virus in any patient [26]. In a subsequent report, two of the nine patients enrolled in this study were alive and stable at four years and four months and three years and seven months after treatment, suggesting a possible therapeutic efficacy associated with virotherapy [27]. Evidence of viral replication was subsequently examined in human high grade glioma patients. Results from plaque assays revealed the presence of infectious viral particles in resected tumors from two of the twelve treated patients. [28]. HSV DNA was detected by PCR and immunohistochemistry of tissue samples revealed positive staining for HSV proteins in tumor tissues from some patients, and virus in excess of input dose was recovered in two patients. In a subsequent trial, virus was injected into the tumor bed after surgical debulking, and there was no evidence of virus-associated toxicity [29]. Tumor samples from 4 patients supported virus replication in culture. The expected median survival for patients with primary and recurrent GBM is approximately 14 and 3–6 months, respectively. Of the six patients diagnosed with recurrent GBM, one showed no evidence of recurrence twenty two months post treatment, and two patients diagnosed with primary GBM were alive at 18 and 15 months.

HSV1716 has also been tested by direct intratumoral injection in patients with non-CNS solid tumors including melanoma and head/neck squamous cell carcinoma. Flattening of palpable melanoma tumor nodules was observed in some patients, and evidence of virus replication was confirmed by immunohistochemical staining of the injected nodules [30]. Injections into tumors or normal oral mucosa in twenty patients with squamous cell carcinoma were also well tolerated [31]. HSV was detected in the blood of five patients at in some patients up to 3 weeks post-injection. Unlike the preclinical studies that suggested this cancer type is susceptible to HSV1716 oncolysis [32], no antitumor activity was apparent, suggesting *in vivo* barriers to virus spread must be identified and overcome for this disease such as virus receptor expression [33, 34]. HSV1716 is currently being studied in patients 13 to 30 years of age with non-CNS solid tumors at Cincinnati Children's Hospital Medical Center (www.clinicaltrial.gov NCT00931931) based on preclinical efficacy of oncolytic HSV in models of sarcomas and neuroblastoma [18, 35–40].

G207

G207, is a doubly attenuated oncolytic virus that has deletions at both R_L-1 loci (encoding ICP34.5) and an in-frame and gene disrupting insertion of *E. coli* Beta-galactosidase gene within the ICP6 gene (*UL39*). The presence of two different attenuating mutations and the presence of wide type TK, which permits ganciclovir sensitivity of the recombinant virus, contribute towards the safety of this virus [41], though due to both gene mutations it has been found to be less efficacious than other virus mutants [42]. The insertion of β-galactosidase permits easy detection of cells harboring the virus in infected tissue. Treatment of nude mice harboring subcutaneous or intracerebral gliomas with G207 resulted in significant antitumor efficacy. G207 treatment destroyed human glioma cells in monolayer cultures, and intratumoral treatment of glioma bearing nude mice harbouring subcutaneous or intracerebral U-87MG gliomas decreased tumor growth and/or prolonged survival. Additionally, G207 was found to be avirulent upon intracerebral inoculation of mice and

HSV-sensitive non-human primates [41]. Cellular effects of oncolytic G207 therapy were studied in intracranial gliomas implanted in mice that revealed widespread regions of viral infection and replication (plaques) with reduced proliferation indices and higher apoptotic counts in infected areas of the lesions. Along with direct tumor cell killing, this study also found a significant decline in the number of blood vessels in the plaques, suggesting that G207 had both tumoricidal and antiangiogenic effects [43].

More recently the effect of G207 on the developing mammalian brain was tested by intracerebral injections in neonatal mice. The results suggested that G207 may have significant adverse effects on neurodevelopmental outcomes of very young patients with brain tumors, and implied that infants in such a study will need to be closely monitored for the development of hydrocephalus [44]. Safety of intracerebral inoculation of G207 in humans was determined in a dose escalation study in patients suffering from recurrent malignant glioma. Cohorts of three patients were injected with increasing doses of G207 directly into the contrast-enhancing portions of intracranial gliomas [45]. In a subsequent study, safety of two inoculations of G207 totaling 1.5×10^9 pfu injected before and after tumor resection was investigated in patients with GBM. This study also underscored the safety of multiple doses, including direct inoculation into the brain surrounding the tumor resection cavity.

NV1020

NV1020 is a non-selected clone of R7020, a virus that was initially developed by Bernard Roizman's lab as a potential vaccine for HSV1 and HSV2 infections [46]. This virus is derived from an HSV-1 F strain deleted for one copy of ICP34.5 and contains sequences that encode the HSV-2 glycoproteins D, G, I and E. Because HSV-1 ICP34.5 deletion mutants had shown promise as anti-neoplastic agents, R7020/NV1020 was first tested as an oncolytic virus in chemotherapy/radiation-resistant epidermoid carcinoma and androgen-independent prostate adenocarcinoma cell lines *in vitro* and *in vivo* [47]. In addition to these tumor types, antitumor efficacy of NV1020 has been demonstrated against a wide variety of tumors including bladder, mesothelioma, gastric, prostate, and hepatoma carcinomas, and pediatric sarcomas [35, 37, 42, 48–50]. Interestingly, NV1020 retained its effectiveness even in animals with pre-existing immunity against HSV-1 [51].

NV1020 has been tested in human patients with colorectal cancer metastatic to the liver [52]. This was the first clinical study to test an intravascular delivery approach for an oncolytic HSV-1. Two patients experienced reduction in tumor burden, seven patients showed temporarily stable disease, while twelve patients experienced disease progression. [52]. A follow up study of these patients revealed evidence of HSV replication in liver biopsies, along with an average reduction in colorectal cancer biomarker carcinoembryonic antigen [53]. Taken together, the clinical evidence to date indicates that NV1020 is safe to delivery intra-arterially and suggests that it may be efficacious in treating colorectal cancer that has metastasized to the liver.

HF10

Unlike the other viruses discussed above, HF10 is not genetically engineered but is a non-selective clone from the non-neuroinvasive HSV-1 strain HF [54]. Apart from loss of *U_L56*, sequencing of HF10 revealed an overall 99.1% similarity to HSV-1 strain 17, with mutations in genes involved in regulation of syncytia formation including U_L1, U_L20, U_L22, U_L24, U_L27, and U_L53 [55]. HSV strains can spread from cell-to-cell via infection across the junctions between the membranes of adjacent cells (wild-type strains (syn^+)) or by fusion of the infected cell with adjacent uninfected cells leading to the formation of multinucleated polykaryocytes or syncytia (syncytial mutant strains (syn)). Interestingly, HF10 induces syncytia formation in vitro, and did not result in the cytopathic effect observed in hrR3 infected cells (57).

Significant antitumor efficacy of HF10 has been observed in a variety of tumor models including breast, oral squamous cell carcinoma, peritoneal, and bladder cancers and malignant melanoma tumors in mice [56–59]. Other recent studies have shown that paclitaxel, an established chemotherapeutic, enhances the effect of HF10 in mice with peritoneally disseminated colon cancer [60]. HF10 treatment has been studied in breast cancer patients with metastatic nodules [61]. The virus was well tolerated in all patients with no adverse effects or any evidence of shedding or activation in any patient. This small study suggested that humans tolerate the virus well. HF10 has also been tested in human patients with recurrent head and neck squamous cell carcinoma [62]. Collectively, all of these trials show that the virus is well tolerated with minimal adverse effects, highlighting the need for further investigation of its efficacy. HF10 is currently being tested in Japan for safety and efficacy in patients with breast and pancreatic cancer as well as head and neck squamous cell carcinoma. A clinical trial recruiting patients with refractory head and neck cancer is open at the Oregon Health and Science University and at the University of Pittsburgh, Pennsylvania (www.clinicaltrial.gov NCT01017185).

