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ONCOLYTIC HERPES SIMPLEX VIRUS 1 (HSV-1) VECTORS: INCREASING TREATMENT EFFICACY AND RANGE THROUGH STRATEGIC VIRUS DESIGN

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SUMMARY

Viruses have long been considered potential anticancer treatments. Wild-type viruses have been tested as anticancer agents in clinical trials since the 1960s. The possibility of viral oncolysis as an alternate cancer therapy was transformed by the emergence of modern genetic engineering. The herpes simplex virus (HSV) family offers particular advantages for use as a viral oncolytic. The engineered vectors that make up oncolytic HSVs (oHSVs) have demonstrated remarkable safety in clinical trials, with some evidence of efficacy. The past decade has seen a focus on increasing the efficacy of oncolytic vectors by adding exogenous transgenes to enhance tumor destruction. The current paper describes the various strategies for engineering HSV for increased cancer tissue specificity and efficacy. Presented are the rationale, preclinical data and clinical data where available. This is meant to illustrate a basic framework for the development of a novel therapy meant to exploit the viral life cycle for the killing of cancer.

INTRODUCTION

Over the past two decades, herpes simplex virus 1 (HSV-1) oncolytic agents have transitioned from an area of theoretical interest to a major focus in the search for novel cancer therapies. This shift from esoteric theory to clinical relevance is in large part attributable to the technological breakthroughs of genetic engineering, which have allowed investigators to design progressive generations of oncolytic HSV (oHSV) vectors, constantly improving their ability to selectively infect and treat a wide range of cancers. This article will review the evolution of these agents from casual experimentation of a century ago to various emerging strategies in contemporary vector design. Topics addressed include: 1) the historical roots of viral oncolysis; 2) the development of the first generations of modern oHSV vectors through deletion of viral genes; 3) means of engineering oHSVs to achieve increased efficiency of cancer cell infection; and 4) “arming” of oHSV vectors with transgenes that augment the agents’ efficacy through the localized expression of bioactive proteins.

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DISCLOSURES

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VIRAL ONCOLYSIS: A HISTORICAL PERSPECTIVE

The earliest reports of what are now known to be viral diseases showing the ability to lead to cancer regression predate the discovery of viruses. In the late 19th century, reports surfaced of patients bearing significant cancers experiencing transient remissions following influenza (1–3). Levaditi et al. later demonstrated that vaccinia virus inhibited various mouse and rat tumors in 1922, labeling this phenomenon “le tumeur fait function d’eponge” (4).

Alice Moore became the first scientist to apply viral oncolysis to an animal model. Working with Russian Far East encephalitis virus, she showed complete regression in some cases of mouse sarcoma 180 –the first animal model to demonstrate full regression through viral oncolysis (3, 5, 6). In her work with animal modeling, Moore initiated a fundamental shift in the scientific approach to viral oncolysis –a move to generate a novel agent particularly suited for oncolysis rather than simply attempting to replicate a natural sequela to existing pathogens. Working with others at Memorial Sloan-Kettering Cancer Center in New York, Moore began to experiment with serial viral passaging in order to breed viral strains with improved efficacy as viral oncolytic agents, foreshadowing a modern era of oncolytic vectors genetically engineered to attain selective replication in cancer cells (3, 7, 8).

In the same year, Moore first published her application of the Russian Far East encephalitis virus to a rodent model, and Hoster et al. published the first clinical trial assessing the impact of viral infections on cancer. Twenty-one volunteers with Hodgkin’s disease were inoculated with samples of sera and tissue samples from other patients noted to have developed viral hepatitis. Development of viral hepatitis in the volunteers was defined at the time of jaundice onset. Although there were obviously many significant side effects, some patients did show clinical improvement and decreased tumor size after manifesting classic stigmata of viral hepatitis (9). Shortly thereafter, a variety of small clinical trials were published around the country using various viral agents, including West Nile virus, mumps virus and Newcastle disease virus (5, 10–12). In 1956, the National Cancer Institute (NCI) conducted the first large-scale clinical study, administering wild-type adenoviruses to 30 patients with cervical cancer and achieving varying degrees of localized tumor necrosis but no significant tumor regressions or remissions (13).

While these early studies and trials were considered groundbreaking, interest in viruses as potential antineoplastic therapies was abandoned due to unimpressive and short-lived success, as well as unacceptable side effects that eventually ended the trials (3, 10). These viruses failed in that they infected and replicated in non-cancerous cells, and were unable to circumvent detection and clearance by the immune system. Limited contemporary understanding of virology, molecular biology, immunology and genetics, as well as primitive biotechniques, likely contributed to the demise of the first iteration of this field.

