Oncolytic viruses (OVs) comprise a diverse group of biological agents with potential as cancer therapeutics. Numerous clinical trials are under way or have been completed using this approach. In 2015, in a milestone for the field, talimogene laherparepvec became the first OV to gain US Food and Drug Administration approval of the oncolytic herpesvirus talimogene laherparepvec in advanced melanoma, a major breakthrough for the field. Thus, the OV approach to cancer therapy is becoming more interesting for scientists, clinicians, and the public. The main purpose of this review is to give a basic overview of OVs in clinical development and provide a description of the current status of clinical trials.

## Importance
Oncolytic viruses (OVs) are emerging as important agents in cancer treatment. Oncolytic viruses offer the attractive therapeutic combination of tumor-specific cell lysis together with immune stimulation, therefore acting as potential in situ tumor vaccines. Moreover, OVs can be engineered for optimization of tumor selectivity and enhanced immune stimulation and can be readily combined with other agents. The effectiveness of OVs has been demonstrated in many preclinical studies and recently in humans, with US Food and Drug Administration approval of the oncolytic herpesvirus talimogene laherparepvec in advanced melanoma, a major breakthrough for the field. Thus, the OV approach to cancer therapy is becoming more interesting for scientists, clinicians, and the public. The main purpose of this review is to give a basic overview of OVs in clinical development and provide a description of the current status of clinical trials.

## Observations
In 2016 approximately 40 clinical trials are recruiting patients, using a range of OVs in multiple cancer types. There are also many more trials in the planning stages. Therefore, we are now in the most active period of clinical OV studies in the history of the field. There are several OVs currently being tested with many additional engineered derivatives. In OV clinical trials, there are a number of specific areas that should be considered, including viral pharmacokinetics and pharmacodynamics, potential toxic effects, and monitoring of the patients’ immune status. Clinical development of OVs is increasingly focused on their immune stimulatory properties, which may work synergistically with immune checkpoint inhibitors and other strategies in the treatment of human cancer.

## Conclusions and Relevance
Oncolytic viruses are an active area of clinical research. The ability of these agents to harness antitumor immunity appears to be key for their success. Combinatorial studies with immune checkpoint blockade have started and the results are awaited with great interest.

Oncolytic viruses (OVs) comprise a diverse group of biologic agents with potential as cancer therapeutics. Numerous clinical trials are under way or have been completed using this approach. In 2015, in a milestone for the field, talimogene laherparepvec became the first OV to gain US Food and Drug Administration (FDA) approval in the United States. However, the use of viruses for cancer treatment is not new. Throughout the 20th century, case studies and small trials of various viruses in cancer therapy were reported. These investigations conducted with small numbers of patients used wild-type and often crudely prepared viral isolates, and it was not until the 1990s that the era of genetic engineering of viruses to enhance their oncolytic potential began. The first reported genetically engineered OV was based on herpes simplex virus type 1 (HSV1). This development was rapidly followed by many studies illustrating the effectiveness of this approach using a diverse range of viruses and tumor models. The main focus of the field during the early development of OVs was to identify viruses or their engineered variants with tumor-selective replication. However, it has always been appreciated that an immune component is important and may be critical for the therapeutic efficacy of this approach. Indeed, OVs are now broadly considered as immunotherapy agents for which effectiveness in patients depends on activation of host antitumor immune responses.

Early clinical trials of OVs showed encouraging safety profiles, even at high doses, with some promising responses, evidence of intratumoral viral replication, and immune cell infiltrates. The field is now gaining traction after the report of significant benefits in a large randomized phase 3 clinical trial of the engineered immunostimulatory OV talimogene laherparepvec in patients with advanced melanoma and its subsequent approval in the United States and European Union. Many OVs are now under investigation in advanced trials, with some encouraging data. Thus, after 2 decades of clinical trials in humans with cancer, OVs are emerging as therapeutic agents in oncology. This review summarizes recent progress in this area and describes ongoing clinical trials.
Mechanisms of OV Action

A general mechanistic understanding of OV action is emerging in which therapeutic efficacy is achieved by a combination of selective tumor cell killing and establishment of antitumor immunity (Figure). Immune stimulation is caused by release of cell debris and viral antigens in the tumor microenvironment. Tumor selectivity in OV therapy is driven by several factors. The first of these is cellular entry via virus-specific, receptor-mediated mechanisms. A specific viral entry receptor is often highly expressed on tumor cells. However, there are also efforts to improve tumor selectivity by retargeting OVs to enter cells through tumor-specific receptors.