OncoVex$^{GM\text{-}CSF}$

OncoVEX$^{GM\text{-}CSF}$ represents the first “armed” oncolytic HSV-1 to be tested in human patients [63]. The virus was derived from the JS strain, with deletions of both copies of ICP34.5 and an additional deletion of ICP47, which facilitates early expression of U_S11. U_S11 blocks intracellular PKR phosphorylation, and so this virus displays efficient virus replication in infected cells [64, 65]. Additionally, OncoVEX$^{GM\text{-}CSF}$ expresses the gene encoding GM-CSF driven by the ubiquitously strong CMV promoter, which in theory will augment the activation of a systemic antitumor immune response after oncolysis. Preclinical studies with OncoVEX$^{GM\text{-}CSF}$ revealed efficient tumor killing of both injected and uninjected tumors, resulting in significant tumor shrinkage or in some cases even complete responses. Mice were also found to be protected against future rechallenge by tumor cells, suggesting the induction of a protective anti-tumor immunity in mice [66]. Studies on the role of GM-CSF expression in this context have not been presented. Direct intratumoral injection of OncoVEX$^{GM\text{-}CSF}$ was tested for safety in twenty-six patients with cutaneous or subcutaneous lesions from malignant melanoma, head and neck, breast, and gastrointestinal, cancers refractory to prior treatments [67]. Overall the virus was well tolerated and no

patients dropped from the study. Although complete or partial responses were not observed, stable disease was observed in several patients, and most tumor biopsies showed tumor necrosis [67]. OncoVEX$^{GM\text{-}CSF}$ has also shown safety and efficacy in phase II studies of patients with stage III/IV metastatic melanoma [68, 69], and a multinational phase III trial for malignant melanoma is underway (www.clinicaltrial.gov NCT00769704).

While all the above studies demonstrate the safety of using oncolytic HSV-1 in patients, evidence of dramatic efficacy in these studies has largely been elusive. This failure has been attributed to several potential barriers faced by the therapeutic virus in its voyage against neoplasia. Improved understanding of the factors that negatively influence efficacy will likely lead to improved therapeutic strategies for oncolytic HSVs.

Barriers to Oncolysis

Oncolytic virotherapy in theory should be dose independent, so that even a small initial inoculum of virus can amplify within the permissive cancer cells and result in their lytic destruction. While clinical studies have shown safety of this approach, recent studies of biopsied patient tumor samples obtained after oncolytic HSV-1 treatment revealed the presence of virus only in small discrete pockets within the mass, suggesting that multiple cellular and microenvironmental barriers suppress efficient virus spread *in vivo* [70]. The importance of efficient viral replication to achieve therapeutic benefit from virotherapy has long been recognized [9]. A recent study investigating the direct effect of G207 on intracranial tumors in mice also revealed the effect of treating tumors with a limited viral dose. While infection of tumors resulted in reduced tumor cell proliferation, oncolysis and also destruction of tumor vasculature, these beneficial changes were only observed in areas of active viral replication, leaving non-transduced tumor tissues unaffected. This finding suggests that transduction of a significant volume of tumor tissue is essential to realize the benefits of virotherapy [43]. Oncolytic viruses thus appear to meet several formidable barriers that interfere with their ability to infect, replicate and propagate in tumors, thus limiting their efficacy. Among those barriers under investigation are neutralization by innate factors such as complement in serum, varibilities in virus receptor expression, the presence of resistant subpopulations such as cancer stem cells, physical and chemical barriers within the tumor microenvironment, adaptive immune responses, and intracellular defense pathways. A final barrier to the successful clinical translation of oncolytic HSV virotherapy is the lack of authenic, predictive preclinical tumor models.

Serum Neutralization

Systemic administration of viruses remains the most desirable way to achieve efficient targeting of disseminated invasive/metastatic disease, but there are several hurdles that may limit its efficacy. Upon systemic administration, HSV-1 is very rapidly neutralized by innate anti-HSV-1 antibodies [71]. Ikeda et al demonstrated an efficient inhibition of HSV-1 viral infection by rodent plasma at dilutions as high as 1:32. This effect was partially reversed by *in vitro* depletion of complement with mild heat treatment or *in vivo* depletion by treatment of athymic rats with cobra venom factor (CVF), indicating the inhibitory effects of complement on HSV-1 therapy [14]. Additionally, *in vivo* complement depletion facilitated the initial infection of tumor cells following intra-arterial delivery of hrR3. Binding of

defensins (small host defense peptides) produced by human neutrophils can also impair the ability of HSV-1 to infect human cells [72]. These same defensins have also been reported to be upregulated in human cancers and may contribute towards the neutralization of viral infection in vivo [73]. On the other hand, designer defense-like lytic peptides have been used as oncolytic agents [74]. Future studies are needed to fully elucidate the role of defensins in limiting oncolysis *in vivo*.

Cell binding and intracellular entry

HSV-1 infection is achieved by initial interaction of viral envelope glycoproteins (gC and gB) to cell surface heparin sulfated proteoglycans (HSPGs). This initial interaction is not considered essential but enhances the entry of viral particles. After initial interactions of viral gC and gB envelope proteins with cell surface HSPGs, viral glycoproteins (gD) interact with specific cell surface receptors called herpes viral entry mediators (HVEM), HVE-C (nectin-1) or 3-O-sulfated heparin sulfate to initiate viral entry. Binding of gD to these receptors triggers viral glycoproteins gH and gL to mediate fusion of viral envelope with the cell membrane, permitting entry of the viral capsid into the cytoplasm. Thus the availability of gD receptors on the cell surface dictates the efficiency of viral infection. While most cells express one or more HSV-1 receptors, nectin-1 expression by squamous cell carcinomas has been shown to be a predictor of herpes oncolytic sensitivity [33, 34]. Apart from being an HSV-1 receptor, nectin-1 is an important component of intercellular adherens junctions (Ajs). In some cells, calcium-mediated disruption of Ajs facilitates liberation of nectin-1 that is sequestered within the cell-cell junctions and allows increased oncolytic HSV-1 entry and oncolysis *in vitro* and *in vivo* [34]. Increased expression of nectin-1 on invasive lymph node metastatic cells obtained by serial passaging in mice has also been reported to sensitize these cells to oncolysis [75]. One way to enhance oncolytic HSV-1 targeting is to create viruses with the incorporation of single-chain variable fragment (scFv) incorporated into the viral envelope to retarget virus-cell binding. This strategy was tested in an HSV1716 derivative and found to effectively alter its tropism and permit tumor targeting even via intravenous and intraperitoneal injections in mice [76]. Soluble bi-specific adapter proteins composed of recognition elements for viral envelope glycoprotein gD and cell surface receptors like EGFR or CEA have also shown to facilitate retargeting of HSV infection without drastically changing the entry mechanism [77, 78]. Thus while availability of viral receptors on tumor cells can impede entry, novel mechanisms to exploit entry through other tumor specific cell receptors have shown promise in preclinical models.

Cancer stem cells

Cancer stem cells (CSC) have been recently identified as a small population of neoplastic cells that have the unique ability to initiate tumor growth in vivo. These cells are characterized by an ability to regenerate themselves and are thought to be resistant to most conventional anticancer therapies such as chemotherapy and irradiation. Early published studies on the potential activity of oncolytic viruses, including HSV, to infect and kill cancer stem cells have been recently reviewed [79]. Generation of novel oncolytic viruses with mutations designed to synergize with PI3K/Akt pathway targeted drugs in cancer stem cells is also being tested an effective anti-cancer therapy [80]. Future testing will reveal the efficacy of this approach.