There was a marginal renewal of interest in viral agents as a component of therapy in the late 1970s and 1980s, with isolated animal and clinical studies. In 1974, the oncolytic potential of the mumps virus against 18 different tumor types was studied in Japan (4). Also, a 1982 report described the investigation of 16 different viruses for use in the treatment of sarcoma and Ehrlich ascites tumors in mice (14). While there were side effects from viral infection, more than half of the patients showed either complete tumor regression or decrease in tumor size. Viruses were also studied as a means to generate tumor-specific immunizations. In 1986, Hersey et al. applied a vaccinia virus oncolysate to a clinical trial of tumor vaccination in melanoma patients in Australia (15). Ultimately, though, it would not be until the emergence of modern genetic engineering in the 1990s that viral oncolysis would resurface with renewed potential as a cancer therapy.

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While the potential for viruses to selectively destroy cancers has been a source of intermittent scientific interest and exploration for generations, it is over the past two decades that the modern field of viral oncolysis has emerged in earnest. What separates the modern approach to viral cancer therapeutics from earlier experimentation is the advent of genetic engineering: the ability to manipulate the vector genome to effect increased specificity and efficacy in the infection and destruction of tumor cells.

FOUNDATION – BASIC PROPERTIES OF HSV

HSV is a double-stranded DNA virus with a 152-kb circular genome encoding 84 genes, which occurs naturally in humans, with several natural advantages as a template for viral oncolysis vectors (16). Its relatively large genome allows it to accept multiple transgenes as large as 30 kb. As the HSV genome does not integrate into the host genome, it is generally nonmutagenic to its host. Finally, the existence of multiple antiviral medications with well-established safety and efficacy in the treatment of HSV infections provides a reassuring option for the hypothetical scenario in which oncolytic treatments were to lead to an unexpected pathogenic infection.

HSV-1 does have certain disadvantages as a therapeutic agent. Most intuitively, as a common human pathogen, one might expect the patient/host immune system to neutralize the vector upon administration. However, preclinical studies have found preexisting immunity to wild-type HSV-1 to have minimal if any impact on antitumor efficacy in the context of systemic delivery, and no discernable impact in locoregional treatment models (17–19).

Another theoretical concern involves the possibility of carcinogenic side effects. While the circular intracytoplasmic HSV-1 is incapable of creating host mutation through integration into host DNA, Yang et al. have shown that HSV-1 infection can indirectly lead to mutation by deregulating promoter pathways (20). Such inappropriate promoter induction could theoretically prove carcinogenic over time. While certainly worthy of addressing when considering HSV as a vehicle for the broader field of gene therapy, the short life expectancies of patients bearing the kind of advanced cancers targeted by viral oncolytic agents render such concerns moot in this particular setting.

ORIGINAL DESIGNS – VIRAL GENE DELETION

Attenuating viral replication in noncancerous cells

The initial objective in developing therapeutic agents from HSV species was to eliminate (or at least minimize) the obvious toxicity resulting from infection of noncancerous tissues. Thus, the first generations of modern (i.e., genetically engineered) oHSV vectors were created by simply deleting selected HSV-1 genes whose function was thought to be essential for infection of normal tissues but redundant for the infection of cancer cells (Table I).

Most oHSV vectors contain deletions of HSV genes necessary to supply the genetic material required for viral genome construction, as such enzymes are crucial to viral replication in relatively quiescent normal cells but nonessential in rapidly dividing cancer cells harboring a ready supply of viable genetic machinery. The first recombinant HSV strain directed against cancer, *dl*sptk, was generated through the deletion of the *UL23* gene encoding thymidine kinase (TK) (21). TK processes nucleotides to facilitate replication of DNA. In the absence of HSV TK, HSV-1 infecting normal cells would fail to replicate at a rate sufficient to sustain infection. However, in rapidly dividing cancer cells, where a surplus of thymidine is produced by overactive native cellular replication machinery, viral replication would proceed unhindered, allowing for progressive, lytic infection. In a mouse glioma model

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treatment with *dl*sptk resulted in a significant prolongation of survival as compared to wild-type mice and mice treated with placebo, showing only mild to moderate, self-limiting HSV encephalitis (22).

