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Article

Preclinical Mouse Models for Analysis of the Therapeutic Potential of Engineered Oncolytic Herpes Viruses

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Abstract

After more than two decades of research and development, oncolytic herpes viruses (oHSVs) are moving into the spotlight due to recent encouraging clinical trial data. oHSV and other oncolytic viruses function through direct oncolytic cancer cell–killing mechanisms and by stimulating antitumor immunity. As further viruses are developed and optimized for the treatment of various types of cancer, appropriate predictive preclinical models will be of great utility. This review will discuss existing data in this area, focusing on the mouse tumor models that are commonly used.

Introduction

After two decades of clinical trials in human cancer patients, oncolytic viruses (OVs) are emerging as bona fide therapeutic agents (Andtbacka et al. 2015; Chiocca and Rabkin 2014; Kaufman et al. 2015; Lawler and Chiocca 2015; Lichty et al. 2014). OVs constitute a wide range of viruses (with human or other species specificity), including herpes simplex virus type 1 (HSV-1), adenovirus, reovirus, measles virus, vaccinia virus, and retrovirus, that selectively infect and replicate in tumor cells (reviewed by Chiocca 2002; Russell et al. 2012). Usually, OVs are delivered directly by injection into the tumor site or systemically, either by intravenous injection or within cellular carriers. OVs may be engineered for improved therapeutic efficacy, and preclinical studies have shown that combination with other agents can lead to synergistic antitumor effects.

Mechanisms of tumor selectivity are dependent on the specific viral agent. They may be inherent in some viruses and can also be achieved through engineering the viral genome in various ways; for example, by modifying the viral entry functions so they will target tumor-specific receptors and/or by deleting viral genes required for viral replication but whose function can be complemented by factors present in tumors but not in normal cells. General features of tumor cells that influence viral replication include elevated tumor metabolic activity compared with the majority of normal cells and interferon-mediated antiviral responses, which are often impaired in tumor cells, permitting virus selectivity (Stojdl et al. 2000). Selective viral replication has also been associated with tumor-driver mutations such as *CDKN2A* deletion in malignant glioma (e.g., Aghi et al. 2008).

A number of case studies and small trials that used different strains of viruses for cancer treatment were reported throughout the twentieth century (Chiocca 2002). These reports used wild-type and often crudely prepared clinical or laboratory viral isolates, and it was not until the beginning of the 1990s that the first genetically engineered OV with selective antitumor effects in a preclinical mouse model was reported (Martuza et al. 1991). Since then, a large variety of genetically engineered OVs have been developed and tested, both preclinically and in clinical trials for a variety of cancers.

OV tumor killing can be enhanced by engineering the viral genome to deliver additional payloads to tumor cells. A multitude of approaches have been used, including prodrug metabolizing transgenes (e.g., thymidine kinase [Aghi et al. 1999; Redaelli et al. 2012])

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and proapoptotic genes (e.g., TRAIL [Duebgen et al. 2014]), among others. In addition to their direct tumor cell–killing effects, OVs may also improve long-term antitumor immunity by directing immune responses against tumor antigens, and these effects can be further enhanced by incorporating immune stimulatory transgenes into the OV genome (Chiocca and Rabkin 2014; Lichty et al. 2014).

Numerous early stage clinical trials with OVs have been completed. Overall, these trials demonstrated safety of the OV approach with some occasional promising responses, as extensively reviewed elsewhere (e.g., Kaufman et al. 2015). The field received a major boost in 2015, with the report of the first successful large, randomized, phase III clinical trial of an OV. This trial was conducted in advanced melanoma using talimogene laherparepvec (T-VEC), an engineered immunostimulatory oncolytic type I herpes simplex virus (oHSV), which was engineered to express the immunostimulatory cytokine GM-CSF (Andtbacka et al. 2015; Kaufman et al. 2010; Lawler and Chiocca 2015). T-VEC treatment led to improved durable response rates and was shown to stimulate antitumor T cell responses. The success of T-VEC has been attributed to a combination of direct oncolysis and the induction of antitumor immunity. The effective study of OV action requires animal models that reproduce not only direct tumor-killing effects but also the immune activation effects of each virus. This concept is illustrated in Figure 1. This article will describe preclinical models used to date for assessment of oHSV action and the challenges for the development of effective models necessary to examine these agents and facilitate their development.