Cell surface expression of CD133 is currently thought to be one of the markers for CSCs in certain tumors and has been identified in some glioma and neuroblastoma-derived stem cells [81]. Tumors initiated by these cells recapitulate the histopathological characteristics of human tumors and are hence thought to be better models for testing such agents. In one study, although *ICP6* (*UL39*)–deleted mutants could destroy glioma-derived CSC (GBM-SCs), the deletion of *gamma$_1$ 34.5* significantly attenuated the viral replication and cytotoxicity [82]. However, deletion of the gene encoding ICP47 within the doubly attenuated virus genome could rescue this defect by causing early expression of Us11 and permitting efficient destruction of GBM-SCs [83]. The lack of sensitivity of ICP34.5-deleted viruses has been noted in several other studies and the use of cancer cell specific promoters to drive virulence of oncolytic viruses has been tested [84]. rQnestin34.5 is one such oncolytic virus in which the nestin-HSP68 promoter/enhancer is utilized to drive expression of the gene encoding ICP34.5, in an HSV backbone deleted for both the copies of *gamma$_1$34.5* and ICP6 genes [85]. Nestin is an intermediate filament predominantly expressed in neural stems cells during embryogenesis, and is considered to be upregulated in glioma. A variety of primary tumors of the central nervous system (CNS) display elevated levels of nestin within tumor and/or endothelial cells. This transcriptionally driven oncolytic virus has shown efficient anti-tumor efficacy against CNS and neuroblastoma tumors *in vitro* and *in vivo* [86, 87].

Tumor microenvironment

The extracellular matrix (ECM) in solid tumors is composed of complex secretions of proteins and proteoglycans produced by both neoplastic and normal stromal cells, which continuously regulate and mediate the cross talk between neoplastic and normal cells. While the ECM contributes towards the development of a “fertile field” that supports cancer growth, its composition also has a tremendous impact on cell signaling and modulates the cancer cell’s response to therapy. Changes in cell signaling by the tumor ECM also have been reported to induce resistance to HSV-1. In one study, traditional two-dimensional (2D) cultures and extracellular matrix (ECM) containing three-dimensional (3D) cultures of uveal melanoma cells were infected with a GFP expressing HSV-1 [88]. Despite efficient oncolysis observed in the monolayer cultures, the same cells grown in three dimensional cultures appeared to resist oncolytic HSV-1 infection. This phenomenon was shown not only to be due to impaired virus spread in the ECM but also to ECM-mediated inhibition of viral replication after viral entry into tumor cells. Interestingly, it also appeared that some cells grown in three dimensional cultures could harbor a quiescent infection that could be later reactivated. Collectively, these findings illustrate how the extracellular tumor matrix can interfere with cellular susceptibility to oncolytic HSV-1 infection and virotherapy.

The secreted ECM along with increased vascular leakage associated with solid tumors results in increased water retention, a significant contributor towards increased interstitial fluid pressure. High interstitial pressure has been associated with edema and poor delivery of several therapeutic agents [89, 90]. This increased vascular hyperpermaeability has also been linked to increased induction of anti-viral immune responses and hence reduced viral efficacy *in vivo* [10]. Apart from increased pressure, the interlocked meshwork of secreted proteins within the extracellular matrix can also present a physical barrier that interferes

with efficient dispersal of large molecular weight therapeutics within solid tumors [91, 92]. Because oncolytic virotherapy is based on the initial infection, replication and then spread of the new virions to the entire tumor, the ECM meshwork potentially represents a major hindrance towards the spread of oncolytic HSV-1 virions in some cancer types. This hypothesis is bolstered by the observation of small discrete foci of viral persistence in tumor xenografts treated with adenovirus in mice [93], and also in clinical tissue obtained from human patients treated with G207 [94]. The inhibitory effect of the tumoral ECM can be partially alleviated by direct intratumoral treatment of subcutaneous tumors in mice with ECM modulating enzymes such as collagenase/dispase hyaluronidase and trypsin prior to virus injection [95, 96]. The inhibitory effect of collagen on HSV-1 spread within a solid tumor was demonstrated by Dr. Jain's laboratory using in vivo multi-photon imaging of HSV viral particles with second harmonic generation imaging of fibrillar collagen [97]. This study of melanoma tumors implanted in dorsal skin fold chambers in mice revealed the presence of HSV virions pooled around extracellular spaces devoid of collagen. Further treatment of these tumors with collagenase was found to improve viral dissemination through the tumor and improved outcomes for tumor-bearing mice [97]. More recently we utilized a bacterially expressed enzyme: chondroitinase ABC (Chase-ABC) to investigate the role of chondroitin sulfate proteoglycans in limiting OV spread and efficacy. We found that degradation of glioma extracellular matrix with OV-expressing bacterial Chase-ABC enhanced OV spread and antitumor efficacy [98].

Apart from the secreted extracellular matrix, tumoral vasculature is also a very significant portion of the solid tumor microenvironment. Tumor blood vessels are the routes through which circulating immune cells infiltrate solid tumors. While these infiltrating cells can partner with oncolytic virus-mediated cancer cell destruction and launch a systemic antitumor immune response, the initial entry of host innate immune cells is associated with an anti-viral host response that results in viral clearance. As a consequence, reduction of tumoral vasculature by anti-angiogenic treatment has been shown to augment oncolytic efficacy by limiting host inflammation in the tumor microenvironment [10, 99, 100]. Interestingly, apart from curbing host inflammation, anti-angiogenic agents may lead to increased tumor hypoxia [101]. While hypoxic cells are thought to be relatively resistant to both chemotherapy and radiation, a hypoxic tumor microenvironment has also been shown to support the replication of oncolytic HSV-1 by both increasing cellular MEK activity and GADD34 expression [102, 103].

Immune Responses

The active innate immune response elicited upon oncolytic virus infection probably represents the most formidable barrier to efficient viral replication and propagation in the tumor microenvironment. The significance of the innate immune system as an initial potent line of defense that limits initial HSV-1 infection, replication, and spread has been well recognized and is currently the subject of several studies [104].

The initial oncolytic virus infection of tumors is followed by a rapid decline in viral titers over a period of three days [105]. Because anti-viral antibodies are not produced in that time-frame, the innate immune system, including granulocytes, NK cells, NKT cells, and

macrophages that are recruited to the site of infection, is considered a major player in limiting viral propagation [104]. Depletion of mononuclear cells [106] or antiviral cytokine mediators such as IFN-γ [10, 105] has been shown to cause a significant increase in intratumoral viral titers and anticancer effects.

While neutrophils are the first antiviral responders that are recruited to a site of infection, efficient viral clearance at the cellular level requires both NK cells and monocyte-derived cells. Oncolytic HSV-1 treatment of tumors results in an initial rapid recruitment of circulating peripheral monocytes and leukocytes. The recruitment of these cells correlates with rapid viral clearance and limited anti-tumor efficacy [10, 106, 107]. In fact, recruitment of infiltrating monocytic cells has been shown to coincide with clearance of over 80% of HSV-derived oncolytic viral particles [105, 108]. Increased intra-tumoral presence of macrophage/microglia cells has also been reported in human patients treated with HSV1-derived oncolytic viruses, supporting the very significant role played by these cells in viral clearance [70, 94].

