

# Heterogeneous delivery is a barrier to the translational advancement of oncolytic virotherapy for treating solid tumors

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**Abstract:** Oncolytic viruses are a promising experimental anticancer therapy currently undergoing clinical translation. The development of oncolytic virotherapy offers a potential solution to the elusive “one-shot” cancer cure by providing targeted therapy to selectively infect and kill cancer cells while provoking adaptive anticancer immune responses as protection against distant metastasis and recurrent tumor challenge. While this technology has overcome barriers to safety and efficacy through cancer-specific targeting techniques, in order to reach full therapeutic potential, oncolytic therapies must still overcome barriers to intratumoral delivery and spread that result in heterogeneous intratumoral delivery and nonuniform response. This review will discuss barriers to oncolytic virotherapy translation related to mechanisms of delivering virus via tumor vasculature and distributing virus throughout the tumor microenvironment. Barriers include extravasation into the tumor that is dependent on adequate blood flow, tissue perfusion, and tumoral enhanced permeability and retention for transvascular transport. Subsequently, viruses must undergo interstitial transport against dense stromal barriers and high interstitial fluid pressure to initiate infection. In order to achieve massive tumor regression, infection must spread to cover large volumes of tumor mass. Furthermore, virus bioavailability is quickly dampened upon systemic administration due to neutralization and sequestration. These barriers to delivery limit the amount of virus that effectively reaches and spreads within the tumor, forcing dose increases that impinge upon limits of production and increase possibility of adverse events. Techniques to overcome these barriers are discussed but largely remain to be translated into clinical use.

**Keywords:** oncolytic virotherapy, barriers, delivery, systemic therapy, translation

## Introduction

The need for new cancer therapeutics is ever-present. Oncolytic virotherapy is an experimental cancer therapy that commandeers the ability of a virus to cause cellular death and incite adaptive immune responses to fight cancer. The use of oncolytic virotherapy as a treatment for malignant tumors has been studied both preclinically and in clinical trials. The history of oncolytic virotherapy development and clinical trials has been extensively reviewed.<sup>1,2</sup> Briefly, the field was initially sparked by case reports of individuals with solid cancers who upon coincidental contraction of infectious disease (later identified as viral infection) showed clinical remission.<sup>3</sup> The field lost momentum around the 1970s when development was met with limited efficacy and noncancerous tissue toxicity but has seen resurgence over the last quarter-century due to advances in understanding of genetic engineering and biological systems. Currently, viruses of naturally evolved or engineered cancer tropism are being used

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to specifically infect, replicate in, and kill cancer cells while leaving neighboring healthy cells unharmed due to cancer-specific abnormalities such as defective cellular signaling or receptor expression.<sup>4</sup> Subsequent activation of the immune response during viral infection leads to cross-priming of the T-cells against tumor antigens leading to an antitumor adaptive immune response.<sup>5,6</sup>

There are many different types of oncolytic viruses currently undergoing clinical trial to treat a multitude of different late-stage refractory or recurrent cancers through various approaches of administration and combinations with other therapies (Table S1). Oncolytic viruses currently being translated to clinical practice include DNA viruses: adenovirus, herpes simplex virus, parvovirus, and vaccinia virus, and RNA viruses: coxsackie virus, Newcastle disease virus, Measles virus, poliovirus, reovirus, Seneca Valley virus, and vesicular stomatitis virus. Table S1 presents a list of clinical trials currently registered with the National Institutes of Health ([clinicaltrials.gov](http://clinicaltrials.gov)), summarizing the translational status of oncolytic viruses and demonstrating the breadth and potential of this growing field. As the first oncolytic viruses near completion in Phase III testing, we recognize that the unique duality of the oncolytic paradigm embodies the potential for a one-shot cancer cure by both destroying existing tumor cells through induction of massive tumor cell lysis and maintaining an anticancer immune state to wipe out the total cancer cell population and protect against recurrence.<sup>7</sup> Third-generation oncolytic viruses now in clinical trials have improved safety and efficacy, as they are better able to avoid off-target effects by targeting viral infection through the use of molecular techniques that allow for specific transduction, transcription, and replication.<sup>2,8,9</sup> Oncolytic virotherapy also offers the ability to target the heterogeneous tumor-cell population, including quiescent cancer stem cells that can be a cause of tumor resistance and recurrence with standard therapies.<sup>10–14</sup> Although many early-phase clinical trials have been completed using oncolytic virotherapies, only three have made it to Phase III testing due to variability in efficacy (adenovirus; NCT01869088, herpes simplex virus; NCT00169704/01368276, reovirus; NCT01166542; Table S1). This variability in efficacy is matched with preclinical results that show nonuniformity of intratumoral response to oncolytic therapies and even long-term viral persistence within the tumor in the absence of complete tumor response.<sup>15–17</sup> This identifies a clear barrier in oncolytic virotherapy.

The benefits of these targeted therapies as single-agent cancer therapies are only as good as the ability to adequately deliver the virus to tumor cells. It is becoming increasingly apparent that intratumoral virus delivery and spread is

a limiting factor in the translational success of oncolytic virotherapies. The barrier of delivery is of special importance in the use of systemic administration, which allows for targeted viruses to reach disseminated or numerous tumor deposits. Currently, limited success has been seen with systemic oncolytic virotherapy. Since the earliest reports of intravenously administered oncolytic viruses in humans, only five reasonably well documented complete responses to single agent virotherapy have been achieved (Table 1). Of these studies, detection of virus delivery to the tumor is variable. This highlights the need to optimize delivery of systemically administered therapy to tumor sites. Furthermore, our mathematical model derived to optimize oncolytic virotherapy parameters predicts that improvements in delivery can have large impacts on oncolytic efficacy.<sup>18</sup> To achieve a one-shot cure with oncolytic virotherapy, access to all tumor cells must be maximized, requiring effective and uniform delivery of virus to the tumor. Therefore, this review will discuss issues pertaining to viral delivery, specifically focusing on barriers to intratumoral dissemination that are a major impediment to translational success.

## Barriers to success

While mechanisms to efficiently target viruses to cancer cells have been well established, the ability to effectively and uniformly deliver the virotherapy to the site of the tumor remains a barrier. Uniform coverage of the tumor is hindered by infection voids, locations where virus fails to initiate infection. Infection voids can result from inefficient delivery or extravasation from the blood vessel or the inability to achieve the viremic threshold necessary to seed infection. These voids correlate with regions of tumor-cell viability that remains after single-agent oncolytic virotherapy and limit efficacy (Figure 1). By focusing on the path of an individual virion en route to intratumoral infection, barriers to delivery can be easily identified (Figure 2). Virus arriving at the tumor site must first specifically extravasate into the tumor, bearing dependence on adequate blood flow, tissue perfusion, and tumoral enhanced permeability and retention (EPR) for transvascular transport. Subsequently, virions must undergo interstitial transport against dense stromal barriers and high interstitial fluid pressure to initiate infection. In order to achieve massive tumor regression, infection must spread to cover large volumes of tumor mass. Furthermore, virus bioavailability is quickly dampened upon systemic administration due to neutralization by circulating antibodies, association with factors in the blood, and sequestration by the mononuclear phagocytic system to the liver and spleen. These barriers to delivery limit the amount of

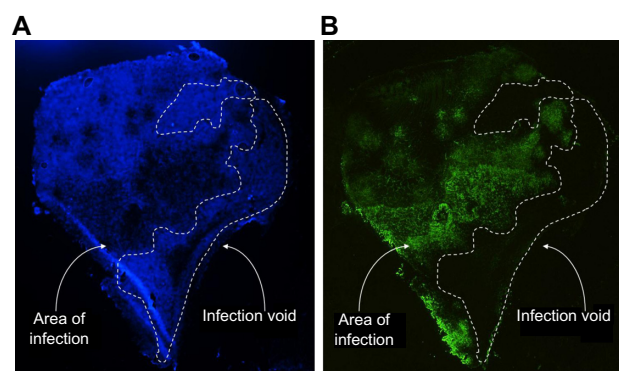
**Table 1** Clinical trials with intravenously administered oncolytic virotherapy monotherapy show variable delivery and limited response