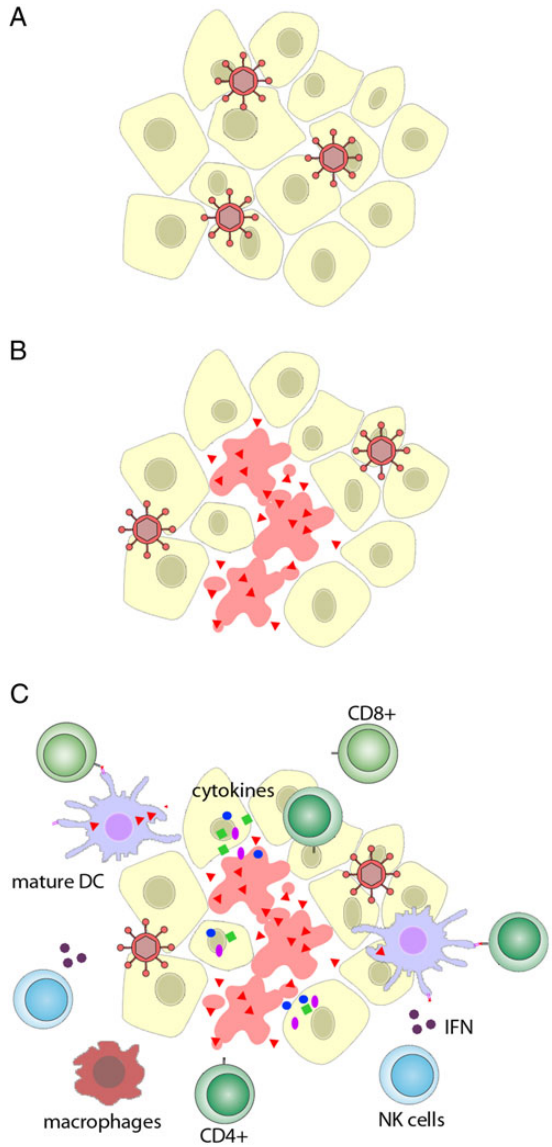

Figure 1 Antitumor effects of oncolytic herpes viruses (oHSV). The diagram illustrates the proposed dual mechanistic action of oHSV. This occurs through (A) an initial stage of infection followed by (B) cell killing. (C) Finally, immune cells are recruited, ultimately leading to the development of antitumor immunity.

Herpes Viruses as Oncolytic Agents

Engineered herpes viruses are some of the best studied oncolytic agents and have been extensively reviewed elsewhere (Grandi et al. 2009; Kanai and Rabkin 2013). Wild-type HSV-1 is an enveloped, double-stranded DNA virus with a linear 152 kb genome encoding approximately 80 genes, whose products are responsible for viral infectivity and/or replication, subversion of host antiviral mechanisms, and assembly of progeny virions. HSV-1 is widespread in the human population, where it usually resides in a latent form in trigeminal ganglia neurons and on reactivation causes the common oral cold sore and can also have more serious complications, including encephalitis (Corey and Spear 1986). HSV-1 has a rapid infectious cycle and can lead to cell lysis within 10 hours and release of new virus particles as the host cell undergoes lysis. In sensory neurons, latency is established with long-term viral genome persistence as a circular episome.

The rapid infection and lysis cycle of HSV prompted its development as an oncolytic agent. To minimize effects on normal cells and harness the oncolytic potential of HSV-1 a number of genetic deletions have been made, including γ34.5 (ICP34.5), which is responsible for neurovirulence and is essential for the dephosphorylation of eIF2α through the protein phosphatase PP1, which is crucial for sustained production of viral proteins in normal cells (reviewed by Peters and Rabkin 2015). All oHSVs that have been used clinically up until now feature γ34.5 deletion. A new virus, rQNestin34.5, planned for potential clinical trials in glioblastoma in the near future, has been developed in which γ34.5 expression has been restored under control of the tumor-specific nestin promoter. This virus was much more potent in glioblastoma mouse models than the parental γ34.5-deleted virus, even showing significant effects when administered to animals after symptoms were apparent (Kambara et al. 2005). Other common alterations in engineered oHSVs include the deletion of *UL39* encoding for the ribonucleotide reductase, ICP6, which creates a safer HSV with improved tumor selectivity (Aghi et al. 2008; Peters and Rabkin 2015). Deletion of ICP6 and γ34.5 were combined to create G207, the first oHSV to enter clinical trials in the United States (Markert et al. 2014; Martuza et al. 1991). The HSV protein ICP47 blocks antigen presentation by inhibition of TAP (transporter associated with antigen presentation) and therefore can block the recognition of HSV-infected cells by cytotoxic T cells (Goldsmith et al. 1998). Hence, deletion of ICP47, present also in G47delta and T-VEC, can enhance immune responses against the virus and subsequently potentially increase antitumor immunity.