While the efficacy and selectivity of *dl*sptk established a proof of principle for the use of HSV-1 genome deletions to achieve tumor selectivity through selective attenuation, the TK deletion was ultimately problematic from the standpoint of clinical application, as it rendered the strain impervious to first-line anti-herpes medications. This resistance represented to many the loss of a crucial safety valve for clinical experimentation with viral therapies. As such, *dl*sptk and its TK deletion were abandoned in favor of HSV-1 recombinants featuring deletion of the *UL39* gene. *UL39* encodes for ICP6, a large (and critical) subunit of the ribonucleotide reductase enzyme. Ribonucleotide reductase functions to generate deoxyribonucleotides necessary for viral replication (23, 24). Deletion of *UL39* would thus be expected to improve targeting of virus to replicating cells, attenuating replication on nondividing cells and thus limiting toxicity (25). Markert et al. applied the prototypical ICP6 knockout HSV-1 strain, hrR3, to a mouse model of glioma and identified a similar therapeutic profile to that seen with *dl*sptk (26). As TK was preserved in this vector, hrR3 also demonstrated a distinct remarkable sensitivity to the common antiviral agent ganciclovir, which can theoretically be used to treat viral dissemination. *UL39* deletion remains central to many HSV-1 recombinants currently under investigation for viral oncolysis.

The next gene to attract attention during this “first generation” of oHSV vector development was *RL1* (also referred to as γ_1 *34.5*). At the time, the precise function of the *RL1* product, ICP34.5, was not known, although it was thought to play a crucial role in neurovirulence. Subsequent research has demonstrated that in wild-type HSV-1 ICP34.5 supports sustained infection by preventing the host cell from shutting off all protein synthesis (an intracellular defense mechanism which prevents the virus from converting the host cell into a factory for virion reproduction). Upon infection by virus of a normal host cell, host RNA-dependent protein kinase (PKR) phosphorylates (and thus inactivates) the α subunit of the translation initiation factor eIF-2α, a critical protein for host cell protein synthesis. ICP34.5 facilitates the dephosporylation (and, as such, reactivation) of eIF-2α (16, 27, 28). In tumor cells sensitive to *RL1* mutants, mitogen-activated protein kinase kinase (MEK) inhibits PKR phosphorylation of eIF-2α, rendering ICP34.5 obsolete (29). The deletion of *RL1* confers an oncotropism to oHSV vectors due to MEK overexpression in a very high proportion of cancer cells. It is this overexpression that renders a cancer cell sensitive to productive infection by an *RL1* mutant (30).

The role of *RL1* in overcoming resistance to HSV infection is, in fact, not limited to neurons, but also plays an important role in infecting a wide range of cells. Thus, not only did the prototypic *RL1* mutant HSV-1716, which contains a double deletion of *RL1* and retains functional copies of both *UL39* and *TK* genes, show efficacy in a glioma model, but it was also found to be an effective oncolytic vector in a variety of non-nervous system tumors (31, 32). As such, it has become clear that the oncotropism of the HSV-1 family of oncolytic vectors is not limited by the neurotropism of the parent wild-type HSV-1 virus.

Other strains developed principally to incorporate the *RL1* mutation included HSV-G207, which contains a combined single deletion of *RL1* and single deletion of *UL39*, and MGH-1, which features a single deletion of *UL39* and double deletion of *RL1* (33, 34).

Viral deletion to augment host response

A third-generation oHSV vector, G47Δ, was created by adding to the *UL39* and *RL1* deletions of G207 a deletion of the HSV-1 gene *α47* (35). This gene encodes for a protein

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that protects the virus from host immune responses by downregulating host cell expression of major histocompatibility complex class I (MHC-I). Deleting *α47* thus restores MHC-I and allows for tumor cells to present antigen to circulating T cells in response to infection. While this deletion might initially appear counterintuitive in that it would theoretically make the viral infection more vulnerable to host immunity, the third-generation agents exploit the host immune response to invoke a mechanism of cell killing beyond direct oncolysis. Todo et al. showed that this deletion had the intended response of increasing tumor reduction by enhancing antitumor immune response (36).

HF-10 – nature's contribution to the repertoire of oHSV vectors

One vector that continues to receive attention in both preclinical and clinical trials was not engineered at all, but rather discovered. In 2003, Takakuwa et al. reported that i.p. injection of HF-10, a spontaneously generated clone of the HSV-1 strain HF identified from their viral stock, safely and effectively treated a murine model of peritoneal carcinomatosis (37). HF-10 has since been shown to have efficacy in colorectal, pancreatic, bladder and breast cancer, as well as melanoma and sarcoma, and has been evaluated in multiple clinical trials (37–49). A mutation in *UL56* is thought to be responsible for attenuation in noncancerous cells (50). However, while experiments using HSV-2 suggest that the gene product of *UL56* plays a critical role in vesicular trafficking, its precise function in HSV-1 is unclear (51, 52). Ultimately, although several genetic rearrangements have been identified in the strain, and phenotypically it was notable for its tendency toward syncytium formation upon infection, the specific mutations and mechanisms accounting for the efficacy of HF-10 as a viral oncolytic agent remain unclear (37).