Family/virus	N <sup>a</sup>	Delivery <sup>b</sup>	PR <sup>c</sup>	CR <sup>c</sup>	Reference
Bunyaviridae					
Bunyamwera	4 (3)	–	0	0	71
Hepadnaviridae					
Hepatitis B	21	–	?	0	72
Flaviviridae					
Ilheus	19 (9)	Yes (3 of 4)	0	0	71
Dengue	5	–	0	0	73
West Nile virus	21 (9)	No (1 of 1)	0	0	71
Paramyxoviridae					
Mumps	200	–	?	3 (breast, breast, Hodgkin's)	74
Newcastle disease virus (PV701)	114	Yes	5	1 (tonsillar)	75
Newcastle disease virus (HUJ)	11	Yes (1 of 2)	0	1 (glioblastoma multiforme)	76
Reoviridae					
Serotype 3 Dearing strain (Reolysin)	33	Yes (3 of 3)	0	0	77
Serotype 3 Dearing strain (Reolysin)	21	Yes (2 of 15)	0	0	78
Poxviridae					
Vaccinia virus (JX-594)	23 (22)	Yes (9 of 22)	1	0	69
Vaccinia virus (AS)	3	–	3	0	79, 80
Vaccinia virus	19 (1)	–	0	0	81
Adenoviridae					
Adenovirus 5 (ONYX-015)	10	Yes (1 of 1)	0	0	82
Adenovirus 5 (ONYX-015)	18	No (1 of 1)	0	0	83
Adenovirus 5 (CG7870)	23	–	0	0	84
Picornaviridae					
Seneca Valley Virus	30	Yes (1 of 1)	0	0	85

**Notes:** <sup>a</sup>Number of patients in trial (number of patients assessable with intravenous administration); <sup>b</sup>delivery indicates presence of replicating virus detected in tumor biopsy when available (number with detectable virus of number of tumor biopsies assessed); <sup>c</sup>partial and complete response following RECIST criteria when able to determine. ? indicates magnitude of response could not be determined with information provided; – indicates unknown.

**Abbreviations:** CR, complete response; PR, partial response; RESIST, Response Evaluation Criteria In Solid Tumors.

virus that effectively reaches and spreads within the tumor, forcing dose increases that impinge upon limits of good manufacturing practice production and increase possibility of adverse events.



**Figure 1** Systemically administered oncolytic virotherapy success is limited by barriers to delivery that result in clinically relevant infection voids.

**Notes:** Immunofluorescent staining shows the correlation between tumor cell viability and regions void of infection. Conversely, areas of viral infection are areas of cell death. Shown here is a tumor section from 5TGM1 murine myeloma solid tumor from a C57Bl6/KaLwRij mouse administered IV VSV-GFP (5e7 TCID<sub>50</sub>). Blue Hoechst nuclear staining (**A**) shows viable tumor tissue and green staining (**B**) shows areas of VSV infection (anti-VSV antibodies), with voids in infection marked with a white dashed line.

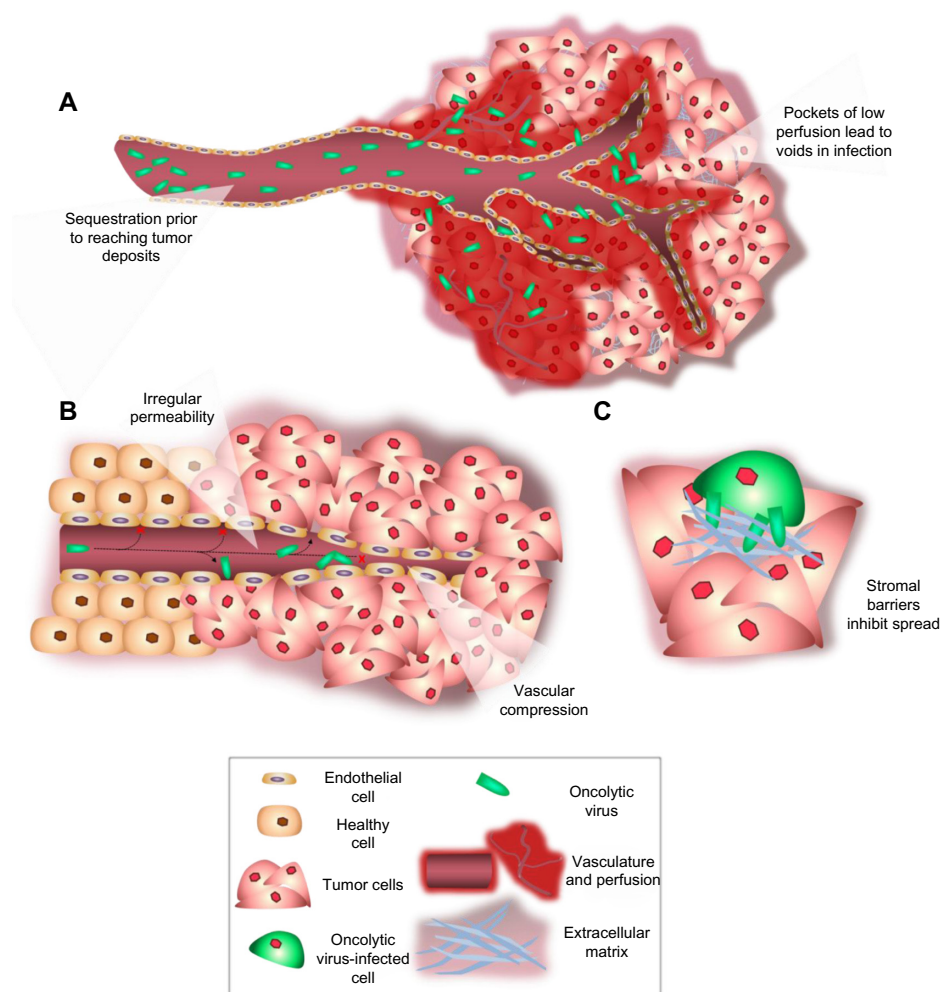
**Abbreviations:** IV, intravenous; VSV, vesicular stomatitis virus; GFP, green fluorescent protein; TCID<sub>50</sub>, 50% tissue culture infectious dose.

## Barriers to transvascular transport

When oncolytic virotherapy is systemically administered, access to the tumor is achieved through the blood supply. Delivery throughout the tumor is therefore dependent on tumor vasculature. Initial tumoral delivery is met by barriers to adequate tumor perfusion and vascular permeability necessary to get the virus into the tumor, including irregular blood flow, vascular occlusion, vascular organization, and permeability.