Intracellular Antiviral Responses

Apart from extracellular host responses, oncolytic viruses are met with innate intracellular defense responses upon entry into cells. Intracellular changes in cell signaling upon oncolytic virus infection have a significant impact on virotherapy, and can transform a cell to be permissive for or resistant to viral replication. The interferon (IFN) system represents the classical anti-viral mechanism activated upon viral entry of cells [109]. Activation of interferon regulatory factor-1 and -3 and the repression of interferon regulatory factor-2 results in the subsequent expression of type I interferons (IFN-I) [110]. Toll-like receptors (TLR) are a family of conserved receptors that function as cellular sensors for viral infection and upon recognition of foreign pathogens can initiate anti-viral responses [111]. The cellular RNA-dependent protein kinase (PKR) pathway also responds to viral infection by phosphorylating and inhibiting cellular eIF2α, leading to a shutoff of host protein synthesis, and hence also viral replication [112]. In addition, TLR activation has also been shown to activate the NF-kB pathway and inducible nitric oxide synthase (iNOS), both of which can further attenuate viral replication [113].

Inadequate preclinical models

A final barrier to the successful clinical translation of oncolytic HSV virotherapy is the lack of animal models that authentically recapitulate human cancers in terms of permissivity for virus replication, immune response, and the tumor microenvironment. Poor models may be significant barriers to progress as they may lull investigators into thinking their data provide rationale for clinical trials rather than identifying other hurdles that must be overcome for successful clinical translation. The major rodent tumor models used for preclinical efficacy studies are human tumor xenografts in immunodeficient mice and genetically engineered mice with high rates of spontaneous tumor formation. While xenografts give the advantage of studying human tumor cells, their undeveloped adaptive and sometimes even inadequate innate immune systems and the artificial tumor microenvironment (implanted tumor cells rather than spontaneously arising tumors) may significantly impact the antitumor effect of virotherapy. Conversely, although spontaneous tumor models have intact immunity and

native microenvironments, animal tumor cells tend to be less permissive for human virus infections, including HSV-1, than human tumor cells. These limitations may in part account for the incongruence between highly encouraging animal data and somewhat limited efficacy observed thus far in clinical trials.

Conclusions

Oncolytic virotherapy with HSV-1-derived mutants holds great promise as a new anticancer therapy, with significant effects found in multiple animal models. Several different variants have been tested or are currently in clinical trials, and to date there has been no evidence of unacceptable toxicity. While there have been hints of efficacy when comparing selected patients with historical experience, dramatic responses so far have been rare.

It is important to consider that many of the trials have been early phase, and the best doses and methods of delivery have not yet been determined. There remain many unanswered questions, even for the currently available vectors. Which tumor types are most susceptible to HSV? What administration routes, doses, and schedules are most effective? What are the biomarkers that could be used to predict which patients are the best candidates? What combinations with other anti-cancer therapeutics will be additive or synergistic? We are still in the very early phases of testing these agents in patients, and clinical trials designed to answer these questions should prove fruitful in further developing the currently available oncolytic HSV mutants as new therapeutics.

It is likely that the efficacy of oncolytic HSV virotherapy can also be improved beyond simply identifying susceptible tumor types and effective doses. Understanding the biologic factors that limit delivery and intratumoral spread of virus should enable the development of strategies that increase efficacy. In addition, a number of “next generation” vectors are under development that are targeted more specifically to tumors or are armed with pro-drug activating, immune modulating, and anti-angiogenic genes to enhance therapy (107). The full clinical therapeutic potential of oncolytic HSV-1 will thus likely be realized from ongoing research at the interface of virology, tumor biology, and immunology.

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References

1. Liu TC, Galanis E, Kirn D. Clinical trial results with oncolytic virotherapy: a century of promise, a decade of progress. Nat Clin Pract Oncol. 2007; 4:101–117. [PubMed: 17259931]
2. Levaditi C, Nicolau S. Affinite du virus herpetique pour les neoplasmes epitheliaux. Compt Rend Soc De Biol. 1922; 87:498–500.
3. Aghi M, Visted T, Depinho RA, Chiocca EA. Oncolytic herpes virus with defective ICP6 specifically replicates in quiescent cells with homozygous genetic mutations in p16. Oncogene. 2008

4. Martuza RL, Malick A, Markert JM, Ruffner KL, Coen DM. Experimental therapy of human glioma by means of a genetically engineered virus mutant. Science. 1991; 252:854–856. [PubMed: 1851332]
5. Goldstein DJ, Weller SK. Herpes simplex virus type 1-induced ribonucleotide reductase activity is dispensable for virus growth and DNA synthesis: isolation and characterization of an ICP6 lacZ insertion mutant. J Virol. 1988; 62:196–205. [PubMed: 2824847]
6. Kasuya H, Nishiyama Y, Nomoto S, Hosono J, Takeda S, Nakao A. Intraperitoneal delivery of hrR3 and ganciclovir prolongs survival in mice with disseminated pancreatic cancer. J Surg Oncol. 1999; 72:136–141. [PubMed: 10562359]
7. Mineta T, Rabkin SD, Martuza RL. Treatment of malignant gliomas using ganciclovir-hypersensitive, ribonucleotide reductase-deficient herpes simplex viral mutant. Cancer Res. 1994; 54:3963–3966. [PubMed: 8033122]
8. Boviatsis EJ, Park JS, Sena-Esteves M, Kramm CM, Chase M, Efird JT, Wei MX, Breakefield XO, Chiocca EA. Long-term survival of rats harboring brain neoplasms treated with ganciclovir and a herpes simplex virus vector that retains an intact thymidine kinase gene. Cancer Res. 1994; 54:5745–5751. [PubMed: 7954393]
9. Todo T, Rabkin SD, Martuza RL. Evaluation of ganciclovir-mediated enhancement of the antitumoral effect in oncolytic, multimutated herpes simplex virus type 1 (G207) therapy of brain tumors. Cancer Gene Ther. 2000; 7:939–946. [PubMed: 10880026]
10. Kurozumi K, Hardcastle J, Thakur R, Yang M, Christoforidis G, Fulci G, Hochberg FH, Weissleder R, Carson W, Chiocca EA, Kaur B. Effect of tumor microenvironment modulation on the efficacy of oncolytic virus therapy. J Natl Cancer Inst. 2007; 99:1768–1781. [PubMed: 18042934]
11. Spear MA, Sun F, Eling DJ, Gilpin E, Kipps TJ, Chiocca EA, Bouvet M. Cytotoxicity, apoptosis, and viral replication in tumor cells treated with oncolytic ribonucleotide reductase-defective herpes simplex type 1 virus (hrR3) combined with ionizing radiation. Cancer Gene Ther. 2000; 7:1051–1059. [PubMed: 10917208]
12. Aghi M, Rabkin S, Martuza RL. Oncolytic herpes simplex virus mutants exhibit enhanced replication in glioma cells evading temozolomide chemotherapy through deoxyribonucleic acid repair. Clin Neurosurg. 2006; 53:65–76. [PubMed: 17380741]
13. Yamada Y, Kimura H, Morishima T, Daikoku T, Maeno K, Nishiyama Y. The pathogenicity of ribonucleotide reductase-null mutants of herpes simplex virus type 1 in mice. J Infect Dis. 1991; 164:1091–1097. [PubMed: 1659596]
14. Ikeda K, Wakimoto H, Ichikawa T, Jhung S, Hochberg FH, Louis DN, Chiocca EA. Complement depletion facilitates the infection of multiple brain tumors by an intravascular, replication-conditional herpes simplex virus mutant. J Virol. 2000; 74:4765–4775. [PubMed: 10775615]
15. Nakamura H, Kasuya H, Mullen JT, Yoon SS, Pawlik TM, Chandrasekhar S, Donahue JM, Chiocca EA, Chung RY, Tanabe KK. Regulation of herpes simplex virus gamma(1)34.5 expression and oncolysis of diffuse liver metastases by Myb34.5. J Clin Invest. 2002; 109:871–882. [PubMed: 11927614]
16. Aghi M, Chou TC, Suling K, Breakefield XO, Chiocca EA. Multimodal cancer treatment mediated by a replicating oncolytic virus that delivers the oxazaphosphorine/rat cytochrome P450 2B1 and ganciclovir/herpes simplex virus thymidine kinase gene therapies. Cancer Res. 1999; 59:3861–3865. [PubMed: 10463570]
17. Pawlik TM, Nakamura H, Mullen JT, Kasuya H, Yoon SS, Chandrasekhar S, Chiocca EA, Tanabe KK. Prodrug bioactivation and oncolysis of diffuse liver metastases by a herpes simplex virus 1 mutant that expresses the CYP2B1 transgene. Cancer. 2002; 95:1171–1181. [PubMed: 12209705]
18. Currier MA, Gillespie RA, Sawtell NM, Mahller YY, Stroup G, Collins MH, Kambara H, Chiocca EA, Cripe TP. Efficacy and safety of the oncolytic herpes simplex virus rRp450 alone and combined with cyclophosphamide. Mol Ther. 2008; 16:879–885. [PubMed: 18388918]
19. Pawlik TM, Nakamura H, Yoon SS, Mullen JT, Chandrasekhar S, Chiocca EA, Tanabe KK. Oncolysis of diffuse hepatocellular carcinoma by intravascular administration of a replication-competent, genetically engineered herpesvirus. Cancer Res. 2000; 60:2790–2795. [PubMed: 10850415]

