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Anti-viral immunity in the tumor microenvironment: implications for the rational design of herpes simplex virus type 1 oncolytic virotherapy

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Abstract

Purpose of review: The design of novel herpes simplex type I (HSV-1)-derived oncolytic virotherapies is a balancing act between safety, immunogenicity and replicative potential. We have undertaken this review to better understand how these considerations can be incorporated into rational approaches to the design of novel herpesvirus oncolytic virotherapies.

Recent findings: Several recent papers have demonstrated that enhancing the potential of HSV-1 oncolytic viruses to combat anti-viral mechanisms present in the tumor microenvironment leads to greater efficacy than their parental viruses.

Summary: It is not entirely clear how the immunosuppressive tumor microenvironment affects oncolytic viral replication and spread within tumors. Recent work has shown that the manipulation of specific cellular and molecular mechanisms of immunosuppression operating within the tumor microenvironment can enhance the efficacy of oncolytic virotherapy. We anticipate that future work will integrate greater knowledge of immunosuppression in tumor microenvironments with design of oncolytic virotherapies.

Introduction

The utility of genetically engineered HSV as “antineoplastic agents” was first demonstrated in 1991 (1) and led to the first FDA approval of a herpesvirus oncolytic agent in 2015. This first report used a virus created in the Knipe laboratory that possessed a small deletion in the middle one-third of the HSV-1 thymidine kinase (tk) gene (2). The tk mutation rendered the

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Conflicts of Interest

Dr. Kousoulas reports non-financial support from Ios Biomedical Group, Inc (IBG), outside the submitted work; In addition, Dr. Kousoulas has a patent “vaccines against genital herpes infections” (Patent number: 10130703) licensed to Ios Biomedical Group, Inc, and a patent “synthetic herpes simplex viruses type-1 for treatment of cancers” (Patent number: 8586028) issued.

Human and Animal rights and Informed Consent

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virus significantly impaired for replication in non-dividing cells such as neurons and was proposed to attenuate its neurovirulence potential. Since then there have been many approaches to the development of HSV-derived viruses for oncolytic virotherapy (3–6).

T-VEC™ is the first FDA approved oncolytic virus (4). T-VEC™ is an attenuated herpes simplex virus-1 (HSV-1) derived from the JS-1 viral strain and engineered to have deletions in the genes encoding the infected cell protein (ICP) γ 34.5 and ICP47 protein (7). T-VEC™ also encodes the human cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF functions as a potent stimulator of antitumor immunity by promoting the recruitment and maturation of macrophages and dendritic cells (DCs) (7). The GM-CSF gene is inserted in place of the two deleted γ 34.5 genes. The γ 34.5 protein, a major viral neurovirulence factor, is required for efficient viral replication in normal tissues (8). γ 34.5 reverses a cellular block to protein synthesis in infected cells (9). HSV-1 infection leads to activation of double-stranded RNA-dependent protein kinase R (PKR) and activated PKR then phosphorylates eukaryotic initiation factor 2 alpha (eIF2 α), significantly reducing viral and host protein synthesis in infected cells (10). The γ 34.5 protein reverses phosphorylation of eIF2 α restoring protein synthesis (11). Additionally, γ 34.5 has been reported to have several other functions including blocking IFN-I responses and autophagy (10, 12–14). The γ 34.5 gene deletion has been included in T-VEC™ and the majority of HSV-1 oncolytic virotherapy (OVT) candidates (15). The ICP47 gene functions to inhibit the transporter associated with antigen presentation (TAP) in HSV-1 infected cells (16) and blocks CD8+ T-cell responses (17). Inactivation of ICP47 was proposed to induce a more effective antitumor immune response (7). Furthermore, the deletion of the ICP47 gene up-regulates the expression the unique short (US) 11 gene that, due to some functional redundancy, compensates γ 34.5-deleted HSV-1 replication in tumor cells (7, 18, 19).

Mechanisms for increasing the safety of HSV oncolytic vectors mainly include the selective replication/infection in cancer cells. Approaches to accomplish this include: 1) deletion or mutation of genes that facilitate replication in normal cells, while allowing replication in cancer cells (2, 20) which can compensate for the missing function. 2) targeting virus infection to specific cancer cell receptors (21–23), 3) miRNA target sites engineered into HSV genes (24, 25), and 4) transcriptional and translational control of essential viral gene expression (26, 27). Many of these approaches also enhance safety by attenuating neurovirulence, a key aspect of herpes simplex pathogenesis (28). More recently, identification of novel determinants of neurovirulence including defects in entry into neurons (29–33), as well as retrograde and anterograde transport (34) in neurons are being incorporated into novel HSV-1-derived oncolytic viruses.

Many characteristics of herpesviruses inform their use as oncolytic virotherapy agents. These include the incredible amount of knowledge regarding the molecular biology of these viruses, the large coding capacity facilitating transgene expression, relative safety, and ease of genetic manipulation. With the approval of T-VEC in 2015 there has been an explosion of next generation approaches to herpesvirus OVT. These next generation candidates seek to address perceived shortcomings in the viral vector, focus tropism, as well as use hypothesis driven approaches to address the unique aspects of tumor microenvironments by arming

viruses with additional transgenes that can modulate and enhance anti-tumor immune responses (5, 35, 36).