### Tumor perfusion

A cause of heterogeneous deposition is the irregularity of blood flow due to inefficient and disorganized tumor vasculature, given that the rate and distribution of blood flow determines the amount of systemically administered virus that can reach specific regions of the tumor. Tumor vasculature is chaotic in terms of microvessel length, diameter, spatial distribution, and blood flow velocity and direction. Tumor vasculature can be further impacted by growing tumor cells and high interstitial pressure that can occlude flow and force vessels apart, thus decreasing microvascular density.<sup>19–21</sup> The result is both spatially and temporally heterogeneous blood perfusion throughout the tumor. Poor perfusion causes



**Figure 2** Barriers to intratumoral delivery and distribution encountered by oncolytic viruses.

**Notes:** Depicted here is vesicular stomatitis virus traversing from systemic administration into the tumor parenchyma. **(A)** Systemically administered virus is depleted by sequestration prior to reaching tumor deposits. Distribution within the tumor is further limited by poor and heterogeneously distributed blood flow and perfusion. **(B)** At the vascular wall, extravasation is limited by permeability of the vessels. High intratumoral pressure compresses tumor vasculature limiting blood flow and delivery. **(C)** Stromal barriers including the extracellular matrix and densely packed cells limit diffusion of virus from the vasculature and from infected cells.

pockets of low oxygenation due to low blood flow that result in transiently or chronically hypoxic tumor regions.<sup>22–24</sup> As hypoxia is representative of perfusion state, it may also be indicative of therapeutic delivery; therefore, understanding of hypoxia is relevant to virotherapy delivery. It is known that areas of transient or chronic hypoxic tissue occur as microregions heterogeneously distributed within the tumor mass and may be located next to areas of normal perfusion.<sup>25,26</sup> Transient hypoxia has been well documented in tumors in which markers of blood flow and perfusion show mismatched delivery after administration at different time points.<sup>23,27,28</sup> Tracking changes in intratumoral partial oxygen pressure also shows cyclical turnover or fluctuations in blood flow that occur over time. Hypoxia is linked to tumor stem cell niches, treatment resistance, and metastasizing disease, as hypoxic tissue is a characteristically difficult target. Intriguingly, new

technologies are available to monitor and target hypoxic regions.<sup>29</sup> Therefore, hypoxia is a clear barrier and target for improving virus delivery.

By normalizing blood flow, the causes of spatial and temporal hypoxia can be reduced, thereby increasing perfusion throughout the entire tumor. Tumor blood flow can be increased for the purpose of therapeutic delivery through manipulation of the systemic vasculature. Physiological changes include increasing blood flow or perfusion pressure to the tumor by increased systemic blood pressure (pharmacologically or through exercise). This approach is based on the principle that irregular, poorly differentiated tumor vasculature does not maintain blood flow volume homeostasis like systemic vasculature does. In this way, while systemic vasculature that is exposed to vasoconstrictors or shear stress experiences increases in peripheral resistance and



arteriolar blood pressure, the tumor vasculature experiences increased perfusion pressure, resulting in increased blood flow into the tumor.<sup>30,31</sup> Small changes in blood flow over short periods of time, as evidenced in multiple tumor types by measuring tumor oxygenation, result in changes in the hypoxic regions surrounding tumor tissue.<sup>22</sup> If these small changes can be therapeutically induced, they can be used advantageously to change areas of perfusion and therefore particle deposition. The vasoconstrictor angiotensin-II has been used in preclinical and clinical studies to increase tumor-specific blood flow and intratumoral delivery of chemotherapies and macromolecular complexes.<sup>30–35</sup> While this idea of improving nanoparticle delivery by influencing blood pressure during infusion was first suggested by Matsumura and Maeda in 1986, its use with oncolytic viruses has yet to be reported.<sup>36</sup>

### Enhanced permeability and retention

Once the virus arrives at the vascular wall, it must exit the vasculature. Passive diffusion from the circulation into the tumor parenchyma is mediated by the enhanced permeability and retention (EPR) effect, a phenomenon that results from irregular, leaky tumor vasculature with enhanced permeability and the lack of lymphatic drainage that allows for particle retention.<sup>36,37</sup> However, the EPR effect is not consistent, with differences in vascular pore size and abrupt changes in tumoral blood perfusion resulting in heterogeneous accumulation.<sup>35,38</sup> While passive EPR-based delivery may be unpredictable, nanodrugs consistently accumulate within a tumor.<sup>35</sup> This is because the enhanced permeability is selective for particles above 40 kDa, which is far surpassed by the average oncolytic therapy.<sup>35</sup> Beyond molecular mass, the dimensions of current oncolytic viruses undergoing translation are important in understanding physical barriers to viral movement (Table 2). Tumor leakiness is related to the size

of tumor vessel interendothelial pores. The size of pores in tumor endothelium is heterogeneous and tumor dependent, ranging from less than 1 nm to greater than 1  $\mu$ m, while the endothelial gaps in healthy tissue are only ~2–6 nm in diameter.<sup>21,39–41</sup> Endothelial gap size determines the pore cutoff size, a functional measure of size exclusion during transvascular transport of nanoparticles. This is important in determining if oncolytic virotherapy is even a viable option for the tumor, as the virus must be able to extravasate between endothelial cells to disseminate throughout the tumor. In some tumors, especially primary brain tumors where the pore cutoff size is as small as 1 nm, systemically administered oncolytic virotherapy will be unable to deliver to the tumor.<sup>42</sup> Interestingly, in animal models it has been shown that this pore cutoff size can change due to location of tumor growth and to growth conditions such as hormone depletion.<sup>40</sup> It should be noted that the primary means of tumor interendothelial junction measurements has been observation of subcutaneously grown murine tumors of human xenografts. Although these observations may differ compared to those seen in patients, the distinctive discrepancy between tumor endothelium and that of healthy tissue allows for tumor-specific pathways of virus extravasation.

Learning how to use the EPR effect to our advantage will assist in overcoming obstacles related to diffusion and heterogeneous deposition. Permeability factors such as vascular endothelial growth factor (VEGF), bradykinin, nitric oxide, prostaglandins, tumor necrosis factor (TNF)- $\alpha$ , TNF- $\beta$ , and interleukin-2 facilitate tumor-selective enhanced vascular permeability and improved delivery of macromolecular drugs in solid tumors.<sup>35</sup> The use of permeability factors may be a promising combinatorial-therapy approach for oncolytic virotherapy. The use of VEGF has been shown to increase vascular leakiness and increased tumor transduction in combination with oncolytic Sindbis virus.<sup>43</sup> Intravenous administration of TNF- $\alpha$  resulted in increased permeability and up to six-fold increase in adenovirus extravasation into tumor tissue.<sup>44</sup> Many of these permeability factors remain to be explored for the purposes of selectively increasing tumor vasculature permeability and subsequent viral delivery. Another means of increasing permeability is the induction of transient hyperthermia. Mild hyperthermia (41°C–42°C) has been used to increase tumor-specific extravasation of nanoparticles to xenograft tumor models by increasing the pore cutoff size and increasing endothelial cell gaps up to 10  $\mu$ m in some tumor models.<sup>45,46</sup> Increased permeability was maintained up to 8 hours after heating.<sup>46</sup> Hyperthermia presents another potential means of modifying tumor

**Table 2** Approximate virion dimensions of oncolytic viruses currently undergoing clinical translation

Oncolytic virus	Dimension <sup>a</sup> (nm)	Reference
Adenovirus	80–100	86
Herpes simplex virus	200–225	86,87
Parvovirus	25–30	88
Vaccinia virus	270–350	89
Newcastle disease virus	150–250	90
Measles virus	50–510	91
Reovirus	~70	92
Coxsackie virus	~30	93
Seneca valley virus	~30	94,95
Poliovirus	~30	96
Vesicular stomatitis virus	180–200 <sup>b</sup>	97,98

**Notes:** <sup>a</sup>Indicates largest dimension in the case of nonspherical virion; <sup>b</sup>nonspherical, bullet-shaped virus with a shorter dimension of 70–80 nm.<sup>97,98</sup>

permeability for oncolytic virotherapy delivery that has yet to be explored.