20. Eshun F, Currier M, Gillespie R, Fitzpatrick J, Baird W, Cripe T. VEGF blockade decreases tumor uptake of systemic oncolytic Herpes virus but enhances therapeutic efficacy when given after virotherapy. Gene Ther. 2010 in press.
21. Chou J, Kern ER, Whitley RJ, Roizman B. Mapping of herpes simplex virus-1 neurovirulence to gamma 134.5, a gene nonessential for growth in culture. Science. 1990; 250:1262–1266. [PubMed: 2173860]
22. Bolovan CA, Sawtell NM, Thompson RL. ICP34.5 mutants of herpes simplex virus type 1 strain 17syn+ are attenuated for neurovirulence in mice and for replication in confluent primary mouse embryo cell cultures. J Virol. 1994; 68:48–55. [PubMed: 8254758]
23. McKie EA, MacLean AR, Lewis AD, Cruickshank G, Rampling R, Barnett SC, Kennedy PG, Brown SM. Selective in vitro replication of herpes simplex virus type 1 (HSV-1) ICP34.5 null mutants in primary human CNS tumours--evaluation of a potentially effective clinical therapy. Br J Cancer. 1996; 74:745–752. [PubMed: 8795577]
24. Lasner TM, Kesari S, Brown SM, Lee VM, Fraser NW, Trojanowski JQ. Therapy of a murine model of pediatric brain tumors using a herpes simplex virus type-1 ICP34.5 mutant and demonstration of viral replication within the CNS. J Neuropathol Exp Neurol. 1996; 55:1259–1269. [PubMed: 8957450]
25. Kesari S, Randazzo BP, Valyi-Nagy T, Huang QS, Brown SM, MacLean AR, Lee VM, Trojanowski JQ, Fraser NW. Therapy of experimental human brain tumors using a neuroattenuated herpes simplex virus mutant. Lab Invest. 1995; 73:636–648. [PubMed: 7474937]
26. Rampling R, Cruickshank G, Papanastassiou V, Nicoll J, Hadley D, Brennan D, Petty R, MacLean A, Harland J, McKie E, Mabbs R, Brown M. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. Gene Ther. 2000; 7:859–866. [PubMed: 10845724]
27. Harland J, Papanastassiou V, Brown SM. HSV1716 persistence in primary human glioma cells in vitro. Gene Ther. 2002; 9:1194–1198. [PubMed: 12170384]
28. Papanastassiou V, Rampling R, Fraser M, Petty R, Hadley D, Nicoll J, Harland J, Mabbs R, Brown M. The potential for efficacy of the modified (ICP 34.5(−)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. Gene Ther. 2002; 9:398–406. [PubMed: 11960316]
29. Harrow S, Papanastassiou V, Harland J, Mabbs R, Petty R, Fraser M, Hadley D, Patterson J, Brown SM, Rampling R. HSV1716 injection into the brain adjacent to tumour following surgical resection of high-grade glioma: safety data and long-term survival. Gene Ther. 2004; 11:1648–1658. [PubMed: 15334111]
30. MacKie RM, Stewart B, Brown SM. Intralesional injection of herpes simplex virus 1716 in metastatic melanoma. Lancet. 2001; 357:525–526. [PubMed: 11229673]
31. Mace AT, Ganly I, Soutar DS, Brown SM. Potential for efficacy of the oncolytic Herpes simplex virus 1716 in patients with oral squamous cell carcinoma. Head Neck. 2008; 30:1045–1051. [PubMed: 18615711]
32. Mace AT, Harrow SJ, Ganly I, Brown SM. Cytotoxic effects of the oncolytic herpes simplex virus HSV1716 alone and in combination with cisplatin in head and neck squamous cell carcinoma. Acta Otolaryngol. 2007; 127:880–887. [PubMed: 17763002]
33. Yu Z, Adusumilli PS, Eisenberg DP, Darr E, Ghossein RA, Li S, Liu S, Singh B, Shah JP, Fong Y, Wong RJ. Nectin-1 expression by squamous cell carcinoma is a predictor of herpes oncolytic sensitivity. Mol Ther. 2007; 15:103–113. [PubMed: 17164781]
34. Yu Z, Li S, Huang YY, Fong Y, Wong RJ. Calcium depletion enhances nectin-1 expression and herpes oncolytic therapy of squamous cell carcinoma. Cancer Gene Ther. 2007; 14:738–747. [PubMed: 17525764]
35. Bharatan NS, Currier MA, Cripe TP. Differential susceptibility of pediatric sarcoma cells to oncolysis by conditionally replication-competent herpes simplex viruses. J Pediatr Hematol Oncol. 2002; 24:447–453. [PubMed: 12218591]
36. Parikh NS, Currier MA, Mahller YY, Adams LC, Di Pasquale B, Collins MH, Cripe TP. Oncolytic herpes simplex virus mutants are more efficacious than wild-type adenovirus Type 5 for the

treatment of high-risk neuroblastomas in preclinical models. Pediatr Blood Cancer. 2005; 44:469–478. [PubMed: 15570577]