Second, rapid cell division in tumor cells with high metabolic and replicative activity may support increased viral replication compared with normal quiescent cells. In addition, tumor-driver mutations specifically increase the selectivity of virus replication in tumor cells.14,15

Third, many tumor cells have deficiencies in antiviral type I interferon signaling, therefore supporting selective virus replication.16 Viral replication within the tumor microenvironment leads to innate and adaptive immune activation. This activation limits virus spread; however, the presence of virus together with cell lysis, with release of tumor antigens and danger-associated molecular patterns, may overcome immunosuppression in the tumor microenvironment and promote antitumor immunity. The success of this approach is influenced by factors including pre-existing antiviral and antitumor immunity and incorporation of immune stimulatory transgenes.

OVs as Cancer Therapeutics

General Properties

A wide range of viruses with diverse properties are under investigation clinically (eTable 1 in the Supplement). Oncolytic viruses range in size and complexity from large, double-stranded DNA viruses such as vaccinia (190 kilobase [kb])17 and HSV1 (152 kb)18 to the tiny parvovirus H1 (5-kb linear, single-stranded DNA).19 Oncolytic viruses cause cell lysis by various means during their life cycle, with the exception of retrovirus, which can be rendered lytic by toxic transgene expression.20

There are a few wild-type viruses in clinical use. These include reovirus, a human virus with low pathogenicity;21,22 coxsackievirus, which is structurally related to polio virus and causes several symptoms in humans;23,24; and viruses with nonhuman hosts, including Newcastle disease virus (avian);25 parvovirus H1 (rat);19 and vesicular stomatitis virus (VSV) (insects, horses, cows, and pigs).26

DAMPs indicates damage-associated molecular patterns; IFN, interferon; JAK-STAT, Janus kinase–signal transducer and activator of transcription; MYD88, myeloid differentiation primary response gene 88; NFKβ, nuclear factor κB; NK, natural killer; PAMPs, pathogen-associated molecular patterns; RF, Interferon regulatory factor; and TRIF, TIR (toll/II-1 receptor)-domain-containing adapter-inducing interferon-β.
Oncolytic vaccines and measles are derived from vaccine strains. There are documented cases of wild-type measles viruses infections and tumor regression. Most measles OVs are based on the attenuated Edmonston measles vaccine strain (MVEdm), which has an excellent safety record after decades of use in humans.

Each type of OV has a specific cellular entry mechanism (eTable 1 in the Supplement), which can affect the efficacy. For example, polio virus enters cells through CD155 (the polio virus receptor), which is abundantly expressed in many tumor types. The adenovirus entry receptor, CAR (coxsackie and adenovirus receptor), is expressed variably in tumor cells; therefore, adenovirus retargeting to other cellular receptors to enhance tumor cell binding has also been widely investigated.

Many OVs have been engineered to improve tumor cell selectivity. Herpes simplex virus type 1 has strong lytic properties and several variants have been constructed, often via deletion of the ICP34.5 neurovirulence and ICP6 (UL39) (ribonucleotide reductase) genes. ICP6 is necessary for generation of the neurotropism pool needed for viral replication in normal, quiescent cells. Deletion of ICP6 provides replicative selectivity for cells with inactivation of the p16INK4a tumor suppressor, one of the most common deficits in cancer. Similarly, revirus has oncolytic selectivity to cells with activation of ras signaling. In the case of adenovirus, replication occurs in S phase, and the wild-type virus encodes a protein (E1A) that functions via retinoblastoma signaling to promote S-phase entry. Because cancer typically possesses retinoblastoma pathway mutations and enriched S-phase populations, and to promote safety and prevent replication in normal cells, the E1A gene (GenBank NC_001405.1) has been deleted from oncolytic adenovirus. Tumor selectivity and potency may also be enhanced by direct intratumoral injection of high viral loads. However, the ability of some OVs, such as vaccinia virus, to spread systemically in the bloodstream may facilitate treatment of metastases, as demonstrated in preclinical models.