## Barriers to interstitial transport

Upon transvascular extravasation or release of progeny from an infected tumor cell, the virions must passively diffuse to uninfected tumor cells and establish infection. The EPR effect acts as a double-edged sword for nanoparticle distribution, since the poorly organized and leaky vasculature results in unregulated extravasation that contributes to increased interstitial fluid pressure (IFP). High IFP can cause intratumoral vessels to collapse, which in combination with dense tumoral extracellular matrix and lack of lymphatic flow results in loss of convective flow throughout the tumor, making macromolecular transport dependent on passive diffusion.<sup>17,47,48</sup> Passive diffusion is limited by the composition and organization of the tumor microenvironment, including the dense extracellular matrix (ECM), and tight packing of tumor and normal stromal cells such as resident macrophages.<sup>38,49</sup> Indeed, a mathematical model developed to provide insight into the causes of insufficient distribution predicted that an improvement in effective diffusion coefficient could result in a substantial increase in number of infected cells.<sup>50</sup> One way to alter the diffusion coefficient is through degradation or manipulation of the ECM and surrounding interstitial space.

## Interstitial fluid pressure

High IFP affects intravenous delivery by limiting movement of large molecules such as oncolytic viruses out of the vascular bed and limiting access to the tumor core where IFP is highest. The high pressures are comparable to that of the microvascular pressure, causing the loss of a pressure gradient necessary for convection-driven diffusion.<sup>51</sup> Mechanical manipulation techniques have been developed to aid in spread against pressure gradients. Therapeutic ultrasound can be used to enhance delivery of therapeutic agents. Ultrasound induced inertial cavitation, which has been shown to successfully deliver macromolecules larger than several millimeters against pressure gradients, has been used to enhance distribution of oncolytic adenovirus in *in vitro* and *in vivo* models.<sup>52–55</sup> For example, focused ultrasound increased systemically administered oncolytic adenovirus delivery to tumor up to 50-fold when coadministered with microbubbles for induction of inertial cavitation.<sup>53</sup> Although this technique increases diffusion into solid tumor, this is not a viable option for disseminated or metastatic cancer where location is unknown or too numerous for ultrasound targeting.

Solid stress alleviation via tumor debulking can also reduce IFP and reduce cellular infringement upon tumor vasculature, thereby improving virus delivery. Solid stress alleviation can be achieved by inducing cell death to reduce crowding in the interstitial space. The average distance between tumor cells is approximately 20 nm, smaller than the dimension of oncolytic viruses, therefore creating a physical barrier (Table 2).<sup>56</sup> Not only do chemotherapies and oncolytic viruses work in synergy to enhance cancer survival, chemotherapies can actually benefit tumoral virus penetration. Channels formed by the spaces left behind by dead tumor cells independent of any ECM alterations will allow for increased tumor penetration by virus particles. Apoptosis-inducing chemotherapies in combination with oncolytic virotherapy have resulted in increased spread within tumors and synergistic antitumor effect. For example, induction of apoptosis using chemotherapies and TNF-related apoptosis-inducing ligand increased oncolytic herpes virus intratumoral spread and produced a more diffuse pattern of infection in mice with both collagen-rich and collagen-poor mammary tumors.<sup>56</sup>

Another approach to decrease IFP uses anti-VEGF or other antiangiogenic therapies to reduce irregular tumor vascularity and allow for tumor neovasculature normalization. This normalization reduces hypoxia and IFP, resulting in an intravascular–interstitial pressure gradient that allows increased delivery of drugs to the tumor and increased survival of patients given chemoradiotherapies.<sup>20,57–60</sup> This technique has yet to be fully developed with oncolytic virotherapies, although work with oncolytic herpes simplex virus has shown that the order of treatment with anti-VEGF bevacizumab in combination with virus treatment matters. While Eshun et al<sup>61</sup> saw an increase in overall tumor response when bevacizumab was given prior to virus compared to either therapy alone, a greater overall response was seen when bevacizumab was given after virus. This may be explained by the dual role VEGF plays in herpes simplex virus receptor expression. Perhaps the decrease in IFP after initiation of infection allowed for greater local spread and tumor control. However, vascular normalization also diminishes the vascular permeability necessary for extravasation. For this reason it will be necessary to balance any improvement in virus delivery gained by vascular normalization leading to increased tumor penetration against the parallel loss in extravasation of macromolecules due to decreased vascular permeability.<sup>62</sup>

## Extracellular matrix

A major contributor to high IFP is the ECM, primarily made up of collagen, elastic fibers, glycosaminoglycans, and

proteoglycans, which creates a gel-like medium difficult for viruses to traverse given that the size of viral vectors is comparable to or larger than the ~70–100 nm space between fibers<sup>17,21,63</sup> (Table 2). It has been demonstrated that the greater the fibrillar collagen content, the lower the interstitial diffusion rate of macromolecules, and because of this higher collagen content, a greater infusion pressure is necessary to initiate flow into the tumor interstitium.<sup>49,64</sup> The ECM composition, and therefore the IFP, can be altered by ECM-degrading enzymes. For instance, collagenases reduce IFP and increase transcapillary pressure gradients, improving uptake and distribution of immunoglobulin G in solid tumors.<sup>65</sup> Overexpression of matrix metalloproteinases in tumors was found to enhance convection and viral distribution.<sup>66</sup> Recombinant viruses expressing matrix-degrading proteins have been developed to successfully increase distribution and penetration in solid tumors. A multitude of oncolytic

viruses in combination with or genetically engineered to express matrix-degrading proteins – including but not limited to bacterial collagenases, matrix metalloproteinases, relaxin, hyaluronidase, and heparanase to increase distribution, control tumor growth, and increase survival of tumor-bearing animals – have been reviewed previously.<sup>67</sup> Table 3 summarizes the main matrix-degrading enzymes used in combination with oncolytic viruses to increase distribution of and tumor response to oncolytic viruses. As an example, increased levels of matrix metalloproteinases increased intratumoral virus delivery, distribution, and tumor response to adenovirus, herpes simplex virus, and vaccinia virus in multiple tumor models.<sup>62,66–68</sup> However, the efficacy of these approaches above traditional oncolytic virotherapy paradigms is minimal, with the increase in delivery and distribution resulting in a nonsignificant survival benefit. Further, the associated risk of potentiating the spread of replicating

**Table 3** Examples of extracellular matrix degradation techniques used in combination with oncolytic viruses in preclinical work that increase intratumoral virus delivery and spread

Matrix-degrading enzyme	Virus	Results	Reference
Collagenase	Ad	Collagenase/dispase and trypsin enhanced virus infection in glioblastoma multiforme-derived tumor models.	99
	HSV	Bacterial collagenase coinfection improved range of viral distribution and enhanced therapeutic outcomes in human melanoma Mu98 models.	100
MMP	Ad	AdMMP8 (nonreplicating) with Adwt300 (replicating) caused reduced tumor growth in A549 and BxPC-3 xenograft tumor models.	101
	HSV	MMP-1 and MMP-8 overexpression in human soft tissue sarcoma enhanced virus delivery and distribution, and tumor response.	66
	Vaccinia	Ectopic MMP-9 increased distribution of HSV in a neuroblastoma model.	102
		MMP-9 increased intratumoral viral dissemination and accelerated tumor regression in a PC-3 tumor model.	103
Relaxin	Ad	Ad-expressing relaxin increased intratumoral viral distribution and penetration, inhibited tumor growth, and increased survival of B16BL6 melanoma mouse model.	104
		Ad-expressing relaxin showed enhanced transduction and spread throughout tumor that correlated with antitumor efficacy and overall survival in metastatic tumor models.	105
		Relaxin-expressing Ad showed better proliferation and eliminated collagens more effectively than Ad in OSCC models.	106
Hyaluronidase	Ad	Coadministration of hyaluronidase and Ad resulted in greater antitumor activity and overall survival in mice with aggressive tumors than Ad alone.	107
		Coadministration of hyaluronidase improves antitumor activity of Ad in xenograft tumor-bearing mice. Hyaluronidase-expressing Ad had enhanced distribution and induced tumor regression in melanoma xenograft models.	108
Heparanase	Ad	Oncolytic Ad in combination with heparanase-expressing Ad resulted in increased penetration in vitro and more profound antitumor effects in a mesothelioma model.	109
Decorin	Ad	Decorin-expressing Ad had enhanced tissue penetration, enhanced viral spread and improved tumor reduction and survival benefit compared to non-decorin-expressing Ad.	110
Elastase	Ad	Macrophage metalloelastase improved overall antitumor efficacy of oncolytic Ad in HCT116 xenografts.	111

**Abbreviations:** Ad, adenovirus; HSV, herpes simplex virus; MMP, matrix metalloproteinase; OSCC, oral squamous cell carcinoma.