AUGMENTING EFFICACY THROUGH MANIPULATION OF REPLICATION, SPREAD AND HOST IMMUNE RESPONSE

While deletion of viral genes has proven remarkably effective in attaining selectivity of infection, a tendency towards incomplete tumor responses in clinical trials of these initial vectors highlighted the need to augment the antitumor effect of these agents (48, 53–60). This prompted the development of oHSV strains, typically derived from the basic constructs outlined above, which increased treatment efficacy through insertion (or strategic reinsertion) of genes to increase their virulence within cancer cells (without compromising the selective attenuation seen in noncancerous cells).

Viral entry: from tumor selection to tumor targeting

A key mechanism by which HSV gains access to host cells involves interactions between glycoproteins located on the virion surface and host cell receptors. These mechanisms remain significant in oncolytic virus recombinants, as well as in wild types. Huang et al. demonstrated that quantifying nectin expression in various thyroid cancer cell lines can be used to predict sensitivity to herpesvirus oncolysis (61). Furthermore, in a murine model of squamous cell carcinoma, Yu et al. found that they were able to increase the sensitivity of established tumors to herpes oncolysis by increasing the tumor surface expression and availability of nectin through calcium depletion (62).

Beyond using cell-surface receptor expression to predict and optimize tumor sensitivity to oncolytic vectors, one can manipulate virus surface glycoproteins to increase virus–tumor cell affinity and facilitate virion entry into tumor cells. Wild-type HSV-1 bears multiple glycoproteins on its surface (most notably gB, gC and gD), which interact with host cell-surface glycosaminoglycans (most notably nectin, heparan sulfate and herpesvirus entry mediator [HVEM]) to facilitate the virion-to-cell attachment and virion penetration (16, 63). Various groups have demonstrated that by altering those surface glycoproteins, HSV strains

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can be engineered to show both greater affinity for a targeted cell type and weakened virulence in normal, nontargeted cells (64).

One promising method for targeting cell-surface receptors involves the application of single-chain antibodies (scFv). Menotti et al. showed that a herpes vector could be engineered to integrate a single variable fragment from a desired antibody into the herpes virion surface molecule glycoprotein D –a coreceptor which engages host cell-surface receptors to facilitate invasion– in order to target it to a molecule specific to the inserted antibody fragment (65). This technique has since been successfully applied to generate vectors showing enhanced efficacy against squamous and breast cancer by virtue of their bearing epidermal growth factor (EGF)- and HER2-targeted glycoproteins, respectively (65–67). The availability of countless antibodies to known tumor markers gives scFv-based targeting an attractive versatility, which could theoretically translate into a wide range of vectors to be selectively applied as individualized cancer therapies.

Virus replication: tumor-responsive promoters restore efficient replication within cancer cells

The remarkable safety of most oHSV vectors derives in part from the deletion of genes deemed essential to viral replication in nondividing cells. However, while these genes may not be absolutely essential to replication within dividing cancer cells, their deletion is still likely to diminish (at least somewhat) the efficiency of replication in any cell. Furthermore, although cancer cells are much more prone to active replication than noncancerous cells, at any given time some portion of cells within a tumor can be found to be in the nonreplicating state. The short duration of oHSV infections thus allows for the possibility that a subset of tumor cells may spend the entire course of infection in quiescence, and thus escape infection and lysis. This inefficiency can be overcome by reinserting the deleted genes in a manner which places them under the control of a promoter selected to activate expression of the critical gene only upon exposure to some cancer-specific trigger. Such regulators are referred to as tumor-responsive promoters.

Tumor-responsive promoters can be designed to direct oHSV vectors to tumors through several mechanisms. Such promoters may be responsive either to properties of a specific cancer cell or to a certain set of environmental conditions known to exist within tumors. Several oHSV vectors employ antigen-responsive promoters which initiate transcription of the reinserted gene upon exposure to a specific cancer-associated antigen. Examples include vectors with antigen-driven expression of reinserted genes upon exposure to carcinoembryonic antigen (CEA), α-fetoprotein (AFP), MUC-1, Musashi and other common tumor markers (68–74) (Table II). Such promoters can be remarkably effective in restoring the potency of oHSV agents within tumor cells. However, such antigens are difficult to identify for many cancers, and the safety and efficacy of the promoter depend largely upon the specificity and consistency of the tumor-associated antigen.

A less tumor type-specific method for tumor targeting involves the use of promoters which are responsive to environmental factors unique to tumor biology at a tissue rather than cellular level. Our laboratory has demonstrated that reinserting the ribonucleotide reductase (RR)-encoding gene *UL39* into a G207 backbone such that its expression is regulated by a hypoxia-responsive promoter can enable effective herpes oncolysis of both breast and colon cancer cells previously found to be resistant to oHSV therapy, and allow for in vivo tumor reduction in a murine metastatic colon cancer model resistant to unarmed G207 (69, 75, 76). Hypoxia-responsive promoters carry the added benefit of enhancing oHSV efficacy in a tissue setting known to otherwise compromise the efficacy of conventional cancer treatments, such as chemotherapy and radiation. Furthermore, hypoxia is a tumor feature shared by many cancer types. This allows for a single vector design to be applied to multiple

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cancers, which can be enormously helpful in streamlining the translation to clinical applications.