The ease with which recombinant HSV can be created has led to a significant increase in the generation and testing of novel recombinant HSV for OVT (21, 35, 37, 38). A critical aspect of the design of OVT vectors is balancing between viral attenuation for safety reasons and enhanced replication and immunogenicity (39). T-VEC was derived from a clinical HSV-1 isolate and chosen for its superior ability to replicate and lyse in a number of cancer cell lines (7). The parental JS1 strain was then attenuated with the γ 34.5 and ICP47 deletions. As described above, these viral gene products play an extensive role in subverting anti-viral immunity. It is not entirely clear how these mutations affect the overall efficacy of HSV-1 OVT. Additionally, γ 34.5 protein is a structural component of the virion particle, functions in assembly and egress, and its deletion reduces overall production of infectious virions (40, 41).

Interestingly, recent approaches have chosen to revert or more precisely engineer some of the mutations in T-VEC™ to achieve greater therapeutic efficacy (15, 42). This brief review will focus on recent work suggesting that, contrary to the past direction of HSV-oncolytic vector design, it may best serve this promising therapy to enable evasion of anti-viral immunity to boost their efficacy.

Anti-viral responses and HSV-1 OVT efficacy

Several recent reports support the argument that perhaps more attenuation is not better and that more effective oncolytic virotherapy agents will be those with greater replicative potential. To facilitate their replication and spread as well as lifelong infection of hosts herpesviruses are prolific modulators of host immunity(43). Restoration of TAP function in T-VEC™ infected cells was hypothesized to enhance its immunogenicity. However, this hypothesis was never formally tested. Contrarily, Pourchet et al. hypothesized that generating viruses that were better able to evade CD8+ anti-viral T-cells may lead to greater anti-cancer benefit (42). To test this hypothesis this group created an HSV-1-derived oncolytic virus that replaced γ 34.5 genes with the bovine herpesvirus (BHV) UL49.5 and US11 genes under the control of an immediate early promoter (BV49.5, for a list of HSV OVT viruses discussed in this text please refer to Table 1). UL49.5 and HSV ICP47 are functional homologs (44). Both BHV UL49.5 and HSV ICP47 disrupt MHCI antigen processing and presentation via TAP disruption. However, while ICP47 competes for peptide binding UL49.5 function leads to proteasomal degradation of TAP (44) and subsequent downregulation of MHCI (45). Mutations in the BHV UL49.5 domain required for TAP binding and degradation have been incorporated into a BHV vaccine reported to possess superior efficacy (46). While HSV ICP47 does not inhibit murine TAP, BHV UL49.5 is capable of binding both murine and human TAP. Thus, using the BHV UL49.5 allowed them to test whether ICP47 makes a positive or negative contribution to the efficacy of the OVT in murine models. In a murine model of bladder cancer (MBT2) BV49.5 was compared to a virus that possessed a point mutation precluding expression of UL49.5 (BV49.5-FS) (42). BV49.5 reached higher titers in tumors and demonstrated greater reduction of tumor growth in injected and non-injected tumors than BV49.5-FS. Importantly, this greater efficacy was

shown to be characterized by greater numbers of activated tumor specific T-cells and the benefit was abolished in mice in which CD8+ T-cells were depleted. Interestingly, when these viruses were altered to express murine GM-CSF the GM-CSF expression did not result in detectable improvement. Further, in the 4T1 murine model of breast cancer, BV49.5 was more effective in reducing the number of metastases in the lungs, which was similarly dependent on UL49.5 expression and CD8+T-cells (42).

It is not clear how exactly disruption of TAP by this virus endows it with greater ability to shrink tumors and facilitate the generation of anti-tumor T-cells. Beyond better replication and spread in tumors which is expected to liberate greater numbers of tumor associated antigens (TAAs), the authors speculate that, based on what is known about antigen processing and presentation in tumors that are TAP deficient (47, 48), perhaps there is generation of unique anti-tumor T-cells recognizing new T cell epitopes. Indeed, novel epitopes generated via immunotherapies including OVT has been shown to be correlated with efficacy (49, 50). The authors note that it is also important to consider the high seroprevalence of HSV-1 in the human population (51). It is expected that the majority of individuals receiving HSV-1-derived oncolytic virotherapy will be HSV-1 seropositive. While Pourchet et al. (42) and others (7, 52) have demonstrated that there is no difference in OVT efficacy between mice that were pre-exposed to HSV-1, the maximum benefit of HSV-1 derived OVT may require the full complement of immune evasion gene products, such as ICP47, in seropositive individuals.