**Table 4** Selected examples of strategies to reduce neutralization and sequestration of oncolytic virotherapy in preclinical work that show increased tumoral deposition and decreased sequestration

Strategy	Mechanism	Virus	Results	Reference
Receptor saturation* <sup>4</sup>	Polyinosinic acid [poly(I)]	Ad	Predosing with poly(I) before Ad infection resulted in inhibition of As expression in macrophage and Kupffer cells, increased circulating Ad, and improved transgene expression in tissue.	112
		MV	Pretreatment of cells with poly(I) reduced MV expression by 99% and 50% in murine and human macrophages, respectively. Predosing of mice with poly(I) reduced MV sequestration and enhanced delivery to ovarian and myeloma xenograft models.	113
	Liposomes	HSV	Treatment with clodronate liposomes depleted peripheral macrophages and increased intratumoral viral titers five-fold.	114
		VSV	Clodronate liposomes mediated elimination of marginal dendritic cells and splenic macrophages associated with increased VSV dissemination.	115
	Viral pre-dosing	Ad	Transgene expression levels from low viral doses were enhanced by coadministering unrelated adenovirus and further enhanced by preadministration.	116
Surface modification* <sup>4,8</sup>	HPMA	Ad	HPMA coating protected against neutralizing antibodies and complement.	117
			HPMA coating made to be bioresponsive allowed for maintained transduction efficiency and enhanced circulation time.	118
		VSV	PEGylation of VSV inhibited serum neutralization and increased circulating half-life.	119
	PEG	Ad	PEGylation of Ad reduced uptake in the spleen and liver.	120–123
			PEGylation of Ad increased circulation half-life, increased accumulation in tumor tissue, and decreased hepatic transduction.	
			Conjugating Ad with high molecular weight PEG reduced hepatocyte transduction and hepatotoxicity, detargeted Ad from Kupffer cells, maintained tumor transduction efficiency, and increased efficacy in hepatocellular carcinoma xenografts.	
	Liposomes	Ad	PEGylation of Ad reduced production of anti-Ad antibodies and increased therapeutic response against metastatic cancer.	
			Liposome-encapsulated Ad resulted in suppressed tumor growth and decreased distribution to liver.	124
		HSV	HSV complexed with liposomes increased survival rates of immunized mice bearing liver metastases.	125
	Polypeptide	Ad	Noncovalent polypeptide coating of Ad reduced antigenicity and facilitated gene transfer by shielding from neutralizing antibodies and blood factors.	126
	Dendrimer	Ad	Poly(amido amine)-dendrimer coating enhanced Ad transduction efficiency in the presence of neutralizing antibodies.	127
			Efficient liver detargeting and tumor retargeting of Ad after coating with dendrimer was shown.	128
Cell carrier* <sup>130–132</sup>	Mesenchymal stem cells	MV	Dendrimer-coated Ad reduced liver pooling and hepatotoxicity and increased transduction efficiency in peripheral xenograft tumors.	129
			Survival of measles-immune mice bearing SKOV3 ovarian tumor xenografts was enhanced by MV-infected MSC but not by naked virus or uninfected MSC. MSC allowed for infection of target cells in the presence of high-titer anti-measles antibody.	133
	Dendritic cells	MV	Carrier-delivered MV infection prevented accumulation of pleural exudate and improved survival of MDA-MB-231 malignant pleural effusion xenograft model.	134
	T-cells	VSV	Delivery to B16ova tumors and antitumor efficacy of VSV by preloaded T-cells is better than systemically administered VSV alone in the presence of neutralizing antibodies.	135
Serum depletion	Immunosuppression (cyclophosphamide)	HSV	Cyclophosphamide pretreatment inhibited HSV-induced infiltration of immune cells allowing increased intratumoral spread and oncolysis.	136
		Ad	The combination of high-dose cyclophosphamide with recombinant adenovirus inhibited neutralizing antibody formation and increased intratumoral virus replication and transgene expression.	137

(Continued)



**Table 4** (Continued)

Strategy	Mechanism	Virus	Results	Reference
		MV	Immunosuppression with cyclophosphamide slowed the appearance of neutralizing antibodies and enhanced oncolytic efficacy of recombinant MV in an immunocompetent model.	138
	Cobra venom factor	HSV	Complement depletion using cobra venom factor facilitates infection of tumor cells by systemically administered HSV.	139
	Anticoagulants (warfarin)	Ad	Warfarin used to block blood factor-dependent virus neutralization reduced transgene expression in the liver and decreased hepatotoxicity. Warfarin combined with Kupffer cell depletion resulted in reduced tumor growth and prolonged survival of tumor bearing animals treated with adenovirus.	140

**Note:** \*Reviewed extensively elsewhere with review citations included; selected examples chosen for illustrative purposes.

**Abbreviations:** Ad, adenovirus; HPMa, N-(2-hydroxypropyl)methacrylamide; HSV, herpes simplex virus; MSC, mesenchymal stem cells; MV, measles virus; MMP, matrix metalloproteinase; PEG, polyethylene glycol; VSV, vesicular stomatitis virus.

viruses must be closely monitored as these techniques continue to be developed.

## Other translational barriers

### Administration, extravasation, and immune modulation

Routes of administration must also be addressed, as they can affect initial viral delivery. Current routes of virus administration include intravenous administration, intratumoral injection, and regional delivery including intracavitary, intrapleural, intraperitoneal, and regional perfusion. While intratumoral or regional administration offer increased delivery specificity, this type of delivery is limited to local spread in the absence of secondary viremia and requires tumors to be injectable, making disseminated disease or metastases difficult targets. Intravenous administration allows for systemic delivery of the virus and is therefore a necessary focus for optimization of the one-shot cure. Systemic administration depends on the ability of the virus to traverse the vascular barrier at sites of tumor growth while evading neutralization and sequestration. Whereas virion size plays a large role in the ability of the virus to traverse the physical barriers primarily focused on in this review, it should be noted that the physical size of the virion is not the determining factor in oncolytic virotherapy efficacy, which is also dependent on factors such as receptor availability, the ability of tumors to support virus infection, and immune status. Viruses are prone to neutralization by pre-existing antibodies, complement proteins, and coagulation and other serum factors that lead to opsonization. Recognition by the mononuclear phagocyte system, including splenic macrophages and hepatic Kupffer cells, results in phagocytosis and rapid clearance from circulation and can result in liver toxicity.<sup>4,68</sup> Approaches to reduce neutralization and sequestration include serum depletion, saturation of the mononuclear phagocyte system

and other virus recognition systems, shielding of viruses through engineered coats or surface modifications, and the use of cell carriers to protect and transport therapies to tumor sites. Many strategies to reduce neutralization and sequestration have been employed in preclinical testing and reviewed previously.<sup>4</sup> Table 4 highlights successful strategies used in the oncolytic virotherapy field to eliminate binding, increase persistence in circulation, reduce sequestration, and improve tumor transduction. Barriers to transvascular transport must be remembered when any type of shielding of the virus is used, as shielding can increase particle dimensions and thereby limit extravasation. Similarly, immune depletion used prior to virotherapy to increase delivery could limit the efficacy of the subsequent immunotherapeutic phase of oncolytic virotherapy. It is therefore necessary to balance improvement in delivery with potential losses in efficacy as these techniques continue to be developed.