Promoters can also be designed to respond to intercellular elements created by a particular genetic transformation found within the targeted tumor. Kuroda et al. demonstrated the efficacy of an oHSV vector, bM24-TE, which exploits dysregulation of the β-catenin/T-cell factor (TCF) in colorectal cancers. The *APC* mutation found in approximately 70% of colon cancers leads to constitutive expression of β-catenin, leading to intracellular levels of this protein in such colon cancer cells being radically higher than those seen in non-cancerous cells. oHSV strain d120 (a virus attenuated by *ICP4* deletion), was modified by reinserting *ICP4* under the control of a promoter which is activated upon exposure to β-catenin/TCF. *ICP4* is an essential gene in viral replication that allows the progression from a first wave of gene expression (the so-called immediate–early or α genes) to the second wave (the expression of the so-called early or βgenes). HSV-1 strains lacking a functional gene to encode for ICP4 are thus replication-defective, i.e., they are able to infect cells upon exposure, but unable to propagate the life cycle beyond that initial phase of infection (16, 77). Applying this agent to various cancer cell lines with and without β-catenin/TCF dysregulation, they demonstrated an obvious increase in efficacy in cancer lines overexpressing β-catenin/TCF as compared to those not carrying this mutation (78).

Pan et al. recently employed the same principle to target a mutation known to play a critical role in a wide range of cancers. They created an oHSV agent, “Signal-Smart 1”, which features a reinsertion of *ICP4* under the control of an Elk-responsive promoter in order to achieve selective expression in cells bearing *RAS* mutations (79) (Elk is constitutively expressed by carcinogenic *RAS* mutations). This intracellular signaling-responsive approach allows for the design of vectors tailored to specific signaling dysregulation, which could eventually be used to direct therapy to pathways known to be critical to resistance to conventional therapies. As such, these agents represent in a sense a new level of targeted therapy –an adjuvant therapy built to directly complement the principal therapy.

It should be noted that this approach of selectively engaging promoters to increase tropism can be applied inversely for the same effect. Along these lines, rather than using cancer-specific triggers to activate promoters of essential genes in cancer cells, expression of critical viral genes can be blocked in noncancerous cells. Lee et al. exploited the differential expression level of microRNA (miRNA) between normal and cancerous cells to repress the expression of the critical *ICP4* gene in normal cells by creating an oHSV vector that incorporated complementary miRNA sequences into the *ICP4* gene such that *ICP4* expression was blocked in cells expressing these specific miRNAs. By choosing as their triggers miRNAs known to be ubiquitously expressed in normal cells (and uniquely absent in the cancer treated), the viability of an otherwise significantly virulent vector was restricted to the targeted tumor cells (80). This strategy of using ubiquitous miRNA to repress oncolytic vectors in non-cancerous cells has also been used with HSV, and is likely to be applied to other families of oncolytic viruses as well. It is particularly attractive in that in (albeit indirectly) targeting cancer cells by their failure to express a normal epigenetic regulator, miRNA-sensitive vectors inherently target dedifferentiating cells. As such, those (more differentiated) cells which prove resistant to treatment would theoretically be more likely to respond to conventional treatments than they were before viral therapy. This is not to say that there are no concerns with this approach. Most notably, the attenuation of these vectors depends upon the supposition of universal expression of the selected miRNAs in healthy cells, which is something far easier extrapolated than proven.

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Viral spread: fusogenic proteins facilitate cell-to-cell vector distribution

One of the major impediments to the propagation of an oncolytic viral infection is the hostility of the extracellular environment. The large size of virions relative to typical therapeutic agents slows diffusion through the dense extracellular matrix within the tumor (81). The time spent in transit through the extracellular milieu increases the vector's exposure to immune mediators capable of sequestering virus and allows time for viral degradation (82). The efficiency of cell-to-cell spread of viral infection can be enhanced dramatically by circumventing this hostile extracellular environment, which can be accomplished through the use of fusogenic proteins.