While the previous study analyzed the contribution of evasion of adaptive anti-viral immune responses via disruption of TAP most studies have focused on innate immunity in the tumor microenvironment. A recent study used a novel oncolytic virus to evade NK cell immune responses in the tumor microenvironment and enhance the efficacy of HSV-1 OVT (36). The authors overexpressed E-cadherin using the HSV-1 pIE4/5 promoter in an HSV-1 backbone with deletions in γ 34.5 and ICP6, the viral ribonucleotide reductase. E-cadherin is a cellular protein involved in cellular adherence, as well as acting as a ligand for the inhibitory NK cell receptor KLRG1+ (53). Overexpression of E-cadherin on infected cells was hypothesized to fulfill two functions: 1) inhibit NK cell activity and 2) facilitate cell-to-cell spread by concentrating nectin-1, the HSV-1 entry receptor (54) and a cofactor in E-cadherin cell-cell adherence (55). Glioblastoma cells (Gli36, U251, U87, GBM30) infected with the resulting virus, OV-CDH1, were less susceptible to lysis by KLRG1+ NK cells compared to cells infected with control virus. However, when KLRG1- cells were used, no differences in cell killing were detected (36). In vitro, OV-CDH1 exhibited enhanced plaque sizes in monolayers of human glioblastoma cell lines, Gli36 or U251 compared to control virus plaque sizes. Additionally, the expression of E-cadherin was shown to lead to improvement of viral entry and maximum viral titers. The in vivo efficacy of OV-CDH1 was evaluated using both xenograft (GBM30, U87dEGFR) and immunocompetent (Gl261N4) mouse models of glioblastoma. OV-CDH1 was superior to control viruses at reducing tumor rates of growth and providing significant survival benefit in all models tested. Further, in the immunocompetent model, viral titers of OV-CDH1 virus were one log greater than control virus. Interestingly, while depletion of NK cells resulted in greater efficacy for both OV-CDH1 and control viruses a significant difference between virus treatments remained

suggesting that perhaps the ability of OV-CDH1 to achieve greater spread was a more important contributor to the efficacy of OV-CDH1 than NK cell evasion.

Several other recent reports in glioblastoma support the idea that early suppression of, at least, innate responses can positively affect the outcome of OVT. A single dose of TGF β 1 (intravenous) prior to OVT of nude mice bearing aggressive U87dEGFR tumors was shown to increase oncolytic HSV viral titers in tumors (56). TGF β is a pleiotropic, secreted cytokine that has been shown to be a significant contributor to immunosuppression in the tumor microenvironment (57). In both xenograft (GB3) and syngeneic (4C8) glioblastoma mouse models TGF β 1 treatment was found to decrease NK cell, macrophage and microglia infiltration into tumors (56). This reduction was correlated with reduced tumor growth rates and a significant increase in median survival time after OVT. While TGF β 1 is a broad spectrum suppressor involving multiple cell types the group was able to replicate these findings with targeted depletion of NK cells using anti-asialo-GM1 antibody (56). These findings suggest that the cell population targeted with TGF β 1 is NK cell.

In another recent study, in both a xenograft (U87dEGFR) and syngeneic (KR158dEGFR) models of glioblastoma, infiltration of activated NK cells began at two hours post treatment with HSV OVT (rQNestin34.5) (58). Specific NK cell subsets expressing the activatory NK cell receptors NKp30 and NKp46 were identified as responsible for killing of HSV OVT infected cells. This group showed that OVT efficacy and viral titers were significantly enhanced with antibody depletion of NK cells. Interestingly, it was further shown that NK cells orchestrate macrophage activation in this model, evidenced by attenuation of macrophage activation in NK depleted animals.

A role for macrophage and microglia cells in anti-viral responses limiting viral replication and OVT efficacy has been reported in a number of studies. Addition of microglia to in vitro culture of U87 glioblastoma cells reduced viral growth and oncolytic potential (59). This reduction was demonstrated to be the result of phagocytosis of virus by microglia and subsequent block of viral gene expression in these cells. The authors found that inhibition of viral replication after phagocytosis by microglia was dependent on STAT1 and STAT3 activities. Importantly, the treatment of mice with STAT inhibitors in the U87 GBM xenograft model led to increased viral replication and decreased tumor growth rates. Another recent study has identified a role for macrophage and microglia cells in limiting OVT efficacy in GBM (60). In this study inhibition of viral replication was shown to be mediated by TNF α secreted by macrophage or microglial cells in vitro. Importantly, this group demonstrated that inhibition of TNF α in an athymic U87dEGFR human GBM model increased viral replication which correlated with an increased survival benefit(60).

An interesting approach by Thorne et al, using The Cancer Genome Atlas (TCGA) reported that low levels of CCN1 mRNA were positively associated with better survival outcome in glioblastomas (61). CCN1 is a secreted protein found in the tumor microenvironment and involved in upregulation of IFN-I responses and inflammation (62). This group hypothesized that neutralization of CCN1 may have some therapeutic value during HSV-1 oncolytic virotherapy. Anti-CCN1 treatment of subcutaneous tumors led to decreased macrophage and NK cell tumor infiltration. Mechanistically, the group demonstrated that binding of CCN1 to

integrins on the cell surface of macrophages upregulated the expression of pro-inflammatory genes. Anti-CCN1 treatment reversed much of the macrophage mediated viral clearance in vitro. In subcutaneous glioblastoma tumors treated with anti-CCN1 prior to OVT all mice demonstrated partial response or stable disease before progression as compared to two mice showing partial response in control groups.