One of the most fascinating aspects of virology is the study of host responses to viral infection. This is played out at the molecular, cellular, and systemic levels, involving the innate and adaptive immune systems. In fact, the host response to OV administration may define the therapeutic success or failure of this approach. In a number of experimental systems, the host response can eliminate the propagation of OVs (Chew et al. 2009;

Melchjorsen et al. 2009). The trick to successful virotherapy may be to ensure that sufficient viral replication occurs to harness the host immune responses to obtain a systemic durable antitumor effect. Initial host cell antiviral responses to oHSV infection are mediated by activation of interferon signaling, as well as NF-κB and nitric oxide synthase. Type I interferons (IFN-α and -β) are secreted in response to OV infection by activation of STING (stimulator of interferon genes) and can lead to the shutting down of viral protein synthesis and the alerting of neighboring cells to the presence of virus (Kalamvoki and Roizman 2014; Parker et al. 2015). Type II interferon (IFN-γ) is produced by immune and inflammatory cells such as macrophages recruited to the tumor site (Zaidi and Merlino 2011). These effects have been well studied in some animal models, as described herein. Several lines of evidence described in this review support the concept that oHSV can act as an in situ antitumor vaccine and stimulate a systemic immune response and antitumor immunity. Further studies in animal models are necessary to understand the interaction of oHSV with the adaptive immune system and predict the effectiveness of these agents.

Animal Models

To fully optimize oHSV-based therapies and provide meaningful mechanistic insights, it is important to have representative animal models of oncolysis in various tumor types. A large number of preclinical studies have shown efficacy of oHSV in rodent models (mainly mouse). An overview of the types of mouse model available to researchers is shown in Table 1. Preclinical studies have generated much promising data, which, in the cases where clinical trials were subsequently performed, did not translate into humans, with the notable exception of T-VEC (Turnbull et al. 2015). Thus, models with greater predictive potential would be useful. This creates some obvious conflicts because researchers who have created a novel virus may be reluctant to test it in the most challenging models, which may limit the perceived impact of their studies. Preclinical studies of oHSV face the challenge that human HSV-1 is not a natural pathogen in rodents and does not replicate well in rodent cells (Corey and Spear 1986).

Immunocompromised Models

By far the most commonly used models in the oHSV field are immunocompromised (nude) mice bearing implanted established human tumor cell lines. An overview of the range of models and examples of oHSVs used in the immunocompromised setting is shown in Table 2. These can be implanted with tumor cells subcutaneously in the flank or orthotopically. Orthotopic tumors offer the obvious advantages of appropriate microenvironmental conditions but are not always possible; therefore, subcutaneous models are commonly used. These models are generally relatively easy to work with and utilize tumor cells that can be labelled to facilitate imaging. Even though there are difference between the mouse and human immune systems, these models have been of great use in establishing proof-of-principle of the safety and efficacy of oHSV and in studying the

Table 1 Mouse models used in cancer and in oncolytic herpes viruses studies

Type of model	Advantages	Disadvantages	oHSV studies
Immunocompromised	• Can use any species cell of origin • Human cells readily support oHSV replication	• No adaptive immune system • Cannot preimmunize	+++
Subcutaneous	• Simplicity • Ease of virus delivery • Ease of measurement	• Incorrect tumor microenvironment	+++
Orthotopic	• Appropriate tissue site • More realistic microenvironment	• May be challenging to introduce tumors or virus to tumor site	+++
Established cell line	• Simplicity • Reproducibility • Easy to engineer	• Mutations may not be representative of parental tumor type	+++
Patient-derived low passage	• Authentic genetics • "Stem cell" components	• May have long latency • May be more challenging to engineer	++
PDX	• Authentic genetics • Adapted to mouse microenvironment	• May have long latency	No
Immunocompetent	• Presence of full immune system • Mouse syngeneic cell lines are readily available	• Not suitable for human tumor cells • Limited viral replication	++
Genetic models	• Appropriate tissue location • Realistic and defined driver mutations • Can preimmunize	• May not interact with immune system realistically because of lack of passenger mutations (i.e., neoantigens) • Animal monitoring and latency	No
Humanized	• Can use human cells • Human immune system	• Expensive, time consuming • Patient/blood matching challenging	No

PDX, patient-derived xenograft. +++, widely reported; ++, some studies have been performed.