Fusogenic glycoproteins (originally discovered in certain naturally occurring pathological viruses) cause infected cells to fuse their cell membranes with those of surrounding cells, forming a growing syncytium through which replicating virus can freely diffuse. Fu et al. constructed a vector that inserted a transgene encoding for the gibbon ape leukemia virus (GALV) fusogenic membrane glycoprotein into the vector genome. To ensure that fusogenic glycoprotein expression was limited to cancer cells, it was placed under the control of a late viral promoter, so that expression would only occur in cells which supported a full course of infection. Infection in vitro and in vivo against multiple cancer types with this virus led to improved antitumor efficacy compared to the parent (i.e., nonfusogenic) strain (83). Multiple vectors featuring transgenes encoding for fusogenic glycoproteins have since demonstrated impressive efficacy in a variety of cancer types and models, including both subcutaneous and diffuse peritoneal colon cancer, metastatic ovarian cancer and renal cell cancer (43, 84–90).

ARMING VECTORS: INSERTION OF GENES ENCODING FOR ANTICANCER PROTEINS

In addition to manipulating the infection cycle, one can also augment the anticancer efficacy of an oncolytic viral agent by using it to engage an additional anticancer mechanism. By incorporating into the vector a transgene which expresses a protein whose function supports tumor destruction, two parallel mechanisms of tumor destruction (viral lysis and the mechanism mediated by the transgene protein) can be achieved through a single agent. Genes are thus inserted into the oHSV genome such that, upon infection of the target cell, a protein is expressed which triggers a local anticancer process. Because these "arming" genes do not interfere with the mutations used to attenuate infection in noncancerous cells, they can be inserted under the control of native viral promoters rather than engineered tumor-responsive promoters.

The first exogenous genes incorporated into oncolytic vectors were immunomodulatory cytokines employed to stimulate the host immune response to tumor cells. Ironically, the first report of an oHSV vector armed with a cytokine-coding gene described this vector as an experimental method used to probe the role of innate immunity in the response to viral oncolysis. Adreansky et al. compared the standard tumor response in a murine glioma model infected with a basic double-*RL1*-negative virus, to the tumor response seen when the same virus was armed with either IL-10 or IL-4. It was found that although IL-10 significantly limited therapeutic response, insertion of a gene coding for IL-4 led to a significant increase in tumor reduction (91). The recognition that local delivery of cytokines could augment or inhibit tumor therapy, possibly by engaging the host's own immune system against infected tumor cells, led to widespread development and investigation of vectors containing cytokine-coding genes to augment virus treatment efficacy, as well as to better understand the potential role of immunotherapy in the field of oncolytic virotherapy (92–97). Arming oHSV vectors with immunostimulatory genes allows treatment with a single agent to

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function through two separate treatment modalities: 1) as an infective agent that eradicates tumors through direct viral oncolysis; and 2) as an immunotherapy which re-engages and augments native anticancer immune responses. Immunotherapy is a particularly attractive modality to complement viral oncolysis, as the very presence of the virus alone naturally instigates a rich and complex immune response. The addition of a transgene which expresses an immunomodulatory protein can then be used to harness and exploit that response. As such, this approach has been widely applied in preclinical models and clinical trials. oHSVs armed with immunomodulators continue to be popular among oHSV laboratories, and continue to be studied in ongoing clinical and preclinical investigations of oHSV therapy (94, 96, 98–103).

Another way in which vectors have been armed with host genes in order to engage host systems involves the use of genes which code for antiangiogenic proteins. Increased local angiogenesis is a natural sequela of wild-type HSV infection, which appears to persist in at least some oHSV strains, which can act counter to the agent's antitumor effect. Aghi et al. demonstrated that restoring thrombospondin, an antiangiogenic protein downregulated in the setting of HSV infection, by inserting the thrombospondin gene into the G207 vector eliminated the angiogenic response to treatment, which translated into a more sustained treatment response in a murine glioma model (104). Additional examples of antiangiogenic protein genes which have been incorporated into oHSV vectors include genes coding for platelet factor 4, endostatin, endostatin–angiostatin fusion protein and double-negative fibroblast growth factor (FGF) (105–108).

A particularly interesting example of a host system engaged by armed oHSVs is the case of the metalloproteinases, proteins expressed by a variety of cells which degrade certain adhesion proteins within the extracellular matrix. Curiously, both tissue metal-loproteinase-expressing genes and tissue metalloproteinase inhibitor-expressing genes have been shown to increase tumor reduction when added to oHSV vectors. Mahler et al. reasoned that since tumor-secreted metalloproteinases facilitate tumor invasion into surrounding tissues, engineering a vector to express a metalloproteinase inhibitor would enhance its antitumor effect. Applying such a vector (rQT3) to murine models of neuroblastoma and peripheral malignant nerve sheath tumors, they found that, compared to the parent virus, treatment with the metalloproteinase inhibitor-armed vector led to increased tumor reduction and a significant antiangiogenic response (109). However, in the case of sarcoma, the dense extracellular matrix likely interferes with the cell-to-cell spread of replicating oncolytic virus. As such, Mok et al. reasoned that augmenting local expression of metalloproteinase inhibitors would improve the distribution of virus throughout the tumor. They found that transfecting sarcoma cells with genes expressing metalloproteinases did indeed increase the intratumoral distribution of virus and tumor reduction in a murine sarcoma model (110). This highlights the fact that the impact of arming an oHSV with a particular gene frequently depends upon the tumor targeted by that vector. While, in contrast to tumor-selective promoters, expression may be fairly uniform across cancer types, the effect of local expression of a particular protein depends in part on the tumor biology of the cancer treated. Thus, while certain oHSVs demonstrate efficacy in a wide variety of cancers, there is likely much to be gained from specifically tailoring vectors to the disease they target.