A similar approach was taken with BAI1, a G-protein-coupled receptor involved in adhesion, which was found to have reduced expression in many solid tumors (63). BAI1 is expressed on macrophages and microglia, is a pattern recognition receptor, and the extracellular portion modulates phagocytosis (64, 65). This group hypothesized that expressing the extracellular fragment of BAI1 (Vstat120) using an oncolytic herpesvirus vector (RAMBO) could interfere with the antiviral activity of BAI1 (63). In an athymic model of glioblastoma (U87dEGFR) treated with RAMBO they reported decreased numbers and activation of infiltrating macrophages compared to those treated with control virus rHSVQ1. Further, the presence of Vstat120 rescued the suppression of viral replication in macrophages in vitro. In intracranial tumors, RAMBO demonstrated significantly more viral gene expression than rHSVQ. Corroborating the study of Meisen et al., this group found a similar contribution of TNF α expression to OVT efficacy. Specifically in the presence of anti-TNF α antibody rHSVQ1 replicated to levels similar to RAMBO in glioma cells co-cultured with macrophage, indicating that TNF α plays a large contribution in limiting the replication of rHSVQ1 in this system.

In a xenograft sarcoma model (A673) where the immunosuppressive TME is characterized by the infiltration and activation of M2 macrophage Denton et al, found that removing these cells via clodrosome or trabectedin resulted in greater efficacy of OVT (rRp450) with no accompanying increase in viral replication (66). M2 macrophages are major mediators of immunosuppression in this model (67). While trabectedin did not reduce A673 growth as a monotherapy, in combination with OVT trabectedin greatly reduced tumor growth and enhanced survival. Interestingly, the authors report that trabectedin reduced MDSCs and NK cells in addition to macrophages. Additionally, in this model the administration of OVT appeared to increase the number of M2-like tumor associated macrophages as well as MDSC. These results suggest that OVT in combination with pharmaceuticals that modulate the cellular constituents of the tumor microenvironment, specifically macrophage polarization, may enhance the therapeutic potential of HSV-1 OVT.

Conclusions

Herpesviruses establish latent infection for the life of the host as part of their life cycle and therefore are expected to promote quantitatively and qualitatively different immune responses than acute pathogens such as adenoviruses or poxviruses. With their armament of immune modulatory gene products, herpesviruses orchestrate innate and adaptive immune responses to suit the establishment and maintenance of latent infection and subsequent reactivation to maintain the latent reservoir. Indeed, as lack of effective antiviral T-cell responses lead to unchecked virus replication, the development of anti-viral T-cell responses can be seen as part of the life cycle of the herpes virus. In this sense, using herpesviruses as vectors for generating immune responses against infectious disease agents and tumors is a

rational application of their unique biology. To take advantage of the biology of herpesvirus infection for such applications a growing number of studies support the retention or even enhancement of immune evasion function to promote greater replication and spread in tumors.

It is a bit of a paradox that an immunosuppressed tumor microenvironment may be hostile to efficient viral replication. However, suppression of anti-tumor immunity does not necessarily equal suppression of anti-viral immunity. A complex interplay of viral and host factors will need to be understood in order to generate more efficacious HSV-1 OVT. In the many cases of NK cell and macrophage depletion presented above it is fair to ask – are we removing a population of cells that participates in mediating immunosuppression in the tumor microenvironment, or are we allowing the virus to better replicate and spread through the tumor? Likely both make contributions. Many of the depletion experiments described above reported an increase in viral replication in tumors and we can presume that this mediates in part, the enhanced efficacy of treatment. However, Denton et al. did not report an increase in viral replication after depletion of macrophages or NK cells in their sarcoma model. In this case perhaps reduction of immunosuppression in the tumor microenvironment in addition to enhanced immunogenicity is the more important factor contributing to the development of anti-tumor responses.

Interestingly, most of the studies of the kind reported here involve the subversion of innate immune responses to restore or enhance efficacy of HSV-1 OVT. In the cases of glioblastoma xenografts in athymic mice the enhancement of efficacy can only be due to innate immunity rather than the development of an anti-tumor T-cell response. However, the efficacy of most immunotherapies is CD8+ T-cell mediated. Therefore, a focus on mechanisms that facilitate the development of cell-mediated anti-tumor immunity is expected to make an outsized contribution to the translational potential of this work. In this respect it has been reported that NK cells can impair the development of antiviral T-cell responses including both effector and memory T-cells (68). Perhaps in some cancers subversion of NK cell immunity in combination with OVT will improve the development of anti-tumor T-cell responses.

We anticipate that next generation HSV-1 oncolytic virotherapies will focus on maintaining much of the replicative potential of HSV-1 while limiting its ability to spread to neurons where they can establish latency, potentially recombine with wild-type virus strains and subsequently reactivate to cause disease. As mentioned above, our lab has identified mutations that block entry of HSV-1 into neurons (29). This virus, VC2, has shown promise as an anti-HSV-1 and HSV-2 live-attenuated vaccine (30, 31, 69, 70). VC2, maintains its replicative potential and in a mouse model of melanoma demonstrates significant efficacy as well as a potent ability to disrupt the tumor microenvironment (Rider et al. manuscript in preparation). Others have included mutations that disrupt transport in neurons (34) in combination with miRNA target sites inserted into essential genes which precludes their expression in healthy tissues (71). This virus has been reported to demonstrate potent anti tumor activity in vivo (71).

It is becoming increasingly clear that understanding mechanisms of immunosuppression in the tumor microenvironment and the ways in which oncolytic herpesviruses affect and are affected by this environment will be important to informing the rational design of next generation oncolytic viruses. Adding complexity, it is important to recognize that the diversity of cancers and the mechanisms of immunosuppression within those cancers makes generalization difficult. As such, there is likely not a “one size fits all” solution with oncolytic virotherapy. The support of studies detailing mechanisms of immunosuppression for individual malignancy types will be critical for aiding in the selection and design of HSV OVT vectors.