Table 2 Examples of oHSV in preclinical immune competent models

Tumor model	Virus/combination	Outcome	Reference
Glioblastoma (orthotopic) U87ΔEGFR (cell line) balb/c-nu/nu mice	RAMBO HSVQ expressing VSTAT120 Cilengitide (integrin inhibitor)	Combination 39.5 days ($p < 0.005$ vs control) RAMBO 29 days Cilengitide and control 19 days	Fujii et al. (2013)
Glioblastoma (orthotopic) U87ΔEGFR (cell line) nu/nu mice	RqNestin - HSVQexpressing ICPγ34.5 under control of nestin promoter	rQNestin 7/9 mice LTS (> 90 days; $p < 0.0005$) HSVQ1 2/10 mice LTS Control 0 LTS (median = 20 days)	Kambara et al. (2005)
Glioblastoma (orthotopic) GBM8/BT74 (patient-derived stem-like cells) nu/nu mice	G47Δ Temozolomide O6benzylguanine	G47D/GBM8 plus TMZ 4/8 LTS median survival = 228 days; G47Δ alone vs G47Δ + TMZ, hazard ratio of survival = 7.1; $p = 0.003$	Kanai et al. (2012)
Cervical cancer (SQ) C33a in SCID mice Me180 in nude mice	G207 (ICP34.5 deleted, ICP6 insertion mutation) External beam radiotherapy (XRT)	Control (n = 11) 0 survivors XRT (n = 9) 0 G207 (n = 12) 0 XRT + G207 (n = 12) 5	Blank et al. (2002)
Glioblastoma (orthotopic) U87MG nu/nu mice	R3616 (inactivated γ34.5) Ionizing radiation	Control 0/19 survivors Radiation 3/30 R3616 4/33 R3616 and radiation 22/33	Advani et al. (1998)
Cholangiocarcinoma (SQ) KMBC, YoMi, SK-ChA-1 cells nu/nu mice	NV1023 (γ 34.5/UL24/UL56/US11/ICP47 deletions) External beam radiotherapy (XRT)	Tumor volume reduction (%, compared with control) KMBC YoMi SK-ChA-1 NV1023 50.0 37.1 27.5 XRT 53.5 24.6 68.1 NV1023 + XRT 80.0 85.9 75.1	Jarnagin et al. (2006)
Prostate cancer LNCaP human cell line (SQ) TRAMP-C2 mouse cell line nu/nu	G207 (ICP34.5 deleted, ICP6 insertion mutation) ionizing radiation	There was no therapeutic benefit from combining radiation with G207 in either a human or a mouse tumor model system.	Jorgensen et al. (2001)
Glioblastoma (orthotopic) U87ΔEGFR (cell line) athymic rats	hrR3 CPA CVF	Combination of intra-arterial hrR3, CVF, and CPA significantly increased the survival of athymic rats harboring intracerebral human glioma xenografts compared with other treatments.	Ikeda et al. (2000)
Glioblastoma (orthotopic) U87 (cell line) nude mouse	G207 (ICP34.5 deleted, ICP6 insertion mutation) TMZ	Combination 100% survival at 90 days G207 46 days TMZ 48 days	Aghi et al. (2006)
NSCLC (SQ) NCI-H460 (cell line) SCID mice	HSV-1716 Mitomycin C	Final mean burden of flank tumor (g) Control 1.406 ± 0.079 Combination 0.793 ± 0.047 (43.6% reduction) HSV-1716 1.127 ± 0.139 (19.8%) MMC 1.122 ± 0.070 (20.2%)	Toyoizumi et al. (1999)
Ovarian cancer (SQ) HRA Nude mice	HR522 (hrR3 derivative with syncytial phenotype) GCV	Survival over 60 days Combination (n = 9) 71% HR522 (n = 9) 22% GCV (n = 7) 0%	Nawa et al. (2003)
Melanoma (SQ) human MDA-MB-435S (435S) SCID mice	MGH2 (*ICP6/γ34.5* deletions, two prodrug-converting transgenes insertion) Paclitaxel-TRAIL (PT)	HSV and HSV in combination with PT significantly delayed the tumor growth compared with vehicle ($p < 0.001$). PT before virus injection was the most effective.	Nagano et al. (2008)

XRT, X-ray treatment; SQ, subcutaneous; NSCLC, non-small cell lung cancer; SCID, severe combined immunodeficient; TMZ, temozolomide; CPA, cyclophosphamide; CVF, cyclophosphamide, vinorelbine and 5-fluorouracil; LTS, long-term survivors; MMC, mitomycin C; GCV, ganciclovir; PT, paclitaxel/TRAIL.

interactions of oHSVs with the innate host immune system (Fu et al. 2016; Odegard et al. 2016; Palacios et al. 2014).