Overcoming the barriers placed by the immune system and taking advantage of viral-initiated antitumor immunity will be critical to the success of systemic virotherapy and has been extensively reviewed.<sup>5</sup> Because the role of the host antiviral and virus-initiated antitumor immune response in the therapeutic outcome of oncolytic virotherapies is another potential barrier to translation, it will be briefly discussed here. The host antiviral immune response decreases the amount of virus available to establish intratumoral infection. As discussed previously, soluble immune mediators, along with nonspecific blood-factor binding and nonspecific extravasation, limit viral efficacy following systemic delivery by reducing the bioavailability of the therapy. However, immunosuppression that accompanies cancer or the induction of an immunosuppressed state may both aid and prevent oncolytic efficacy. Thus, immunosuppression leads to enhanced virus delivery and more extensive intratumoral virus spread, but at the same time it limits cross-priming of the immune system,

**Table 5** Stages of oncolytic virotherapy delivery with barriers that limit therapeutic efficacy

Location	Stages of delivery	Barrier	Solutions
Systemic circulation	1. Systemic administration	Sequestration	Saturate receptors
Tumor vasculature	2. Delivery via circulating blood	Neutralization	Surface modification
	3. Extravasation at vascular wall	Poor perfusion	Target circulation
Tumor microenvironment	4. Dissemination throughout tumor	EPR	Target permeability
	parenchyma	ECM	Degrade matrix
		IFP	Debulk, pressure gradients

**Note:** Preclinical work has offered potential solutions to each barrier in order to increase intratumoral delivery, distribution, and efficacy of oncolytic virotherapy.

**Abbreviations:** ECM, extracellular matrix; EPR, enhanced permeability and retention; IFP, interstitial fluid pressure.

thereby reducing any immunotherapeutic benefit gained from the therapy. However, cross-priming of the antitumor immune response after oncolytic virus administration may be more the exception than the rule, and the host immune response may remain completely focused on the virus, not seeing the tumor antigens at all. Therefore, viral immunodominance may be an important obstacle to effective oncolytic immunotherapy. Considerable additional research in this area is needed.

### Dose and manufacturing

As oncolytic therapies approach approval through late-phase clinical trials, focus must also be placed on issues of translation outside of safety and therapeutic efficacy. These include the production and cost bottleneck. In order to achieve a clinically relevant infection, viral delivery must exceed the threshold necessary to establish infection. As seen in the Phase I clinical trial of intravenously administered JX-594, virus was recoverable from tumor biopsies only at doses above  $10^9$  infectious units, demonstrating that tumoral deposition and most likely destruction is dose dependent.<sup>4,69</sup> Since delivery is dose dependent, current delivery barriers severely drive dose up, impinging on the limits of production capability. Currently, production facilities that employ good manufacturing practice have the capability to produce high-titer virus preparations, but it is costly and time intensive. Manufacture requires high-grade purification techniques to remove cellular proteins and nucleic acid contaminants while maintaining gentle conditions for shear-sensitive viruses such as measles virus.<sup>70</sup> With the idea that more virus delivered to the tumor will lead to more tumor infected, we can actively seek to maximize efficacy of current doses or even decrease the dose required by optimizing the current barriers that stand in the way of therapeutic efficacy, namely, deficiencies in delivery and distribution that result in nonuniform response.

### Public acceptance

Perhaps unique to oncolytic viruses are other areas of safety that must be addressed, including pre-existing immunity,

pathogenicity, and transmission. The idea of a replicating therapy is bound to bring controversy and apprehension to public acceptance. While certain genetic modifications provide built-in mechanisms of protection against infectious outbreaks by increasing tumor specificity and reducing the ability for the virus to spontaneously revert to a harmful strain, modifications such as matrix-degrading enzymes may have contradicting effects that increase likelihood of extra-tumoral dissemination of replicating viruses. It is difficult to tell what public perception of oncolytic virotherapies will be, as there are currently no therapies approved by the US Food and Drug Administration and little knowledge in the general public. It can be predicted that public acceptance of the use of replicating virotherapy will be met with hesitation, similar to that seen with the use of viruses for gene therapies and vaccines. However, patient acceptability and adherence may be improved compared to standard anticancer regimens as the field of oncolytics moves towards a one-shot cure that will reduce the need for repeat dosing and hospital stays.

### Conclusion

Oncolytic viruses present a new paradigm in cancer treatment that allows for selective cancer-cell killing with built-in maintenance mechanisms to protect against tumor recurrence and to target distant metastases. As the technology advances along the path of translation, we are learning that although oncolytic virotherapy has shown efficacy in early-phase clinical trials, oncolytic virotherapies are still limited by deficiencies in tumor coverage. By looking at the specific path that is taken by a virion after systemic administration, we have elucidated key barriers to uniform delivery and distribution throughout the tumor, including sequestration and neutralization prior to reaching the tumor site, homogeneous distribution via the tumor circulation followed by transvascular transport, and interstitial transport to establish sites of infection, summarized in Table 5. Preclinical work has been done to address many of the barriers pertaining to stages of delivery and shows potential

to overcome barriers to uniform delivery. Many of these techniques, however, have yet to be translated or used in current oncolytic virotherapy clinical trials (Table S1). This highlights a translational gap that will need to be addressed as this field seeks to optimize virotherapies. Taking the insight learned in preclinical and early-phase clinical trials regarding variable response rates matched with nonuniform therapeutic distribution, extensive work has been done to improve the delivery and spread of oncolytic viruses throughout the tumor. Knowledge of the delivery barriers and techniques to overcome them is the first step in translating a truly optimized oncolytic virotherapy.

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## Supplementary materials

**Table S1** Current clinical trials registered with the US National Institutes of Health (clinicaltrials.gov) investigating the use of replicating oncolytic viruses as a cancer treatment gives an insight into the translational status of this therapy

Virus	Therapy name	Modification	Cancer	Route	Additional interventions	Phase	Status	Start date	Primary outcome	NCT identifier
Adenovirus	ICOVIR-5	Ad-DM-EZF-K-Delta24RGD	Melanoma (advanced/metastatic)	IV		I	Recruiting	January 2013	MTD	01864759
	CELYVIR	ICOVIR-5 + MSC	Solid tumors (metastatic and refractory)	IV	MSC infected with ICOVIR5	I/II	Recruiting	January 2013	AE	01844661
	CGTG-102	Ad5/3-D24-GM-CSF	Solid tumors (refractory, injectable)	IT/IV	Cyclophosphamide	I	Ongoing	April 2012	Dose	01598129
	CG0070	GM-CSF	Nonmuscle invasive bladder cancer	Bladder instillation		II/III	Opening soon	November 2013	CR, DCR	01438112
		GM-CSF	Superficial transitional cell carcinoma of the bladder (after BCG failure)	Intravesical (directly into bladder)		I	Unknown	April 2005	MTD/MFD	00109655
	ColoAd1	Ad11p/Ad3 chimeric	Ovarian cancer (platinum resistant)	IP		I/II	Opening soon	January 2014	MTD, dose	02028117
		Ad11p/Ad3 chimeric	Colon cancer (resectable)	IT/IV		I	Recruiting	June 2013	Delivery and spread	02053320
		Ad11p/Ad3 chimeric	Solid tumor of epithelial origin/metastatic colorectal cancer (nonresponsive)	IV		I/II	Recruiting	September 2012	MTD/MFD, dose	02028442
DNX2401		D24RGD-4C	Glioblastoma (recurrent)	IT	Tumor resection	I	Ongoing	December 2008	MTD	00805376
		D24RGD-4C	Glioblastoma (recurrent)	IT	Temozolomide	I	Recruiting	September 2013	AE	01956734
		D24RGD-4C	Glioblastoma (recurrent)	Convection enhanced delivery by four catheters		I/II	Recruiting	June 2010	MTD, AE, preliminary efficacy	01582516
Recombinant human Ad5		D24RGD-4C	Ovarian cancer (recurrent)	IP		I	Completed	June 2007	MTD, toxicity	00562003
		Recombinant human Ad 5 E1B deleted	Hepatocellular carcinoma (advanced)	Hepatic intra-arterial	Transartery chemoembolization	III	Recruiting	January 2013	OS	01869088
Ad-RTS-hIL-12	Ad-RTS-hIL-12	Ad-RTS-hIL-12	Breast cancer (recurrent/metastatic)	IT	Palifosfamide-Tris	II	Recruiting	March 2013	Safety and tolerability, PFS	01703754
BG00001	Replication defective + hIFN- $\beta$		Pleural malignancies, metastatic or pleural mesothelioma	Intrapleural		I	Ongoing	March 2006	Toxicity	00299962
Ad5-SGE-REIC/Dkk3	Ad5-SGE-REIC/Dkk3		Prostate cancer (localized)	IT		I/IIa	Opening soon	October 2013	MTD/MFD	01931046