37. Currier MA, Adams LC, Mahller YY, Cripe TP. Widespread intratumoral virus distribution with fractionated injection enables local control of large human rhabdomyosarcoma xenografts by oncolytic herpes simplex viruses. Cancer Gene Ther. 2005; 12:407–416. [PubMed: 15665822]
38. Mahller YY, Vaikunth SS, Currier MA, Miller SJ, Ripberger MC, Hsu YH, Mehrian-Shai R, Collins MH, Crombleholme TM, Ratner N, Cripe TP. Oncolytic HSV and erlotinib inhibit tumor growth and angiogenesis in a novel malignant peripheral nerve sheath tumor xenograft model. Mol Ther. 2007; 15:279–286. [PubMed: 17235305]
39. Mahller YY, Rangwala F, Ratner N, Cripe TP. Malignant peripheral nerve sheath tumors with high and low Ras-GTP are permissive for oncolytic herpes simplex virus mutants. Pediatr Blood Cancer. 2006; 46:745–754. [PubMed: 16124003]
40. Eshun FK, Currier MA, Gillespie RA, Fitzpatrick JL, Baird WH, Cripe TP. VEGF blockade decreases the tumor uptake of systemic oncolytic herpes virus but enhances therapeutic efficacy when given after virotherapy. Gene Therapy. In Press.
41. Mineta T, Rabkin SD, Yazaki T, Hunter WD, Martuza RL. Attenuated multi-mutated herpes simplex virus-1 for the treatment of malignant gliomas. Nat Med. 1995; 1:938–943. [PubMed: 7585221]
42. Bennett JJ, Delman KA, Burt BM, Mariotti A, Malhotra S, Zager J, Petrowsky H, Mastorides S, Federoff H, Fong Y. Comparison of safety, delivery, and efficacy of two oncolytic herpes viruses (G207 and NV1020) for peritoneal cancer. Cancer Gene Ther. 2002; 9:935–945. [PubMed: 12386832]
43. Huszthy PC, Immervoll H, Wang J, Goplen D, Miletic H, Eide GE, Bjerkvig R. Cellular effects of oncolytic viral therapy on the glioblastoma microenvironment. Gene Ther. 2010; 17:202–216. [PubMed: 19829315]
44. Radbill AE, Reddy AT, Markert JM, Wyss JM, Pike MM, Akella NS, Bharara N, Gillespie GY. Effects of G207, a conditionally replication-competent oncolytic herpes simplex virus, on the developing mammalian brain. J Neurovirol. 2007; 13:118–129. [PubMed: 17505980]
45. Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, Palmer CA, Feigenbaum F, Tornatore C, Tufaro F, Martuza RL. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. Gene Ther. 2000; 7:867–874. [PubMed: 10845725]
46. Meignier B, Longnecker R, Roizman B. In vivo behavior of genetically engineered herpes simplex viruses R7017 and R7020: construction and evaluation in rodents. J Infect Dis. 1988; 158:602–614. [PubMed: 2842408]
47. Advani SJ, Chung SM, Yan SY, Gillespie GY, Markert JM, Whitley RJ, Roizman B, Weichselbaum RR. Replication-competent, nonneuroinvasive genetically engineered herpes virus is highly effective in the treatment of therapy-resistant experimental human tumors. Cancer Res. 1999; 59:2055–2058. [PubMed: 10232586]
48. Cozzi PJ, Malhotra S, McAuliffe P, Kooby DA, Federoff HJ, Huryk B, Johnson P, Scardino PT, Heston WD, Fong Y. Intravesical oncolytic viral therapy using attenuated, replication-competent herpes simplex viruses G207 and Nv1020 is effective in the treatment of bladder cancer in an orthotopic syngeneic model. FASEB J. 2001; 15:1306–1308. [PubMed: 11344122]
49. Cozzi PJ, Burke PB, Bhargav A, Heston WD, Huryk B, Scardino PT, Fong Y. Oncolytic viral gene therapy for prostate cancer using two attenuated, replication-competent, genetically engineered herpes simplex viruses. Prostate. 2002; 53:95–100. [PubMed: 12242723]
50. Wong RJ, Kim SH, Joe JK, Shah JP, Johnson PA, Fong Y. Effective treatment of head and neck squamous cell carcinoma by an oncolytic herpes simplex virus. J Am Coll Surg. 2001; 193:12–21. [PubMed: 11442249]
51. Delman KA, Bennett JJ, Zager JS, Burt BM, McAuliffe PF, Petrowsky H, Kooby DA, Hawkins WG, Horsburgh BC, Johnson P, Fong Y. Effects of preexisting immunity on the response to herpes simplex-based oncolytic viral therapy. Hum Gene Ther. 2000; 11:2465–2472. [PubMed: 11119418]

52. Kemeny N, Brown K, Covey A, Kim T, Bhargava A, Brody L, Guilfoyle B, Haag NP, Karrasch M, Glasschroeder B, Knoll A, Getrajdman G, Kowal KJ, Jarnagin WR, Fong Y. Phase I, open-label, dose-escalating study of a genetically engineered herpes simplex virus, NV1020, in subjects with metastatic colorectal carcinoma to the liver. Hum Gene Ther. 2006; 17:1214–1224. [PubMed: 17107303]

53. Fong Y, Kim T, Bhargava A, Schwartz L, Brown K, Brody L, Covey A, Karrasch M, Getrajdman G, Mescheder A, Jarnagin W, Kemeny N. A herpes oncolytic virus can be delivered via the vasculature to produce biologic changes in human colorectal cancer. Mol Ther. 2009; 17:389–394. [PubMed: 19018254]

54. Nawa A, Luo C, Zhang L, Ushjima Y, Ishida D, Kamakura M, Fujimoto Y, Goshima F, Kikkawa F, Nishiyama Y. Non-engineered, naturally oncolytic herpes simplex virus HSV1 HF-10: applications for cancer gene therapy. Curr Gene Ther. 2008; 8:208–221. [PubMed: 18537595]

55. Ushijima Y, Luo C, Goshima F, Yamauchi Y, Kimura H, Nishiyama Y. Determination and analysis of the DNA sequence of highly attenuated herpes simplex virus type 1 mutant HF10, a potential oncolytic virus. Microbes Infect. 2007; 9:142–149. [PubMed: 17218138]

56. Teshigahara O, Goshima F, Takao K, Kohno S, Kimata H, Nakao A, Nishiyama Y. Oncolytic viral therapy for breast cancer with herpes simplex virus type 1 mutant HF 10. J Surg Oncol. 2004; 85:42–47. [PubMed: 14696086]

57. Ogawa F, Takaoka H, Iwai S, Aota K, Yura Y. Combined oncolytic virotherapy with herpes simplex virus for oral squamous cell carcinoma. Anticancer Res. 2008; 28:3637–3645. [PubMed: 19189645]

58. Takakuwa H, Goshima F, Nozawa N, Yoshikawa T, Kimata H, Nakao A, Nawa A, Kurata T, Sata T, Nishiyama Y. Oncolytic viral therapy using a spontaneously generated herpes simplex virus type 1 variant for disseminated peritoneal tumor in immunocompetent mice. Arch Virol. 2003; 148:813–825. [PubMed: 12664303]

59. Tolba KA, Bowers WJ, Hilchey SP, Halterman MW, Howard DF, Giuliano RE, Federoff HJ, Rosenblatt JD. Development of herpes simplex virus-1 amplicon-based immunotherapy for chronic lymphocytic leukemia. Blood. 2001; 98:287–295. [PubMed: 11435295]

60. Shimoyama S, Goshima F, Teshigahara O, Kasuya H, Kodera Y, Nakao A, Nishiyama Y. Enhanced efficacy of herpes simplex virus mutant HF10 combined with paclitaxel in peritoneal cancer dissemination models. Hepatogastroenterology. 2007; 54:1038–1042. [PubMed: 17629034]

61. Kimata H, Imai T, Kikumori T, Teshigahara O, Nagasaka T, Goshima F, Nishiyama Y, Nakao A. Pilot study of oncolytic viral therapy using mutant herpes simplex virus (HF10) against recurrent metastatic breast cancer. Ann Surg Oncol. 2006; 13:1078–1084. [PubMed: 16865590]

62. Fujimoto Y, Mizuno T, Sugiura S, Goshima F, Kohno S, Nakashima T, Nishiyama Y. Intratumoral injection of herpes simplex virus HF10 in recurrent head and neck squamous cell carcinoma. Acta Otolaryngol. 2006; 126:1115–1117. [PubMed: 16923721]

63. Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, McGrath Y, Thomas SK, Thornton M, Bullock P, Love CA, Coffin RS. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. Gene Ther. 2003; 10:292–303. [PubMed: 12595888]