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References

1. 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(5007):854–6. [PubMed: 1851332]
2. Coen DM, Kosz-Vnenchak M, Jacobson JG, Leib DA, Bogard CL, Schaffer PA, et al. Thymidine kinase-negative herpes simplex virus mutants establish latency in mouse trigeminal ganglia but do not reactivate. *Proc Natl Acad Sci U S A*. 1989;86(12):4736–40. [PubMed: 2543985]
3. Ma W, He H, Wang H. Oncolytic herpes simplex virus and immunotherapy. *BMC Immunol*. 2018;19(1):40. [PubMed: 30563466]
4. Bommareddy PK, Patel A, Hossain S, Kaufman HL. Talimogene Laherparepvec (T-VEC) and Other Oncolytic Viruses for the Treatment of Melanoma. *Am J Clin Dermatol*. 2017;18(1):1–15. [PubMed: 27988837]
5. Harrington K, Freeman DJ, Kelly B, Harper J, Soria JC. Optimizing oncolytic virotherapy in cancer treatment. *Nat Rev Drug Discov*. 2019;18(9):689–706. [PubMed: 31292532]
6. Shae D, Baljon JJ, Wehbe M, Becker KW, Sheehy TL, Wilson JT. At the bench: Engineering the next generation of cancer vaccines. *J Leukoc Biol*. 2019.
7. Liu BL, Robinson M, Han ZQ, Branston RH, English C, Reay P, et al. ICP34.5 deleted herpes simplex virus with enhanced oncolytic, immune stimulating, and anti-tumour properties. *Gene Ther*. 2003;10(4):292–303. [PubMed: 12595888]
8. 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(4985):1262–6. [PubMed: 2173860]
9. Chou J, Roizman B. The gamma 1(34.5) gene of herpes simplex virus 1 precludes neuroblastoma cells from triggering total shutoff of protein synthesis characteristic of programmed cell death in neuronal cells. *Proc Natl Acad Sci U S A*. 1992;89(8):3266–70. [PubMed: 1314384]
10. Wilcox DR, Longnecker R. The Herpes Simplex Virus Neurovirulence Factor gamma34.5: Revealing Virus-Host Interactions. *PLoS Pathog*. 2016;12(3):e1005449.
11. He B, Gross M, Roizman B. The gamma(1)34.5 protein of herpes simplex virus 1 complexes with protein phosphatase 1alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 2 and preclude the shutoff of protein synthesis by double-stranded RNA-activated protein kinase. *Proc Natl Acad Sci U S A*. 1997;94(3):843–8. [PubMed: 9023344]
12. Verpooten D, Ma Y, Hou S, Yan Z, He B. Control of TANK-binding kinase 1-mediated signaling by the gamma(1)34.5 protein of herpes simplex virus 1. *J Biol Chem*. 2009;284(2):1097–105. [PubMed: 19010780]