In addition to using proteins that directly interact with host systems, oHSVs can be armed with enzymes that convert prodrugs into active forms to allow for ultralocalized chemotherapy. As modern oHSVs are typically ganciclovir-sensitive, it was initially hypothesized that systemic treatment with ganciclovir during a course of viral oncolysis would enhance cytotoxicity in infected cells. Initial experiments using ganciclovir in this manner showed that while cytotoxicity upon infection was augmented by ganciclovir treatment in some cancers, this benefit was negated by a concurrent reduction in viral

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propagation (111, 112). Nonetheless, the concept that the selective infection of viral oncolytic agents could be exploited to yield localized activity of systemically administered drugs was validated. Several vectors have since been engineered to express prodrugs that convert systemically administered prodrugs allowing for ultralocalized concentrations of (activated) drugs at levels previously unattainable without unacceptable systemic toxicity. Most commonly, vectors are armed with enzymes that convert prodrugs into activated forms of classic chemotherapeutic agents, such as cyclophosphamide, taxanes or alkylating agents (102, 113–118).

This approach can also be used to localize radiation therapy. Rather than prodrug conversion, HSV-facilitated localized radio-therapy utilizes cell membrane symporters to transport radioactive particles into the targeted cell. Using conventional genetic engineering techniques, multiple groups have found that transfection of the naturally occurring noradrenaline transporter gene (*NAT*) leads to expression of a functional symporter capable of importing radioactive iodine-131-*meta*-iodobenzylguanidine (MIBG) into the transfected cells (119–121). Quigg et al. demonstrated that arming an oHSV vector with *NAT* leads to the production of an NAT symporter. As such, infected cells were able to effectively concentrate ambient MIBG, leading to increased antitumor efficacy for the *NAT*-armed vector as compared to the unarmed control (122).

It should be emphasized that arming vectors to express prodrug-converting enzyme or radioactive particle transporters accomplishes something very different from traditional combination therapy (i.e., the simple addition of conventional chemo- or radiotherapy to a primary viral therapy). These are ultralocalizers, which expose the uninfected (i.e., virus-resistant) cells surrounding the infected cancer cells to chemo- or radiotherapeutic agents at concentrations unattainable through conventional therapeutic modalities. This represents the most promising basic aspect of armed vectors, the ability to exploit the vectors' ability to infect sensitive cells to deliver with extreme precision a second treatment specifically chosen to treat the nonsensitive cells nearby.

CONCLUSIONS

The field of oncolytic viral therapy has advanced significantly in the last decade. The improvements in viral genetic engineering have transformed this field from one employing natural viruses to one utilizing multiply altered viruses that are more specific to cancer and less toxic to man. These recombinant viruses have also reached clinical testing in man (Table III), and have proven to be safe and tolerable in phase I and II trials. Sufficient antitumor efficacy encourages future large-scale trials and further design refinements in this novel class of cancer treatments.

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Table I

Common oncolytic HSV vectors.

Virus (Ref.)	Mutations	Transgene	Comments
*Dl*sptk (26)	TK	–	First modern engineered oHSV vector Resistant to aciclovir
hrR3 (24, 123–126)	*UL39*	*lac Z*	Reporter gene, monitors viral replication Specifically infects dividing cancer cells
HSV1716 (18, 31, 32, 56, 127–129)	*RL1* (both copies)	–	Safety confirmed in phase I trial
NV1020 (101, 130–137)	*RL1* (one copy) *UL24*, *UL56*	HSV-2 segment	Originally developed as an HSV vaccine
G207 (133, 134, 136–146)	*UL39* *RL1* (both copies)	*lac Z*	Safety confirmed in phase I trial Effective against a broad array of tumors
G47Δ(36, 147–154)	*UL39* *RL1* (both copies) *α47*	*lac Z*	Enhanced antitumor immune response
NV1034 (97, 99, 100)	*UL56* *α47*	GM-CSF *lac Z*	Efficacy demonstrated in SCC xenografts
NV1042 (97, 99, 155)	*UL56* *α47*	IL-12 *lac Z*	Inhibits SCC xenograft growth Elicits memory tumor immunity
NV1066 (136, 156–161)	*RL1* (one copy) *α0*, *α4*, TK	GFP	Useful in detecting small tumor deposits Inhibits esophageal tumor xenografts
OncoVEX (162, 163)	*RL1*, *CP47*−	GM-CSF	

GM-CSF, granulocyte–macrophage colony-stimulating factor; SCC, squamous cell carcinoma.