Clinical Application

A diverse range of viruses has been investigated as potential cancer therapeutics. The individual characteristics of OVs currently in clinical trials are summarized below and outlined in Table 1.

Herpesvirus

Oncolytic viruses derived from engineered HSV1 (oHSVs) have been tested widely in patients. A major focus of the field has now moved to talimogene laherparepvec, an immunostimulatory oHSV that expresses granulocyte-macrophage colony-stimulating factor (GM-CSF). Intratumoral injection of talimogene laherparepvec led to significant improvement in durable response rate (DRR) in patients with melanoma (16.3%) compared with controls (2.1%). Effects were most pronounced in patients with stage IIIIB, IIC, IVMa, or treatment-naive disease. Tumor regression was seen in distant noninjected lesions, suggesting the establishment of systemic antitumor immunity. Talimogene laherparepvec is derived from a clinical HSV1 strain (JS1) deleted for ICP34.5 and ICP47, which normally acts to block HSV1 major histocompatibility class I antigen presentation on the infected cell surface resulting in immune evasion. The reason for the success of this agent is likely a combination of the choice of melanoma (an immunogenic tumor) and immunostimulation via GM-CSF as well as the use of a clinical HSV1 strain backbone, which may allow improved replication in patients compared with other oHSVs built on a laboratory HSV1 strain background.

Adenovirus

Oncolytic adenoviruses were some of the earliest OVs to enter clinical trials. The history of adenovirus as an oncolytic agent has been well reviewed. An E1A/E1B-deleted virus (ONXY015) has been extensively tested and approved for treatment of head and neck cancer in China under the name H101. An integrin-binding retargeted adenovirus, Δ24RGD (DNX2401), has been examined in clinical trials in which the maximum tolerated dose (MTD) was not reached and some responses were observed. Trials are also under way with enadenotucire, which is built on the Ad11/3 serotype rather than the common Ad5 serotype, rendering it less susceptible to rapid neutralization in the bloodstream.

Vaccinia Virus

At the present time, JX-594 (Pexa-Vec) is being tested in multiple tumor types. Pediatric measles vaccine strain engineered to express human GM-CSF and has been tested in more than 300 patients. It is well tolerated and increased survival in patients with liver cancer after intravenous injection. Prostvac, a prime-boost regimen targeting prostate-specific antigen in prostate cancer, uses engineered vaccinia as a primary immunotherapy, followed by boosters using fowlpox virus. Both vectors express prostate-specific antigen and a panel of costimulatory molecules: ICAM-1 (intercellular adhesion molecule 1), B7.1, and LFA3. Subcutaneous injection of Prostvac initiates an antitumor immune response, is well tolerated, and significantly increased overall survival in phase 2 trials. This dual virus approach overcomes the rapid appearance of neutralizing antibodies against vaccinia. A phase 1 dose-escalation trial of Prostvac in combination with ipilimumab showed no additional toxic effects and some promising responses (MTD was not reached).

Measles Virus

Measles causes fusogenic syncytia formation and cell death. A measles virus expressing the human sodium/iodide symporter SLC5A5 (MV-NIS) is currently in a range of clinical trials. MV-NIS allows imaging of infected cells and monitoring of treatment progression as well as radiovirotherapy with I-131-labeled sodium iodide. Clinical data have confirmed safety and demonstrated imaging of virus infection and tumor regression with this approach.

Coxsackievirus

Coxsackievirus has oncolytic properties in cancer cell lines and leads to a robust immune response. Wild-type coxsackievirus A21 is in clinical trials under the name Cavatak. Numerous trials are ongoing and are built on favorable 2015 phase 2 data in stage IIIC and stage IV melanoma. One study reported a preliminary DRR of 21% with regression of distant noninjected lesions.