The most commonly used models use established cancer cell lines. These offer the advantages of simplicity and reproducibility but are not always fully representative of human tumors because of selection for growth in cell culture. Indeed, such cell lines may not faithfully preserve the genetics of the original tumor, and they also have biological differences (Domcke et al. 2013). For example, in the case of glioblastoma, established cell lines are not invasive in vivo, forming a circumscribed well-defined tumor with very little or no invasion of normal brain (Candolfi et al. 2007). Although some tumors are readily transplantable orthotopically, thus allowing cells to grow in the appropriate microenvironment, others are more challenging and are grown subcutaneously in the mouse flank and not in their natural tissue of origin. Despite these caveats, such models have been important in proof-of-principle studies for many oHSVs in tumors. However, truly predictive models would benefit from the use of fresh or low-passage patient-derived samples, which can be done either by the use of low-passage, patient-derived, cancer stem-like cells or by growing patient tumor samples in the flanks of animals immediately after resection and maintaining them by passaging in vivo. These patient-derived xenograft (PDX) models maintain tumor genetics and pathologic characteristics, making them a more challenging and realistic model for evaluation of therapeutic approaches, but they have not been reported in the literature as of yet with oHSV. Nonetheless, some studies have been performed with low-passage, patient-derived tumor cells. For example, neurosphere-type cultures from glioblastoma were responsive to oHSV (Wakimoto et al. 2009). These models have an invasive phenotype and preserve patient tumor genotype and, to some extent, tumor heterogeneity.

Immunocompromised mouse models have illustrated that there is rapid mobilization of innate immune cells in response to oHSV. Cells recruited to infected tumors include neutrophils, natural killer (NK) cells, macrophages (and microglia in the brain) (Alvarez-Breckenridge et al. 2012; Fulci et al. 2007). Immunocompromised models have a clear limitation in understanding the interaction of oHSV with the host because of their severely limited adaptive immune system (Belizário 2009). Nonetheless, they have been successfully used to investigate interactions between oHSV and angiogenesis, macrophages/microglia (Meisen et al. 2015), and NK cells (Alvarez-Breckenridge et al. 2012). These studies have shown that innate immunity is a major potential oHSV resistance mechanism, which can restrict the ability of the virus to replicate and spread within tumors (Ikeda et al. 1999; Wakimoto et al. 2003).

Moreover, immunocompromised models have been widely used to test therapeutic combinations with various small molecules, including chemo- and radiotherapy (Ottolino-Perry et al. 2010). The combination between OVs and standard chemotherapeutics has been widely tested using various immunocompromised cancer models (Aghi et al. 2006). In prostate tumors, OV plus docetaxel abolished the post-treatment regrowth of the tumor (Muthana et al. 2013) and was effective in combination with mitomycin C in non–small cell lung cancer (Toyoizumi et al. 1999). In head and neck squamous cell carcinomas, ovarian cancer, and glioma the combination of oHSV and bortezomib synergistically enhanced virus replication both in vivo and in vitro because of HSP90 induction and therefore improved the efficacy of oHSV therapy (Yoo et al. 2014). Other examples include combinations with cilengitide (Kurozumi et al. 2007) and histone deacetylase 6 inhibitors (Nakashima et al. 2015). Animal models have demonstrated the effectiveness of the combination of oHSV and irradiation in various tumor types, leading to curative effects in some models (Advani et al. 1998; Blank et al. 2002; Jarnagin et al. 2006; Jorgensen et al. 2001). To better simulate the clinical scenario, recurrent glioblastoma can be resected and the resistant cells further treated in situ—for instance, using mesenchymal stem cells loaded with oHSV-TRAIL (Duebgen et al. 2014), which delivers tumor necrosis factor–related apoptosis-inducing ligand (TRAIL) that has antitumor potential (Valley et al. 2012). oHSV-TRAIL amplified the effect of oHSV and induced apoptosis even in cells nonpermissive to oHSV and normally resistant to TRAIL (Duebgen et al. 2014). Drug combinations are also effective in oHSVs engineered to express prodrug metabolizing transgenes such as thymidine kinase (Nawa et al. 2003) and cytosine deaminase (Guffey et al. 2007).

Thus, immunocompromised rodent models have been used widely to explore a variety of approaches with oHSV. However, their limited adaptive immune system renders these models unsuitable for studies involving T cells, and they therefore cannot tell us anything meaningful regarding the in situ vaccine properties of these agents.