64. Cassady KA, Gross M. The herpes simplex virus type 1 U(S)11 protein interacts with protein kinase R in infected cells and requires a 30-amino-acid sequence adjacent to a kinase substrate domain. J Virol. 2002; 76:2029–2035. [PubMed: 11836380]

65. Cassady KA, Gross M, Roizman B. The herpes simplex virus US11 protein effectively compensates for the gamma1(34.5) gene if present before activation of protein kinase R by precluding its phosphorylation and that of the alpha subunit of eukaryotic translation initiation factor 2. J Virol. 1998; 72:8620–8626. [PubMed: 9765401]

66. Cassel WA, Garrett RE. Newcastle Disease Virus As An Antineoplastic Agent. Cancer. 1965; 18:863–868. [PubMed: 14308233]

67. Hu JC, Coffin RS, Davis CJ, Graham NJ, Groves N, Guest PJ, Harrington KJ, James ND, Love CA, McNeish I, Medley LC, Michael A, Nutting CM, Pandha HS, Shorrock CA, Simpson J, Steiner J, Steven NM, Wright D, Coombes RC. A phase I study of OncoVEXGM-CSF, a second-generation oncolytic herpes simplex virus expressing granulocyte macrophage colony-stimulating factor. Clin Cancer Res. 2006; 12:6737–6747. [PubMed: 17121894]

68. Senzer NN, Kaufman HL, Amatruda T, Nemunaitis M, Reid T, Daniels G, Gonzalez R, Glaspy J, Whitman E, Harrington K, Goldsweig H, Marshall T, Love C, Coffin R, Nemunaitis JJ. Phase II clinical trial of a granulocyte-macrophage colony-stimulating factor-encoding, second-generation oncolytic herpesvirus in patients with unresectable metastatic melanoma. J Clin Oncol. 2009; 27:5763–5771. [PubMed: 19884534]
69. Kaufman HL, Kim DW, DeRaffele G, Mitcham J, Coffin RS, Kim-Schulze S. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol. 2010; 17:718–730. [PubMed: 19915919]
70. Markert JM, Liechty PG, Wang W, Gaston S, Braz E, Karrasch M, Nabors LB, Markiewicz M, Lakeman AD, Palmer CA, Parker JN, Whitley RJ, Gillespie GY. Phase Ib trial of mutant herpes simplex virus G207 inoculated pre-and post-tumor resection for recurrent GBM. Mol Ther. 2009; 17:199–207. [PubMed: 18957964]
71. Ikeda K, Ichikawa T, Wakimoto H, Silver JS, Deisboeck TS, Finkelstein D, Harsh GRt, Louis DN, Bartus RT, Hochberg FH, Chiocca EA. Oncolytic virus therapy of multiple tumors in the brain requires suppression of innate and elicited antiviral responses. Nat Med. 1999; 5:881–887. [PubMed: 10426310]
72. Daher KA, Selsted ME, Lehrer RI. Direct inactivation of viruses by human granulocyte defensins. J Virol. 1986; 60:1068–1074. [PubMed: 3023659]
73. Lundy FT, Orr DF, Gallagher JR, Maxwell P, Shaw C, Napier SS, Gerald Cowan C, Lamey PJ, Marley JJ. Identification and overexpression of human neutrophil alpha-defensins (human neutrophil peptides 1, 2 and 3) in squamous cell carcinomas of the human tongue. Oral Oncol. 2004; 40:139–144. [PubMed: 14693236]
74. Steinstraesser L, Hauk J, Schubert C, Al-Benna S, Stricker I, Hatt H, Shai Y, Steinau HU, Jacobsen F. Suppression of soft tissue sarcoma growth by a host defense-like lytic Peptide. PLoS One. 2011; 6:e18321. [PubMed: 21483840]
75. Yu Z, Li S, Huang YY, Lin SF, Fong Y, Wong RJ. Sensitivity of squamous cell carcinoma lymph node metastases to herpes oncolytic therapy. Clin Cancer Res. 2008; 14:1897–1904. [PubMed: 18347193]
76. Conner J, Braidwood L, Brown SM. A strategy for systemic delivery of the oncolytic herpes virus HSV1716: redirected tropism by antibody-binding sites incorporated on the virion surface as a glycoprotein D fusion protein. Gene Ther. 2008; 15:1579–1592. [PubMed: 18701918]
77. Nakano K, Kobayashi M, Nakamura K, Nakanishi T, Asano R, Kumagai I, Tahara H, Kuwano M, Cohen JB, Glorioso JC. Mechanism of HSV infection through soluble adapter-mediated virus bridging to the EGF receptor. Virology. 2011; 413:12–18. [PubMed: 21382632]
78. Baek H, Uchida H, Jun K, Kim JH, Kuroki M, Cohen JB, Glorioso JC, Kwon H. Bispecific adapter-mediated retargeting of a receptor-restricted HSV-1 vector to CEA-bearing tumor cells. Mol Ther. 2011; 19:507–514. [PubMed: 20924362]
79. Cripe TP, Wang PY, Marcato P, Mahller YY, Lee PW. Targeting cancer-initiating cells with oncolytic viruses. Mol Ther. 2009; 17:1677–1682. [PubMed: 19672244]
80. Kanai R, Wakimoto H, Martuza RL, Rabkin SD. A Novel Oncolytic Herpes Simplex Virus that Synergizes with Phosphatidylinositol 3-Kinase/Akt Pathway Inhibitors to Target Glioblastoma Stem Cells. Clin Cancer Res. 2011
81. Vangipuram SD, Wang ZJ, Lyman WD. Resistance of stem-like cells from neuroblastoma cell lines to commonly used chemotherapeutic agents. Pediatr Blood Cancer. 2010; 54:361–368. [PubMed: 19927294]
82. Friedman GK, Langford CP, Coleman JM, Cassady KA, Parker JN, Markert JM, Yancey Gillespie G. Engineered herpes simplex viruses efficiently infect and kill CD133+ human glioma xenograft cells that express CD111. J Neurooncol. 2009; 95:199–209. [PubMed: 19521665]
83. Wakimoto H, Kesari S, Farrell CJ, Curry WT Jr, Zaupa C, Aghi M, Kuroda T, Stemmer-Rachamimov A, Shah K, Liu TC, Jeyaretna DS, Debasitis J, Pruszak J, Martuza RL, Rabkin SD. Human glioblastoma-derived cancer stem cells: establishment of invasive glioma models and treatment with oncolytic herpes simplex virus vectors. Cancer Res. 2009; 69:3472–3481. [PubMed: 19351838]

84. Hardcastle J, Kurozumi K, Chiocca EA, Kaur B. Oncolytic viruses driven by tumor-specific promoters. Curr Cancer Drug Targets. 2007; 7:181–189. [PubMed: 17346110]

85. Kambara H, Okano H, Chiocca EA, Saeki Y. 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. 2005; 65:2832–2839. [PubMed: 15805284]

86. Mahller YY, Williams JP, Baird WH, Mitton B, Grossheim J, Saeki Y, Cancelas JA, Ratner N, Cripe TP. Neuroblastoma cell lines contain pluripotent tumor initiating cells that are susceptible to a targeted oncolytic virus. PLoS ONE. 2009; 4:e4235. [PubMed: 19156211]

87. Kambara H, Saeki Y, Chiocca EA. Cyclophosphamide allows for in vivo dose reduction of a potent oncolytic virus. Cancer Res. 2005; 65:11255–11258. [PubMed: 16357128]

88. Valyi-Nagy K, Dosa S, Kovacs SK, Bacsa S, Voros A, Shukla D, Folberg R, Valyi-Nagy T. Identification of virus resistant tumor cell subpopulations in three-dimensional uveal melanoma cultures. Cancer Gene Ther. 2009