13. Yordy B, Iijima N, Huttner A, Leib D, Iwasaki A. A neuron-specific role for autophagy in antiviral defense against herpes simplex virus. *Cell Host Microbe*. 2012;12(3):334–45. [PubMed: 22980330]
14. Trgovcich J, Johnson D, Roizman B. Cell surface major histocompatibility complex class II proteins are regulated by the products of the gamma(1)34.5 and U(L)41 genes of herpes simplex virus 1. *J Virol*. 2002;76(14):6974–86. [PubMed: 12072498]
15. Kanai R, Zaupa C, Sgubin D, Antoszczyk SJ, Martuza RL, Wakimoto H, et al. Effect of gamma34.5 deletions on oncolytic herpes simplex virus activity in brain tumors. *J Virol*. 2012;86(8):4420–31. [PubMed: 22345479]
16. Hill A, Jugovic P, York I, Russ G, Bennink J, Yewdell J, et al. Herpes simplex virus turns off the TAP to evade host immunity. *Nature*. 1995;375(6530):411–5. [PubMed: 7760935]
17. Goldsmith K, Chen W, Johnson DC, Hendricks RL. Infected cell protein (ICP)47 enhances herpes simplex virus neurovirulence by blocking the CD8+ T cell response. *J Exp Med*. 1998;187(3):341–8. [PubMed: 9449714]
18. Mohr I, Sternberg D, Ward S, Leib D, Mulvey M, Gluzman Y. A herpes simplex virus type 1 gamma34.5 second-site suppressor mutant that exhibits enhanced growth in cultured glioblastoma cells is severely attenuated in animals. *J Virol*. 2001;75(11):5189–96. [PubMed: 11333900]
19. Mulvey M, Camarena V, Mohr I. Full resistance of herpes simplex virus type 1-infected primary human cells to alpha interferon requires both the Us11 and gamma(1)34.5 gene products. *J Virol*. 2004;78(18):10193–6. [PubMed: 15331752]
20. Markert JM, Malick A, Coen DM, Martuza RL. Reduction and elimination of encephalitis in an experimental glioma therapy model with attenuated herpes simplex mutants that retain susceptibility to acyclovir. *Neurosurgery*. 1993;32(4):597–603. [PubMed: 8386343]
21. Menotti L, Avitabile E, Gatta V, Malatesta P, Petrovic B, Campadelli-Fiume G. HSV as A Platform for the Generation of Retargeted, Armed, and Reporter-Expressing Oncolytic Viruses. *Viruses*. 2018;10(7).
22. Goins WF, Hall B, Cohen JB, Glorioso JC. Retargeting of herpes simplex virus (HSV) vectors. *Curr Opin Virol*. 2016;21:93–101. [PubMed: 27614209]
23. Zhou G, Roizman B. Construction and properties of a herpes simplex virus 1 designed to enter cells solely via the IL-13alpha2 receptor. *Proc Natl Acad Sci U S A*. 2006;103(14):5508–13. [PubMed: 16554374]
24. Mazzacurati L, Marzulli M, Reinhart B, Miyagawa Y, Uchida H, Goins WF, et al. Use of miRNA response sequences to block off-target replication and increase the safety of an unattenuated, glioblastoma-targeted oncolytic HSV. *Mol Ther*. 2015;23(1):99–107. [PubMed: 25200130]
25. Marzulli M, Mazzacurati L, Zhang M, Goins WF, Hatley ME, Glorioso JC, et al. A Novel Oncolytic Herpes Simplex Virus Design based on the Common Overexpression of microRNA-21 in Tumors. *J Gene Ther*. 2018;3(1).
26. Lee CY, Bu LX, DeBenedetti A, Williams BJ, Rennie PS, Jia WW. Transcriptional and translational dual-regulated oncolytic herpes simplex virus type 1 for targeting prostate tumors. *Mol Ther*. 2010;18(5):929–35. [PubMed: 20179676]
27. Campbell SA, Mulvey M, Mohr I, Gromeier M. Attenuation of herpes simplex virus neurovirulence with picornavirus cis-acting genetic elements. *J Virol*. 2007;81(2):791–9. [PubMed: 17079296]
28. Roizman B, Knipe D, Whitley RJ. Herpes Simplex Viruses In: David M Knipe PH, editor. *Fields Virology*. Fifth ed: Lippincott Williams & Wilkins; 2006 p. 2501–602.
- 29. Jambunathan N, Charles AS, Subramanian R, Saied AA, Naderi M, Rider P, et al. Deletion of a Predicted beta-Sheet Domain within the Amino Terminus of Herpes Simplex Virus Glycoprotein K Conserved among Alphaherpesviruses Prevents Virus Entry into Neuronal Axons. *J Virol*. 2015;90(5):2230–9. [PubMed: 26656706] This publication identifies mutations in the amino terminus of HSV-1 gK which prevent entry into neurons via axonal termini.
30. Bernstein DI, Pullum DA, Cardin RD, Bravo FJ, Dixon DA, Kousoulas KG. The HSV-1 live attenuated VC2 vaccine provides protection against HSV-2 genital infection in the guinea pig model of genital herpes. *Vaccine*. 2019;37(1):61–8. [PubMed: 30471955]