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Table II

Tumor-responsive promoters used to enhance oHSV efficacy.

Trigger (Ref.)	Virus	Cancer	Promoter gene	Comments
DF3/MUC1 (164)	3616	Colon, melanoma, pancreas	*RL1*	Tumor-responsive promoters applied through helper virus
Hypoxia (76)	G207 + 10xHRE	Colon to liver	*RR1*	
CEA (68)	G207	CR	*RR1*	
AFP (69)	G207+	Liver (HCC)	*RR1*	
Androgen (prostate-specific) (74)	CgalΔ3	Prostate	*ICP4*	
Nestin (70)	rQNestin34.5	Brain	*RL1*	Nestin has been shown to play a critical role in HSV infection across a wide range of cell types
Musashi1 (71, 72)	dvM345: G207 + M345 KeM34.5: (G207)	Glioma, NSCLC Glioma	*RL1*	–
T-cell factor (78)	bM24-TE (hrR3)	Colon, HCC	*ICP4*	Response depended on the specific mutation in *APC* (between 1st and 2nd 20-amino-acid repeats)
URR16 (165)	HSPV-1	Oral cancer	*ICP4*	URR16 is an oral cancer-specific marker
B-Myb (166)	Myb34.5	Colon	*RL1*	–

HCC, hepatocellular carcinoma; CR, colorectal cancer; NSCLC, non-small cell lung cancer.

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Table III

Clinical trials of oncolytic HSV agents.

				Results		
Phase	**Tumor type**	**Vector**	**Number of patients**	**Number of patients with response[1]**	**Adverse events**	**Study**
I	GBM	G207	21	0	None	Markert et al. (2000, USA) (60)
I	Melanoma	1716	5	1 (20%)	None	MacKie et al. (2001, UK) (59)
II	GBM	1716	12	N/A	None	Papanastassiou et al. (2002, UK) (58)
I	GBM	1716	12	2 (17%)	None	Harrow et al. (2004, UK) (57)
I	Oral SCC	1716	20	2 (10%)	No	Mace et al. (2008) (56)
I	Colorectal liver metastases	NV1020	12	2 (17%) (11/12 showed decrease in CEA levels)	10 grade 3–4 events in 3 patients	Fong et al. (55) and Kemeny et al. (54) (2006, 2009, USA)
I	Recurrent breast cancer	HF10	6	1 (15%) (some histological changes noted in tumor following treatment)	None	Teshigahara et al., Watanabe et al., Kohno et al., Nakao et al. and Kimata et al. (2004–2007, all in Japan) (45–49)
I	Pancreatic cancer		3	(0/3) Decrease in CA19-9 found in 1/3 patients		
I	Recurrent glioma	1716	9	0	No	Rampling et al. (2000, UK) (53)
I	SCC metastases	OncoVEX	8	N/A	N/A	Hu et al. (2003, UK) (167)
II	Melanoma	OncoVEX	50	13 (26%) (8 CR, 5 PR)	14 grade 3, no grade 4	Senzer et al. (2009, USA/UK (168)
I/II	SCC, head and neck	OncoVEX (with chemoradiation therapy)	17	13 (76%)	17 grade 3–4 AEs[2]	Harrington (2009, UK) (169)
III	Metastatic melanoma (stage III and IV)	OncoVEX–GM-CSF	Estimated enrollment 430, still recruiting (tx and control with GM-CSF only)	N/A	N/A	http://www.biovex.com/oncovex.html www.clinicaltrials.gov

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Phase	Tumor type	Vector	Number of patients	Results		Study
				Number of patients with response[1]	Adverse events	
III	SCC, head and neck	OncoVEX–GM-CSF	Estimated enrollment	N/A	N/A	http://www.biovex.com/oncovex.html www.clinicaltrials.gov

[1] Response defined as decrease in size of tumor (either on physical exam or radiological evaluation) following treatment.

[2] Protocol including adverse events (AEs) attributable to concurrent chemoradiation therapy. N/A, not available; CR, complete response (local); PR, partial response; GBM, glioblastoma multiforme; SCC, squamous cell cancer; CEA, carcinoembryonic antigen.