Immunocompetent Models

Effects of oHSV on the adaptive immune system are well established and have been brought to the limelight by the clinical success of T-VEC (Andtbacka et al. 2015). To examine oHSV interaction with the adaptive immune system, immunocompetent models are required. A number of studies over many years have used these models; examples of studies on oHSV in immunocompetent models are shown in Table 3. These have shown that oHSV, particularly when "armed" with immune-stimulatory transgenes, can stimulate a host antitumor T cell response and immunologic antitumor memory.

Large numbers of transplantable syngeneic tumor rodent models that recapitulate important features of human disease and are useful for testing therapeutic efficacy are available. However, there are complications in these types of studies because humans and mice have differences in the pathogenesis of HSV infection and immune responses (Blacklaws et al. 1987). Also, oHSV can have different effects based on both virus and mouse strain. For instance, HSV can induce demyelination in BALB/c, SJL/J, A/J, and PL/J mice, but not in BL/6 mice, where viral DNA and antigen-positive cells cannot be identified after infection (Kastrukoff et al. 2012). In addition, the oHSV field faces the challenge that, because of naturally occurring species barriers, human HSV does not replicate well in mouse cells, and there may in fact be potent natural mechanisms that mediate this process (Ahn et al. 1996). Recent studies have shown that human HSV infection of rodent cells leads to necroptosis, a caspase-independent form of cell death, driven by receptor-interacting protein kinase (RIP3) and its ligand mixed lineage kinase-like (MLKL), contributing to antiviral host defense (Mocarski et al. 2015). After human HSV-1 infection of mouse cell lines, ICP6 interacts with RIP3 through their RIP homotypic interaction motifs (RHIM), which induces RIP3 activation and subsequent necroptosis, and mice lacking RIP3 are permissive to HSV-1 replication and pathogenesis (Mocarski et al. 2015; Wang et al. 2014). Thus, this necrotic death pathway represents a cross-species barrier for oHSV in a non-natural rodent host.

Early syngeneic mouse models of human cancer involved in vivo induction of tumorigenesis with mutagens such as N-ethyl-nitrosourea (Huszthy et al. 2012). This generates tumors that, despite being genetically and histopathologically heterogeneous, can be propagated in culture, labelled for imaging purposes,

Table 3 Examples of studies on oncolytic herpes viruses in immunocompromised models

Tumor model	Virus/combination	Outcome (median survival)	Reference
Glioblastoma (orthotopic) D74(HveC) cell line Fischer 344 Rats	HrR3 Cyclophosphamide (immune suppressive agent)	Survival not measured Tumor macrophage/microglia infiltrates reduced Enhanced viral replication	Fulci et al. (2007)
Prostate cancer (flank) TRAMP-C2 mouse C57/BL6 mice	G207 (ICP34.5 deleted, ICP6 insertion mutation) Ionizing radiation	The combination of G207 and radiation did not enhance efficacy in the TRAMP syngeneic tumor model.	Jorgensen et al. (2001)
Neuroblastoma N18 cell line A/J mice	G207 (ICP34.5 deleted, ICP6 insertion mutation)	Intraneoplastic inoculation of G207 prolongs the survival of A/J mice bearing intracerebral N18 tumors ($p = 0.018$). Antitumor immunity was associated with an elevation of specific CTL activity.	Todo et al. (1999)
Colorectal carcinoma CT26 cell line BALB/c mice	G207 (ICP34.5 deleted, ICP6 insertion mutation)	Infection with G207 resulted in tumor growth reduction of both the inoculated tumors (Rt) and their noninoculated contralateral counterparts (Lt) when compared with mock-inoculated controls ($p < 0.0005$ [Rt] and $p < 0.001$ [Lt] on day 21 after infection). Treatment of subcutaneous CT26 tumors by intratumoral inoculation of G207 induced a tumor-specific T cell response.	Toda et al. (1999)
Melanoma mouse M3 cells DBA/2 mice	G207 (ICP34.5 deleted, ICP6 insertion mutation)	Intratumoral inoculation with G207 resulted in a significant reduction in tumor growth of both the inoculated tumor (Rt) and the contralateral tumors (Lt) compared with mock-inoculated controls ($p < 0.0005$ [Rt], $p < 0.05$ [Lt] on day 17 after infection; unpaired t test).	Toda et al. (1999)
Neuroblastoma Neuro-2A cells A/J mice	FusOn-H2 (derived from the wild-type HSV-2 strain 186)	FusOn-H2 effectively destroyed tumors in vivo. Adoptive transfer of splenocytes from mice receiving virotherapy to naive mice resulted in a measurable antitumor effect.	Li et al. (2007)
Glioma D74/HveC glioma cells Fischer rats	hrR3 cRGD	Combination (n = 7) 21 days median survival hrR3 (n = 3) 17 days median survival	Kurozumi et al. (2007)
Neuroblastoma Neuro-2a cells A/J mice	M012 (ICP34.5 deleted, expressing cytosine deaminase (CD), derived from R3659) 5-fluorocytosine (5-FC)	M012 combined with 5-FC administration had significant reduction of tumor burden vs tumors treated with R3659 combined with 5-FC or treated with M012 alone.	Guffey et al. (2007)