(Continued)

Table S1 (Continued)

Virus	Therapy name	Modification	Cancer	Route	Additional interventions	Phase	Status	Start date	Primary outcome	NCT identifier
Coxsackie virus	Ad-hCMV-TK, Ad-hCMV-Fit3L	Ad-hCMV-TK, Ad-hCMV-Fit3L	Malignant glioma, glioblastoma multiforme	IV	Given at time of resection followed by valacyclovir, temozolomide, and radiotherapy	I	Recruiting	December 2013	DLT, dose	01811992
	VCN-01	PH20 expressing Ad	Pancreatic adenocarcinoma (advanced)	IT	Gemcitabine	I	Recruiting	January 2014	AE, MFD, DLT	02045589
		PH20 expressing Ad	Solid tumor (locally advanced, metastatic)	IV	Gemcitabine	I	Recruiting	January 2014	AE, MFD, DLT	02045602
	CAVATAK	Coxsackievirus TypeA21	Malignant melanoma (stage IIIc-IV)	IT		II	Recruiting	October 2011	Immune-related PFS	01227551/01636882
		Coxsackievirus TypeA21	Malignant melanoma	IT		I	Completed	August 2005		00235482
		Coxsackievirus TypeA21	Melanoma (stage IV)	IT		I	Completed	February 2007	Safety and tolerability	00438009
		Coxsackievirus TypeA21	Solid tumor cancers (stage 4 melanoma, breast, prostate)	IV		I	Completed	March 2008	Safety and tolerability	00636558
	G207	Neuroattenuated HSV-1	Recurrent brain cancer (glioma, astrocytoma, glioblastoma)	IC	Tumor resection	Ib/II	Completed	December 2001	Safety, tolerability, efficacy	00028158
		Neuroattenuated HSV-1	Malignant glioma	IT	Radiation therapy	I	Completed	May 2005	AE	00157703
	NV1020	Attenuated HSV-1	Colorectal cancer metastatic to the liver	Hepatic artery infusion	Second-line chemotherapy	I/II	Completed	July 2004	AE/DLT, tumor response	00149396
HSV	HF10	Attenuated HSV-1	Head and neck cancer (refractory) or solid tumors with cutaneous/superficial lesions (squamous cell, breast carcinomas, malignant melanoma)	IT		I	Recruiting	August 2009	Local tumor response, AE	01017185
	HSV-1716	HSV-1 deleted in RL1 that encodes ICP34.5	Glioma (high grade, refractory/recurrent)	IT/peritumoral	Surgery, dexamethasone	I	Recruiting	December 2013	MTD	02031965
		HSV-1 deleted in RL1 that encodes ICP34.5	Malignant pleural mesothelioma	Intrapleural		I/IIa	Recruiting	October 2012	Safety and tolerability	01721018
		HSV-1 deleted in RL1 that encodes ICP34.5	Non-CNS solid tumors of adolescents and young adults	IT		I	Recruiting	March 2010	Safety and tolerability	00931931

T-Vec (talimogene laherparepvec, OncoVEX <sup>GM-CSF</sup> )	JSI 34.5-hGMCsf 47- pA-	Malignant melanoma (stage IIIc-IV)	IT	II	Completed	October 2005	Tumor response rate, survival time, PFS	00289016
	JSI 34.5-hGMCsf 47- pA-	Melanoma (stage IIIC, IIIC, IV)	IT	III	Ongoing	April 2009	CR/PR	00769704/ 01368276
	JSI 34.5-hGMCsf 47- pA-	Pancreatic cancer (unresectable)	IT	I	Completed	November 2006	AE	00402025
	JSI 34.5-hGMCsf 47- pA-	Melanoma (unresected stage IIIB-IV)	IT	Ib/II	Recruiting	February 2013	Safety and tolerability, OS	01740297
	JSI 34.5-hGMCsf 47- pA-	Melanoma (unresected stage IIIB-IVM1a)	IT	II	Opening soon		Biodistribution and shedding	02014441
OrienX010	hGM-CSF	Solid tumors (melanoma, liver, pancreatic, lung)	IT	I	Recruiting	May 2012	MTD, DLT	01935453
rRp450	HSV-1 rRp450	Liver metastases and primary liver tumors	Hepatic intra- arterial	I	Recruiting	October 2012	Safety and tolerability	01071941
M032	HSV-1 IL-12	Glioma (recurrent, progressive)	IT	I	Opening soon	April 2014	MFD/MTD	02062827
Measles virus	MV-CEA, MV-NIS	Ovarian epithelial cancer or primary peritoneal cancer (progressive, recurrent, refractory)	IP	I	Ongoing	April 2004	MTD, toxicity	00408590
	MV-NIS	Malignant pleural mesothelioma	Intrapleural	I	Recruiting	November 2011	AE	01503177
	MV-NIS	Squamous cell carcinoma of head and neck cancer (recurrent or metastatic)	IT	I	Recruiting	April 2013	MTD, AE	01846091
	MV-NIS	Multiple myeloma (recurrent or refractory)	IV	I	Recruiting	May 2007	MTD	00450814
	MV-CEA	Glioblastoma multiforme (recurrent)	IT	I	Recruiting	October 2006	AE, toxicity, MTD, viremia, viral gene expression, shedding	00390299
Newcastle disease virus	NDV-HUJ	Glioblastoma, sarcoma, neuroblastoma (resistant)	IV	I/II	Opening soon	September 2010	PFS	01174537
Parvovirus	Parvoryx	Glioblastoma multiforme (progressive primary or recurrent)	IT/IV	I/II	Recruiting	September 2011	Safety and tolerability	01301430
Poliovirus	PVSRIPO	Glioblastoma multiforme (recurrent supratentorial)	IT	I	Recruiting	January 2012	MTD	01491893
Reovirus	REOLYSIN®	Malignant glioma (recurrent)	IT	I	Completed	July 2006	MTD/DLT, tumor response rate	00528684

(Continued)

Table S1 (Continued)