Polio Virus

Polio virus has demonstrated oncolytic properties in preclinical studies and has attracted attention owing to initial results in brain tumors and wide media exposure. These studies were performed using PVS-RIPO, which has been engineered to abolish the neurovirulence...
<table>
<thead>
<tr>
<th>Virus</th>
<th>Modification</th>
<th>Published Clinical Studies*a</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Herpesvirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1716</td>
<td>ICP34.5 deletion</td>
<td>Melanoma (exploratory), 25 single intratumoral injection; tumor shrinkage, necrosis replication, safety; high-grade glioma (phase 1 trial), 26 resection cavity injection; safety and responses reported</td>
</tr>
<tr>
<td>HF10</td>
<td>UL56 deletion; single copy of UL52</td>
<td>Pancreatic cancer (phase 1), 27 multiple intratumoral injections; safe with potential responses (stable disease) reported</td>
</tr>
<tr>
<td>G207</td>
<td>ICP34.5 deletion; UL39 disruption</td>
<td>Recurrent malignant glioma (phase 1), 28 resection cavity injection plus irradiation; safety and some radiographic responses</td>
</tr>
<tr>
<td>Talimogene laherparevvec</td>
<td>ICP34.5 deletion; US11 deletion; human GM-CSF insertion</td>
<td>Advanced melanoma (phase 3), 29 intralesional talimogene laherparevvec vs subcutaneous GM-CSF, 16.3% DRR; safety established; effects most pronounced in patients with stage IIIIB, IIIC, or IVN1a disease</td>
</tr>
<tr>
<td><strong>Adenovirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG0070</td>
<td>E3 deletion; GM-CSF insertion</td>
<td>Nonmuscle invasive bladder cancer (phase 1) 30; viral replication suggested, minor toxicity; complete response rate 48.6% and median response duration 10.4 mo</td>
</tr>
<tr>
<td>ICOVIR5</td>
<td>Modified DNX-2401-2E2 promoter opimitized</td>
<td>Diffuse intrinsic pontine glioma (exploratory); mesenchymal stem cell loaded virus; intra-arterial injection, safe 31</td>
</tr>
<tr>
<td>VCN-0141</td>
<td>PH20 hyaluronidase insertion; RGD targeting</td>
<td>None reported</td>
</tr>
<tr>
<td>DNX-2401</td>
<td>Δ24RGD insertion</td>
<td>Recurrent malignant gynecologic disease (phase 1) 32; intraperitoneal delivery; established safety, feasibility, and potential antitumor response; MTD not reached</td>
</tr>
<tr>
<td>Enadenotucirev*</td>
<td>Chimeric Ad11/3 group B</td>
<td>None reported</td>
</tr>
<tr>
<td>Ads-vCD/mutTKR39rep-hIL12**</td>
<td>Ad serotype 5; insertion of IL12; insertion of yeast cytosine deaminase; insertion of TKR39 (thymidine kinase mutant)</td>
<td>None reported</td>
</tr>
<tr>
<td>AdsPTD(Cga-E1AmiR122)**</td>
<td>Ad serotype 5; E1B deleted; conditional neuroendocrine E1A expression with miR122 in UTR; PTD motif in capsid</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Measles Virus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MV-NIS</td>
<td>Edmonston vaccine measles strain; insertion of sodium-iodide symporter</td>
<td>Drug-resistant ovarian cancer (phase 1) 33; intraperitoneal delivery every 4 wk for 6 cycles; well tolerated; promising survival data, evidence for immune stimulation; radioactive iodine 123 uptake observed by PET/CT and associated with longer progression-free survival</td>
</tr>
<tr>
<td><strong>Vaccinia Virus</strong></td>
<td></td>
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</tr>
<tr>
<td>PexaVec (JX594)</td>
<td>TK deletion; GM-CSF insertion</td>
<td>Advanced hepatocellular carcinoma (randomized phase 2) 34; dose-related response; oncolytic and immunotherapeutic effects observed; treatment-refractory colorectal cancer (phase 1b) 35; multiple dosing; no toxic effects and MTD not reached; 10 patients (67%) had radiographically stable disease</td>
</tr>
<tr>
<td>Psostvac</td>
<td>Expresses PSA; expresses TRICOM (3 costimulatory genes); administered with a subsequent fowlpox boost; engineered to express PSA and TRICOM (artificial gene construct)</td>
<td>Castration-resistant prostate cancer (phase 1 and numerous phase 2 [reviewed in Singh et al 36]); well tolerated, encouraging survival data, combined with various agents including radiotherapy and docetaxel; phase 3 trial completed in 2015 (results not available)</td>
</tr>
<tr>
<td><strong>Reovirus</strong></td>
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<tr>
<td>Reolysin</td>
<td>Wild-type virus, serotype 3</td>
<td>Advanced cancer (phase 1) 37; multiple intravenous injections; safe and well tolerated; evidence of response, particularly in patients with viral shedding; Metastatic melanoma (phase 2) 38; multiple intravenous injections; well tolerated; no objective responses</td>
</tr>
<tr>
<td><strong>Polio Virus</strong></td>
<td></td>
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<tr>
<td>PVS-RIPO**</td>
<td>Attenuated polio virus Sabin type 1; IRES replaced with HRV2 IRES to prevent neurotoxic side effects</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Coxsackievirus</strong></td>
<td></td>
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</tr>
<tr>
<td>Cavatak**</td>
<td>Wild-type coxsackievirus A21</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Vesicular Stomatitis Virus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VSV-hIFNβ</td>
<td>Insertion of IFNβ</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Parvovirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ParvOryx**</td>
<td>Parvovirus H1 (wild-type)</td>
<td>None reported</td>
</tr>
<tr>
<td><strong>Retrovirus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toca511**</td>
<td>Murine leukemia virus; insertion of yeast cytosine deaminase</td>
<td>None reported</td>
</tr>
</tbody>
</table>