31. Stanfield BA, Stahl J, Chouljenko VN, Subramanian R, Charles AS, Saied AA, et al. A single intramuscular vaccination of mice with the HSV-1 VC2 virus with mutations in the glycoprotein K and the membrane protein UL20 confers full protection against lethal intravaginal challenge with virulent HSV-1 and HSV-2 strains. *PLoS One*. 2014;9(10):e109890.
32. Saied AA, Chouljenko VN, Subramanian R, Kousoulas KG. A replication competent HSV-1(McKrae) with a mutation in the amino-terminus of glycoprotein K (gK) is unable to infect mouse trigeminal ganglia after cornea infection. *Curr Eye Res*. 2014;39(6):596–603. [PubMed: 24401006]
33. David AT, Saied A, Charles A, Subramanian R, Chouljenko VN, Kousoulas KG. A herpes simplex virus 1 (McKrae) mutant lacking the glycoprotein K gene is unable to infect via neuronal axons and egress from neuronal cell bodies. *MBio*. 2012;3(4):e00144–12.
- 34. Richards AL, Sollars PJ, Pitts JD, Stults AM, Heldwein EE, Pickard GE, et al. The pUL37 tegument protein guides alpha-herpesvirus retrograde axonal transport to promote neuroinvasion. *PLoS Pathog*. 2017;13(12):e1006741. This publication identifies mutation in HSV-1 UL37 which disrupt retrograde transport to the nucleus after viral entry into neurons.
35. Wirsching HG, Zhang H, Szulzewsky F, Arora S, Grandi P, Cimino PJ, et al. Arming oHSV with ULBP3 drives abscopal immunity in lymphocyte-depleted glioblastoma. *JCI Insight*. 2019;4(13).
- 36. Xu B, Ma R, Russell L, Yoo JY, Han J, Cui H, et al. An oncolytic herpesvirus expressing E-cadherin improves survival in mouse models of glioblastoma. *Nat Biotechnol*. 2018. In this study the authors generate an “armed” virus capable of expressing a ligand for an inhibitory NK cell receptor in infected cells. This enhances efficacy of oncolytic virotherapy.
37. Watanabe D, Goshima F. Oncolytic Virotherapy by HSV. *Adv Exp Med Biol*. 2018;1045:63–84. [PubMed: 29896663]
38. de Graaf JF, de Vor L, Fouchier RAM, van den Hoogen BG. Armed oncolytic viruses: A kick-start for anti-tumor immunity. *Cytokine Growth Factor Rev*. 2018;41:28–39. [PubMed: 29576283]
39. Marelli G, Howells A, Lemoine NR, Wang Y. Oncolytic Viral Therapy and the Immune System: A Double-Edged Sword Against Cancer. *Front Immunol*. 2018;9:866. [PubMed: 29755464]
40. Loret S, Guay G, Lippe R. Comprehensive characterization of extracellular herpes simplex virus type 1 virions. *J Virol*. 2008;82(17):8605–18. [PubMed: 18596102]
41. Brown SM, MacLean AR, Aitken JD, Harland J. ICP34.5 influences herpes simplex virus type 1 maturation and egress from infected cells in vitro. *J Gen Virol*. 1994;75 (Pt 12):3679–86. [PubMed: 7996163]
- 42. Pourchet A, Fuhrmann SR, Pilonis KA, Demaria S, Frey AB, Mulvey M, et al. CD8(+) T-cell Immune Evasion Enables Oncolytic Virus Immunotherapy. *EBioMedicine*. 2016;5:59–67. [PubMed: 27077112] This study describes restoration of TAP inhibition by HSV as enhancing the efficacy of HSV-1 oncolytic virotherapy.
43. Griffin BD, Verweij MC, Wiertz EJ. Herpesviruses and immunity: the art of evasion. *Vet Microbiol*. 2010;143(1):89–100. [PubMed: 20303681]
44. Koppers-Lalic D, Reits EA, Rensing ME, Lipinska AD, Abele R, Koch J, et al. Varicelloviruses avoid T cell recognition by UL49.5-mediated inactivation of the transporter associated with antigen processing. *Proc Natl Acad Sci U S A*. 2005;102(14):5144–9. [PubMed: 15793001]
45. Wei H, Wang Y, Chowdhury SI. Bovine herpesvirus type 1 (BHV-1) UL49.5 luminal domain residues 30 to 32 are critical for MHC-I down-regulation in virus-infected cells. *PLoS One*. 2011;6(10):e25742.
46. Chowdhury SI, Wei H, Weiss M, Pannhorst K, Paulsen DB. A triple gene mutant of BoHV-1 administered intranasally is significantly more efficacious than a BoHV-1 glycoprotein E-deleted virus against a virulent BoHV-1 challenge. *Vaccine*. 2014;32(39):4909–15. [PubMed: 25066735]
47. Lampen MH, Verweij MC, Querido B, van der Burg SH, Wiertz EJ, van Hall T. CD8+ T cell responses against TAP-inhibited cells are readily detected in the human population. *J Immunol*. 2010;185(11):6508–17. [PubMed: 20980626]
48. Oliveira CC, van Hall T. Alternative Antigen Processing for MHC Class I: Multiple Roads Lead to Rome. *Front Immunol*. 2015;6:298. [PubMed: 26097483]