TRAMP, transgenic adenocarcinoma of the mouse prostate; cRGD, cyclic arg-gly-asp peptide; CTL, cytotoxic T lymphocyte.

and reintroduced as transplantable tumors in animals of the same strain. These models have been used to show immune stimulation by oHSV, even if rodents are not the natural host for HSV-1 and replication is limited (Fomchenko and Holland 2006). In addition to the drawback of low levels of virus replication, syngeneic rodent tumors derived either spontaneously or from mutagen-treated animals are often poorly characterized genetically and may not have typical mutational drivers as seen in the human disease. Also, their transplantable nature may not allow time for the development of a fully developed stroma and microenvironment, incorporating representative immune infiltrates (Fomchenko and Holland 2006). There may also be important differences in immune system function that do not represent the human situation. For example, murine IFNs, but not human IFNs, can significantly reduce the expression of virus-specific proteins in IFN-treated cells (Wintergerst et al. 1996). In humans, but not in mice, NK cells have been shown to have a role in the defense against HSV (Chew et al. 2009), and the contribution to innate anti-HSV immunity in humans has been suggested to be the predominant immune protection mechanism (Brutkiewicz and Welsh 1995).

Immunocompetent rodent models have also been used to study many of the features seen in immunocompromised models, including combinations with other therapies and the importance of innate immunity after OV infection (Altfeld and Gale 2015). In glioma, the chemotherapy agent cyclophosphamide (CPA) enhances viral replication by reducing innate immune responses, partially reversing the inactivation of oHSV viral transduction by human plasma (Ikeda et al. 1999), and by enhancing adaptive antitumor immunity induced by OVs (Bartlett et al. 2013). Pretreatment with CPA enhanced oHSV replication and oncolysis and reduced the virus-mediated increase in $CD68^+$ and $CD163^+$ cells and intratumoral IFN-γ (Fulci et al. 2006). CPA was also used with an HSV-2–based OV (FusOn-H2), which was constructed by deleting the N-terminal region of the ICP10 gene, against Lewis lung carcinoma, which is semipermissive to infection with FusOn-H2. CPA had a synergistic effect in this model (Li et al. 2007). This virus was associated with high intratumoral neutrophil counts, which were suggested to be part of the antitumor mechanism of this virus (Fu et al. 2011).

In contrast with the approach of suppressing the innate immune system to enhance the effects of oHSV, substantial data

from immunocompetent models show that oHSV can be used to stimulate T cell–mediated antitumor immunity (Benencia et al. 2005; Benencia et al. 2006; Miller and Fraser 2000; Toda et al. 1999, Todo et al. 2001). These models have shown that viral infection and replication, as well as the delivery of immunostimulatory factors, can lead to durable vaccine-type responses. Thus, an ideal therapeutic approach may require an initial immunosuppression to allow viral replication, followed by immunostimulation to confer long-term antitumor immunity and improved tumor reduction.

In recent years, investigators have been able to make a number of genetically engineered mouse models of human cancer. These models have representative driver mutations and grow in the correct tissue setting, and the tumor stroma is able to coevolve with the tumor as it grows, thus providing a potentially more accurate model of human disease (Politi and Pao 2011; Walrath et al. 2010). Disadvantages of this type of approach include a less precise timing of tumor growth and location than possible in transplantable models. Drawbacks of these models for immunotherapy studies are that these tumors may be poorly immunogenic; this could possibly be because of their comparatively low number of mutations compared with chemically induced tumors and a lack of novel neoantigens that could be recognized by the host immune system. There is a lack of studies of the use of genetic models to test oHSV.