Abbreviations: CT, computed tomography; DRR, durable response rate; GM-CSF, granulocyte-macrophage colony-stimulating factor; HSV, herpes simplex virus; IFNβ, interferon β; IRES, internal ribosome entry site; MTD, maximum tolerated dose; PET, positron emission tomography; PSA, prostate-specific antigen; PTD, protein transduction domain; RGD, arginine-glycine-aspartate; UTR, untranslated region; VSV, vesicular stomatitis virus.

*a The clinical trials listed are not exhaustive but provide representative examples of published studies or the most advanced studies published so far.
of the native virus. PVS-RIPO is a cytotoxic and immunostimulatory virus with preliminary reports of durable radiographic and clinical responses in glioblastoma.67 Based on recent phase I data, PVS-RIPO was designated as a breakthrough therapy in recurrent glioblastoma.68

**Retrovirus**
Retroviruses are potentially useful agents because they readily infect mitotic cells and rapidly spread, although without necessarily causing cell lysis.20 Toca511 is based on murine leukemia virus engineered to express the yeast enzyme cytosine deaminase, which converts 5-fluorocytosine to the toxic metabolite 5-fluorouracil. In studies53,54 of mice with implanted gliomas, Toca511 therapy resulted in long-term survival and systemic antitumor immunity mediated by memory T cells. Toca511 is currently in a phase 2/3 clinical trial for malignant glioma and has shown promising interim results.69

**Parvovirus H1**
Parvovirus H1 causes tumor-selective cell killing that is thought to result from a combination of toxic effects of the viral gene product NS1 as well as lytic viral replication.26 A trial of wild-type parvovirus H1 (ParVOrx) in recurrent glioblastoma has been completed and this agent is currently in trials for metastatic pancreatic cancer.

**Vesicular Stomatitis Virus**
Vesicular stomatitis virus selectivity is dependent on deficient interferon signaling26,71 An engineered VSV variant overexpressing interferon β is currently in a clinical trial for liver cancer. It is thought that interferon β may act synergistically with VSV by protecting normal cells from infection and causing tumor cell-specific destruction and antitumor immune responses.

**Newcastle Disease Virus**
Wild-type Newcastle disease virus has oncolytic effects in a variety of tumor types through syncytia formation and apoptosis.25 Early clinical trials showed promise and safety27; however, to our knowledge, no trials involving this virus are presently recruiting patients.

### Considerations for OV Clinical Trials

With the emergence of increasing numbers of OVs and combinatorial studies in the clinical trial arena, it is worth considering issues involved in clinical trial design and execution. Areas evolving as the field develops are delivery, viral pharmacokinetics and pharmacodynamics, and biomarkers.