49. Rajaraman S, Canjuga D, Ghosh M, Codrea MC, Sieger R, Wedekink F, et al. Measles Virus-Based Treatments Trigger a Pro-inflammatory Cascade and a Distinctive Immunopeptidome in Glioblastoma. *Mol Ther Oncolytics*. 2019;12:147–61. [PubMed: 30775418]
50. Gulley JL, Madan RA, Pachynski R, Mulders P, Sheikh NA, Trager J, et al. Role of Antigen Spread and Distinctive Characteristics of Immunotherapy in Cancer Treatment. *J Natl Cancer Inst*. 2017;109(4).
51. Looker KJ, Magaret AS, May MT, Turner KM, Vickerman P, Gottlieb SL, et al. Global and Regional Estimates of Prevalent and Incident Herpes Simplex Virus Type 1 Infections in 2012. *PLoS One*. 2015;10(10):e0140765.
52. Chahlav A, Rabkin S, Todo T, Sundaresan P, Martuza R. Effect of prior exposure to herpes simplex virus 1 on viral vector-mediated tumor therapy in immunocompetent mice. *Gene Ther*. 1999;6(10):1751–8. [PubMed: 10516725]
53. Ito M, Maruyama T, Saito N, Koganei S, Yamamoto K, Matsumoto N. Killer cell lectin-like receptor G1 binds three members of the classical cadherin family to inhibit NK cell cytotoxicity. *J Exp Med*. 2006;203(2):289–95. [PubMed: 16461340]
54. Geraghty RJ, Krummenacher C, Cohen GH, Eisenberg RJ, Spear PG. Entry of alphaherpesviruses mediated by poliovirus receptor-related protein 1 and poliovirus receptor. *Science*. 1998;280(5369):1618–20. [PubMed: 9616127]
55. Tachibana K, Nakanishi H, Mandai K, Ozaki K, Ikeda W, Yamamoto Y, et al. Two cell adhesion molecules, nectin and cadherin, interact through their cytoplasmic domain-associated proteins. *J Cell Biol*. 2000;150(5):1161–76. [PubMed: 10974003]
56. Han J, Chen X, Chu J, Xu B, Meisen WH, Chen L, et al. TGFbeta Treatment Enhances Glioblastoma Virotherapy by Inhibiting the Innate Immune Response. *Cancer Res*. 2015;75(24):5273–82. [PubMed: 26631269]
57. Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, Iglesias M, et al. TGFbeta drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature*. 2018;554(7693):538–43. [PubMed: 29443964]
58. Alvarez-Breckenridge CA, Yu J, Price R, Wojton J, Pradarelli J, Mao H, et al. NK cells impede glioblastoma virotherapy through NKp30 and NKp46 natural cytotoxicity receptors. *Nat Med*. 2012;18(12):1827–34. [PubMed: 23178246]
59. Delwar ZM, Kuo Y, Wen YH, Rennie PS, Jia W. Oncolytic Virotherapy Blockade by Microglia and Macrophages Requires STAT1/3. *Cancer Res*. 2018;78(3):718–30. [PubMed: 29118089]
60. Meisen WH, Wohleb ES, Jaime-Ramirez AC, Bolyard C, Yoo JY, Russell L, et al. The Impact of Macrophage- and Microglia-Secreted TNFalpha on Oncolytic HSV-1 Therapy in the Glioblastoma Tumor Microenvironment. *Clin Cancer Res*. 2015;21(14):3274–85. [PubMed: 25829396]
61. Thorne AH, Meisen WH, Russell L, Yoo JY, Bolyard CM, Lathia JD, et al. Role of cysteine-rich 61 protein (CCN1) in macrophage-mediated oncolytic herpes simplex virus clearance. *Mol Ther*. 2014;22(9):1678–87. [PubMed: 24895995]
62. Lobel M, Bauer S, Meisel C, Eisenreich A, Kudernatsch R, Tank J, et al. CCN1: a novel inflammation-regulated biphasic immune cell migration modulator. *Cell Mol Life Sci*. 2012;69(18):3101–13. [PubMed: 22527715]
63. Bolyard C, Meisen WH, Banasavadi-Siddegowda Y, Hardcastle J, Yoo JY, Wohleb ES, et al. BAI1 Orchestrates Macrophage Inflammatory Response to HSV Infection-Implications for Oncolytic Viral Therapy. *Clin Cancer Res*. 2017;23(7):1809–19. [PubMed: 27852701]
64. Park D, Tosello-Tramont AC, Elliott MR, Lu M, Haney LB, Ma Z, et al. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature*. 2007;450(7168):430–4. [PubMed: 17960134]
65. Das S, Owen KA, Ly KT, Park D, Black SG, Wilson JM, et al. Brain angiogenesis inhibitor 1 (BAI1) is a pattern recognition receptor that mediates macrophage binding and engulfment of Gram-negative bacteria. *Proc Natl Acad Sci U S A*. 2011;108(5):2136–41. [PubMed: 21245295]
66. Denton NL, Chen CY, Hutzen B, Currier MA, Scott T, Nartker B, et al. Myelolytic Treatments Enhance Oncolytic Herpes Virotherapy in Models of Ewing Sarcoma by Modulating the Immune Microenvironment. *Mol Ther Oncolytics*. 2018;11:62–74. [PubMed: 30505937]

67. Denton NL, Chen CY, Scott TR, Cripe TP. Tumor-Associated Macrophages in Oncolytic Virotherapy: Friend or Foe? *Biomedicines*. 2016;4(3).
68. Rydzynski C, Daniels KA, Karmele EP, Brooks TR, Mahl SE, Moran MT, et al. Generation of cellular immune memory and B-cell immunity is impaired by natural killer cells. *Nat Commun*. 2015;6:6375. [PubMed: 25721802]
69. Stanfield BA, Rider PJF, Caskey J, Del Piero F, Kousoulas KG. Intramuscular vaccination of guinea pigs with the live-attenuated human herpes simplex vaccine VC2 stimulates a transcriptional profile of vaginal Th17 and regulatory Tr1 responses. *Vaccine*. 2018;36(20):2842–9. [PubMed: 29655629]
70. Bernstein DI, Cardin RD, Pullum DA, Bravo FJ, Kousoulas KG, Dixon DA. Duration of protection from live attenuated vs. sub unit HSV-2 vaccines in the guinea pig model of genital herpes: Reassessing efficacy using endpoints from clinical trials. *PLoS One*. 2019;14(3):e0213401.
71. Kennedy EM, Farkaly T, Behera P, Colthart A, Goshert C, Jacques J, et al. Design of ONCR-177 base vector, a next generation oncolytic herpes simplex virus type-1, optimized for robust oncolysis, transgene expression and tumor-selective replication [abstract]. Proceedings of the American Association for Cancer Research Annual Meeting 2019 2019 Mar 29-Apr 3;Atlanta, GA. Philadelphia (PA): AACR; *Cancer Res* 2019;79(13 Suppl):Abstract nr 1455.

Table 1.

HSV-1 Oncolytic Vectors discussed in this review.

Oncolytic HSV-1	Strain	Mutations	Transgene inserted	Reference
T-VEC	JS-1	γ 34.5 , ICP47	Human GM-CSF	(7)
BV49.5	Patton	γ 34.5	BHV-1 UL49.5	(42)
OV-CDH1	Q1	γ 34.5 , ICP6-	Human <i>CDH1</i>	(36)
rQNestin34.5	F	γ 34.5 , ICP6	Nestin-Hsp68promoter- γ 34.5	(58)
RAMBO	F	γ 34.5 , ICP6-	Human Vstat120	(63)
rRp450	KOS	ICP6	Rat CYP2B1 gene	(66)