$CD8^+$ T cells play a key role in the control of tumor growth by the immune system. It is thought that stimulation of a vigorous antitumor $CD8^+$ T cell response is critical for the control of tumor growth, and this is dependent on the composition of intratumoral immune infiltrates (Fridman et al. 2012). In general, studies of oHSV that have shown effective immune stimulation have used immunostimulatory transgenes, which suggests these may be necessary for a long-term immune response. For example, IL4 was shown to improve survival in GL261 syngeneic gliomas compared with the parental virus, which had no effect. IL4 expression was associated with increased T cell infiltration and suggests immunostimulation is needed for immune responses to oHSV (Andreansky et al. 1998). A subsequent study showed IL12 expression led to a Th1 response and increased animal survival compared with the parental vector, which had no effects (Parker et al. 2000). Using glioma stem-like cells recovered from a genetic mouse model of glioblastoma, it was shown that an oHSV (G47Δ plus IL12) was effective, whereas the parental virus had very little effect. Survival was likely to be T cell mediated because no significant difference was observed in immunocompromised mice (Cheema et al. 2013). T-VEC, which expresses the immunostimulatory factor GM-CSF, was examined in both immunocompromised and immunocompetent models (Liu et al. 2003). In immunocompromised models, the positive effects of ICP47 deletion were illustrated and suggested to be due to the effects of increased US11 expression, which blocks host cell resistance by PKR. Immune competent Balb/c mice were then used to show immune stimulation in A20 lymphoma subcutaneous xenografts. Impaired tumor growth was seen in tumors injected with GM-CSF–expressing and –nonexpressing viruses. However, a significant reduction in contralateral tumors was observed in GM-CSF–expressing cells, which were protected from tumor rechallenge. The authors found that preexisting anti–HSV-1 immunity did not have any effect on the outcome (Liu et al. 2003). In contrast, another study showed that preimmunization of rats with HSV-1 led to diminished HSV-1–mediated gene delivery, and preexisting HSV-1 immunity decreased, but did not abolish, gene transfer to experimental brain tumors by an HSV-1 vector (Herrlinger et al. 1998).

The relative contribution of viral replication to immune stimulation was examined by Workenhe and colleagues (2014). This study showed that HSV-1–based vectors, in comparison with HSV-2 vectors, showed significantly higher levels of danger-associated molecular patterns (DAMPs), and these correlated with higher numbers of antigen-presenting cells within the tumor and increased antigen-specific $CD8^+$ T cell levels in the peripheral blood. The authors concluded that in the context of oHSV, the initial virus/host interaction is more important than the persistence of replicating virus within the tumor in the induction of antitumor immunity (Workenhe et al. 2014).

At present, there are no immunocompetent rodent models that allow the testing of oHSV-1 by supporting its oncolytic activity driven by rapid replication. A number of approaches could theoretically be developed to overcome this hurdle:

1. Utilize rodent HSV. This would allow viral replication to be studied, however, because of evolutionary divergence, the data from a rodent oHSV may not be readily interpretable.
2. Modify oHSV to overcome species barriers; because some mechanisms are known that block HSV-1 replication in animal models, it could be feasible to modify the virus to overcome these pathways.
3. The host cells could also be modified to be more permissive to HSV-1 replication.
4. Models could be used with a humanized immune system. However, this approach is both economically expensive and experimentally challenging (Brehm et al. 2013).

Conclusion and Perspective

The recent clinical success in oHSV therapy has provided an incredible boost to the oncolytic virus field and will inspire further studies with newly engineered viruses and/or combination therapies. In the last decades, multiple papers have demonstrated the effect of oHSV on both the innate and adaptive immune systems. Our current understanding of the mode of anticancer action of all OVs is based on two temporally linked events: (1) an initial infection of cancer cells by the OV with replication into multiple progeny OVs, cancer cell lysis/necrosis, and subsequent rounds of infection/replication, and (2) a cytotoxic T cell (and possibly other immune cell) response against the remaining cancer cells, triggered by the debris of lysed cells with exposed cancer and viral antigens. The temporal occurrence of the first anticancer event is thought to be important in the first few days after OV infection and may be limited by innate immune responses, whereas, clearly, the second event is critical for long-term maintenance of antitumor immunity. The data presented in this review show that, so far, animal models have been successful in predicting the safety of the oHSVs in current use. The advantage of the mouse model is that it is simple and many combinations and viruses can be easily tested. However, only T-VEC has gone on to be clinically successful, and this illustrates that the models in present use are not predictive of clinical success. This may be because of lack of oHSV replication in mouse cells and/or differences in the mouse and human immune systems. The use of more challenging mouse models (e.g., PDX, genetic, and humanized) could therefore be very useful to establish greater predictive power.

As oHSVs gather momentum in the clinic, it will be of great importance to find ways to use these more sophisticated models to understand in detail about the mechanisms of resistance and sensitivity to allow patient-tailored and effective OV-based approaches.

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