89. Kamoun WS, Ley CD, Farrar CT, Duyverman AM, Lahdenranta J, Lacorre DA, Batchelor TT, di Tomaso E, Duda DG, Munn LL, Fukumura D, Sorensen AG, Jain RK. Edema control by cediranib, a vascular endothelial growth factor receptor-targeted kinase inhibitor, prolongs survival despite persistent brain tumor growth in mice. J Clin Oncol. 2009; 27:2542–2552. [PubMed: 19332720]

90. Fukumura D, Jain RK. Tumor microvasculature and microenvironment: targets for anti-angiogenesis and normalization. Microvasc Res. 2007; 74:72–84. [PubMed: 17560615]

91. Netti PA, Berk DA, Swartz MA, Grodzinsky AJ, Jain RK. Role of extracellular matrix assembly in interstitial transport in solid tumors. Cancer Res. 2000; 60:2497–2503. [PubMed: 10811131]

92. Vargova L, Homola A, Zamecnik J, Tichy M, Benes V, Sykova E. Diffusion parameters of the extracellular space in human gliomas. Glia. 2003; 42:77–88. [PubMed: 12594739]

93. Sauthoff H, Hu J, Maca C, Goldman M, Heitner S, Yee H, Pipiya T, Rom WN, Hay JG. Intratumoral spread of wild-type adenovirus is limited after local injection of human xenograft tumors: virus persists and spreads systemically at late time points. Hum Gene Ther. 2003; 14:425–433. [PubMed: 12691608]

94. Markert JM, Liechty PG, Wang W, Gaston S, Braz E, Karrasch M, Nabors LB, Markiewicz M, Lakeman AD, Palmer CA, Parker JN, Whitley RJ, Gillespie GY. Phase Ib Trial of Mutant Herpes Simplex Virus G207 Inoculated Pre-and Post-tumor Resection for Recurrent GBM. Mol Ther. 2008

95. Kuriyama N, Kuriyama H, Julin CM, Lamborn K, Israel MA. Pretreatment with protease is a useful experimental strategy for enhancing adenovirus-mediated cancer gene therapy. Hum Gene Ther. 2000; 11:2219–2230. [PubMed: 11084679]

96. Ganesh S, Gonzalez-Edick M, Gibbons D, Van Roey M, Jooss K. Intratumoral coadministration of hyaluronidase enzyme and oncolytic adenoviruses enhances virus potency in metastatic tumor models. Clin Cancer Res. 2008; 14:3933–3941. [PubMed: 18559615]

97. McKee TD, Grandi P, Mok W, Alexandrakis G, Insin N, Zimmer JP, Bawendi MG, Boucher Y, Breakefield XO, Jain RK. Degradation of fibrillar collagen in a human melanoma xenograft improves the efficacy of an oncolytic herpes simplex virus vector. Cancer Res. 2006; 66:2509–2513. [PubMed: 16510565]

98. Dmitrieva N, Yu L, Viapiano M, Cripe TP, Chiocca EA, Glorioso JC, Kaur B. Chondroitinase ABC I-mediated enhancement of oncolytic virus spread and antitumor efficacy. Clin Cancer Res. 2011; 17:1362–1372. [PubMed: 21177410]

99. Aghi M, Rabkin SD, Martuza RL. Angiogenic response caused by oncolytic herpes simplex virus-induced reduced thrombospondin expression can be prevented by specific viral mutations or by administering a thrombospondin-derived peptide. Cancer Res. 2007; 67:440–444. [PubMed: 17234749]

100. Liu TC, Castelo-Branco P, Rabkin SD, Martuza RL. Trichostatin A and oncolytic HSV combination therapy shows enhanced antitumoral and antiangiogenic effects. Mol Ther. 2008; 16:1041–1047. [PubMed: 18388912]

101. Kaur B, Khwaja FW, Severson EA, Matheny SL, Brat DJ, Van Meir EG. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. Neuro-oncol. 2005; 7:134–153. [PubMed: 15831232]
102. Fasullo M, Burch AD, Britton A. Hypoxia enhances the replication of oncolytic herpes simplex virus in p53- breast cancer cells. Cell Cycle. 2009; 8:2194–2197. [PubMed: 19502787]
103. Aghi MK, Liu TC, Rabkin S, Martuza RL. Hypoxia enhances the replication of oncolytic herpes simplex virus. Mol Ther. 2009; 17:51–56. [PubMed: 18957963]
104. Wakimoto H, Johnson PR, Knipe DM, Chiocca EA. Effects of innate immunity on herpes simplex virus and its ability to kill tumor cells. Gene Ther. 2003; 10:983–990. [PubMed: 12756419]
105. Fulci G, Breymann L, Gianni D, Kurozomi K, Rhee SS, Yu J, Kaur B, Louis DN, Weissleder R, Caligiuri MA, Chiocca EA. Cyclophosphamide enhances glioma virotherapy by inhibiting innate immune responses. Proc Natl Acad Sci U S A. 2006; 103:12873–12878. [PubMed: 16908838]
106. Fulci G, Dmitrieva N, Gianni D, Fontana EJ, Pan X, Lu Y, Kaufman CS, Kaur B, Lawler SE, Lee RJ, Marsh CB, Brat DJ, van Rooijen N, Stemmer-Rachamimov AO, Hochberg FH, Weissleder R, Martuza RL, Chiocca EA. Depletion of peripheral macrophages and brain microglia increases brain tumor titers of oncolytic viruses. Cancer Res. 2007; 67:9398–9406. [PubMed: 17909049]
107. Wakimoto H, Fulci G, Tyminski E, Chiocca EA. Altered expression of antiviral cytokine mRNAs associated with cyclophosphamide's enhancement of viral oncolysis. Gene Ther. 2004; 11:214–223. [PubMed: 14712306]
108. Lamfers ML, Fulci G, Gianni D, Tang Y, Kurozumi K, Kaur B, Moeniralm S, Saeki Y, Carette JE, Weissleder R, Vandertop WP, van Beusechem VW, Dirven CM, Chiocca EA. Cyclophosphamide increases transgene expression mediated by an oncolytic adenovirus in glioma-bearing mice monitored by bioluminescence imaging. Mol Ther. 2006; 14:779–788. [PubMed: 16996314]
109. Klotzbucher A, Mittnacht S, Kirchner H, Jacobsen H. Different effects of IFN gamma and IFN alpha/beta on "immediate early" gene expression of HSV-1. Virology. 1990; 179:487–491. [PubMed: 2171221]
110. Zhou Y, Ye L, Wan Q, Zhou L, Wang X, Li J, Hu S, Zhou D, Ho W. Activation of Toll-like receptors inhibits herpes simplex virus-1 infection of human neuronal cells. J Neurosci Res. 2009; 87:2916–2925. [PubMed: 19437550]
111. Wuest T, Austin BA, Uematsu S, Thapa M, Akira S, Carr DJ. Intact TRL 9 and type I interferon signaling pathways are required to augment HSV-1 induced corneal CXCL9 and CXCL10. J Neuroimmunol. 2006; 179:46–52. [PubMed: 16884784]
112. Mohr I. Neutralizing innate host defenses to control viral translation in HSV-1 infected cells. Int Rev Immunol. 2004; 23:199–220. [PubMed: 14690861]
113. Marques CP, Cheeran MC, Palmquist JM, Hu S, Lokensgard JR. Microglia are the major cellular source of inducible nitric oxide synthase during experimental herpes encephalitis. J Neurovirol. 2008; 14:229–238. [PubMed: 18569457]