Virus	Therapy name	Modification	Cancer	Route	Additional interventions	Phase	Status	Start date	Primary outcome	NCT identifier
	Serotype 3 dearing		Bone and soft tissue sarcomas metastatic to the lung	IV		II	Completed	June 2007	CR/PR/SD	00503295
	Serotype 3 dearing		Colorectal cancer (KRAS mutant, oxaliplatin refractory/intolerant)	IV	FOLFIRI (irinotecan, leucovorin, fluorouracil)	I	Ongoing	December 2010	MTD/DLT, PK of FOLFIRI with REOLYSIN®	01274624
	Serotype 3 dearing		Non-small-cell lung cancer (KRAS and epidermal growth factor receptor activation)	IV	Paclitaxel and carboplatin	II	Ongoing	March 2009	CR/PR	00861627
	Serotype 3 dearing		Ovarian epithelial, fallopian tube, or primary peritoneal cancer (recurrent or persistent)	IV	Paclitaxel	II	Recruiting	December 2010	PFS, AE	01199263
	Serotype 3 dearing		Pancreatic adenocarcinoma (advanced/metastatic)	IV	Gemcitabine	II	Ongoing	October 2009	CR/PR/SD	00998322
	Serotype 3 dearing		Head and neck carcinoma	IV	Paclitaxel and carboplatin	II	Unknown	August 2008	CR/PR/SD	00753038
	Serotype 3 dearing		Squamous cell carcinoma of the head and neck (platinum-refractory, metastatic/recurrent)	IV	Paclitaxel and carboplatin	III	Ongoing	June 2010	OS	01166542
	Serotype 3 dearing		Melanoma (metastatic)	IV	Paclitaxel and carboplatin	II	Ongoing	September 2009	CR/PR/SD	00984464
	Serotype 3 dearing		Squamous cell carcinoma of the lung	IV	Paclitaxel and carboplatin	II	Ongoing	October 2009	CR/PR/SD	00998192
	Serotype 3 dearing		Prostate cancer (metastatic castration resistant)	IV	Docetaxel and prednisone	II	Recruiting	July 2012	PFS	01619813
	Serotype 3 dearing		Breast cancer (advanced/metastatic)	IV	Paclitaxel	II	Recruiting	August 2012	PFS	01656538
	Serotype 3 dearing		Colorectal cancer (metastatic)	IV	FOLFOX6 and bevacizumab	II	Recruiting	August 2012	PFS	01622543
	Serotype 3 dearing		Non-small-cell lung cancer (previously treated, advanced or metastatic)	IV	Pemetrexed or docetaxel	II	Recruiting	October 2012	PFS	01708993
	Serotype 3 dearing		Melanoma (recurrent, stage IV)	IV		II	Completed	April 2008	Tumor response	00651157
	Serotype 3 dearing		Multiple myeloma (relapsed or refractory)	IV		I	Ongoing	April 2012	AE, MTD	01533194
	Serotype 3 dearing		Solid tumors (pediatric relapsed or refractory)	IV	Cyclophosphamide	I	Ongoing	December 2010	MTD	01240538



SVV	Serotype 3 dearing	Pancreatic cancer (recurrent or metastatic)	IV	Carboplatin and paclitaxel	II	Recruiting	December 2010	PFS	01280058
	Serotype 3 dearing	Ovarian epithelial cancer, primary peritoneal cancer, or fallopian tube cancer (nonresponsive to platinum therapy)	IV		I	Ongoing	April 2008	MTD, CR/PR	00602277
	Serotype 3 dearing	Multiple myeloma (refractory)	IV	Dexamethasone, carfilzomib	I	Opening soon	March 2014	Viral replication, AE	02101944
	SVV-001	Solid tumors with neuroendocrine features (neuroendocrine carcinoid)	IV		I	Unknown	April 2006	DLT, dose	00314925
	SVV-001/NTX-010	Neuroblastoma, rhabdomyosarcoma, or rare tumors with neuroendocrine features (relapsed or refractory)	IV	Cyclophosphamide	I	Recruiting	September 2009	Safety and tolerability, MTD, dose	01048892
	SVV-001/NTX-010	Small cell lung cancer (extensive stage)	IV	Given after platinum-containing cytoreductive induction chemotherapy	II	Ongoing	January 2010	PFS	01017601
	Attenuated	Solid organ tumors (advanced)	IV		I	Recruiting	November 2008	Safety and tolerability	00794131
	Attenuated	Peritoneal carcinomatosis (advanced)	IP		I/II	Recruiting	February 2012	AE	01443260
	Attenuated	Head and neck cancer (locoregionally advanced)	IV	Radiation therapy and cisplatin	I	Recruiting	April 2012	AE	01584284
	Attenuated	Malignant pleural effusions: primary, metastases, and mesothelioma	Intrapleural		I	Recruiting	January 2013	MTD	01766739
Vaccinia virus	TK deleted + GM-CSF	Malignant melanoma (unresectable stage III/IV)	IT		I/II	Completed	March 2007	Tumor response rate, survival time, PFS	00429312
	TK deleted + GM-CSF	Primary hepatocellular carcinoma (unresectable)	IT		II	Completed	August 2008	Dose	00554372
	TK deleted + GM-CSF	Colorectal carcinoma (refractory/intolerant to oxaliplatin, irinotecan, and Erbitux)	IV		I	Ongoing	July 2010	MTD/MFD	01380600
	TK deleted + GM-CSF	Colorectal carcinoma (metastatic, refractory)	IV	Irinotecan	I/II	Recruiting	January 2012	MTD/MFD, safety, radiographic response rate	01394939

(Continued)

Table S1 (Continued)

Virus	Therapy name	Modification	Cancer	Route	Additional interventions	Phase	Status	Start date	Primary outcome	NCT identifier
		TK deleted + GM-CSF	Hepatocellular carcinoma (advanced, failed sorafenib)	IT		II	Ongoing	August 2011	OS	01387555
		TK deleted + GM-CSF	Solid tumors (melanoma, non-small-cell lung cancer, renal cell carcinoma, squamous cell carcinoma of head and neck) (advanced/metastatic/refractory to standard therapy)	IV		I	Ongoing	June 2008	MTD/MFD, safety and toxicity	00625456
		TK deleted + GM-CSF	Hepatic carcinoma (primary or metastatic)	IT		I	Completed	January 2006	MTD/MFD	00629759
		TK deleted + GM-CSF	Colorectal cancer (refractory)	IV		I	Ongoing	September 2010	MTD/MFD, safety	01469611
		TK deleted + GM-CSF	Peritoneal carcinomatosis of ovarian cancer origin	IV		II	Opening soon	January 2014	Radiographic response rate	02017678
		TK deleted + GM-CSF	Solid tumors in pediatric patients (neuroblastoma, phabdomyosarcoma, lymphoma, Wilms' tumor, Ewing's sarcoma) (refractory)	IT		I	Ongoing	August 2010	MTD/MFD, safety and toxicity	01169584
		TK deleted + GM-CSF	Hepatocellular carcinoma (advanced, naïve to sorafenib)	IV		II	Ongoing	June 2012	Tumor response	01636284
		TK deleted + GM-CSF	Primary hepatocellular carcinoma	IV then IT	Prior to standard sorafenib	II	Ongoing	August 2009	Safety and tolerability, AE	01171651
	wDD-CDSR	wDD-CDSR	Melanoma, breast cancer, head and neck squamous cell cancer, liver, colorectal, or pancreatic adenocarcinoma	IV or IT		I	Ongoing	May 2008	MTD/MFD	00574977
VSV	VSV-IFNb	VSV-IFNb	Liver cancer	IT		I	Recruiting	August 2012	MTD/DLT, AE, tumor response, OS	01628640

**Abbreviations:** Ad, adenovirus; AE, adverse event; BCG, bacillus Calmette-Guérin; CEA, carcinoembryonic antigen; CNS, central nervous system; CR, complete response; DCR, durable complete response; DLT, dose-limiting toxicity; GM-CSF, granulocyte macrophage colony-stimulating factor; HSV, herpes simplex virus; IC, intracerebral; IP, intraperitoneally; IL, interleukin; IT, intratumoral or intralesional; IV, intravenously; MFD, maximum feasible dose; MSC, mesenchymal stem cell; MTD, maximum tolerated dose; NCT identifier, clinicaltrials.gov registered trial identification number; NIS, natrium-iodine symporter; OS, overall survival; PFS, progression-free survival; PK, pharmacokinetics; PR, partial response; SD, stable disease; SVV, Seneca Valley virus; TK, thymidine kinase; VSV, vesicular stomatitis virus.

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