### Safety
Although mortality has been reported occasionally, published trial data have not shown significant general safety issues. However, as OVs with greater potency are developed and used in novel combinational treatments, safety remains a concern. Despite engineering for tumor cell specificity, there is the possibility of off-target effects, and genetic manipulation may result in unexpected toxic effects. Other concerns include virus mutation, evolution, and recombination; cytotoxic gene products; and viral transmissibility. Oncolytic HSVs have retained their native thymidine kinase gene, which facilitates virus replication and is also the target of the antiviral drug ganciclovir. The retention of thymidine kinase allows the possibility of controlling infection and is seen as an important advantage in terms of safety. Potential safety concerns are reflected in clinical trial criteria, which do not allow inclusion of immunocompromised patients or those with active viral infections.

### Toxic and Adverse Effects
Local delivery of OVs is generally well tolerated. The most common adverse effects reported are mild flu like symptoms, which may be more severe after systemic administration, and local reaction at the injection site. These reactions can be reduced by acetaminophen administration before treatment.73

### Dose
In contrast to results in conventional drug clinical trials, many OVs do not reach an MTD owing to the concentration of virus stock that is possible to achieve or very high tolerance for the virus. Maximum tolerated dose may need to be re-established for trials using novel therapeutic combinations.

### Viral Pharmacokinetics, Pharmacodynamics, and Biomarkers
Effectiveness of OV therapy is monitored by standard approaches, including imaging and tumor-specific biomarkers. In addition, viral pharmacokinetics and pharmacodynamics (shedding, viremia, replication, genomes, and viral load) are frequently included in OV trials. These approaches allow inpatient tracking of viral fate in patients. Additional viral pharmacokinetic and pharmacodynamic variables include analysis of intratumoral viral replication and immune infiltrates by immunohistochemistry and circulating immune cell status. In multi-institutional trials, it may be optimal to use centralized testing to ensure reproducibility.

### Resistance Mechanisms
One of the most fascinating features of OV therapy is the battle between the virus and the host, which is vital in determining the therapeutic outcome. The major resistance mechanisms in OV therapy result from the ability of the host to rapidly shut down viral replication. Host antiviral mechanisms include the presence of neutralizing antibodies and the rapid mobilization of innate immune cells in response to OVs. Cells recruited after OV treatment include neutrophils, natural killer cells, macrophages, and microglia in the brain.74,75 Innate immunity is a major resistance mechanism, which can restrict the ability of the virus to replicate and spread within tumors. The existence of these innate immune resistance mechanisms has led to the idea that inhibition of immune responses early in treatment may be beneficial, and immunosuppressants such as cyclophosphamide promote viral replication.76 However, this approach should be treated with caution for safety reasons and also to ensure that the ultimate antitumor response is not blocked. Studies77 have shown that vascular endothelial growth factor also plays an important, although virus-
dependent, role in OV action. In the case of oHSV, vascular endothelial growth factor can limit efficacy, potentially because of recruitment of myeloid cells into the tumor microenvironment. Conversely, vascular endothelial growth factor sensitizes tumor vasculature to vaccinia and VSV via a novel mechanism.

Delivery
Therapeutic delivery of OVs is dependent on virus and tumor type. Most often, OVs are injected directly into the tumor site. For example, tumors of the brain are treated using local delivery by multiple injections at a single time point (during surgery). Other, more accessible tumors can be treated with multiple doses and multiple injection sites over time. The experience with talimogene laherparepvec provides an indication that OV administration at a single tumor site can lead to regression of distant tumors, implying that the induction of local antitumor immunity can have systemic effects. Intravenous injection is also commonly used and allows systemic administration to multiple tumor sites. Cellular carriers may also be used, which may protect the virus from recognition by the host immune system before reaching the tumor.

Ongoing OV Trials
A search of clinicaltrials.gov performed April 1, 2016, listed approximately 40 clinical trials currently recruiting patients for treatment with OVs (summarized in Table 2; full list in eTable 2 in the Supplement). There is representation from multiple countries across 4 continents, with most trials being conducted in the United States. These trials have been almost exclusively performed in adults, with studies in young adults and pediatric patients just beginning. Most trials are early-phase, dose-finding, and exploratory studies, although increasing numbers of late-phase trials are anticipated. Trials increasingly incorporate viral pharmacokinetics and pharmacodynamics, and a consistent feature is monitoring of the immune response to virus and tumor. Few viruses in the present trials express human transgenes. Based on encouraging preclinical data, numerous combination studies are under way using small molecules and chemotherapy. As described below, most trials involve combination therapy with immune checkpoint blockade.

Immune Therapy OV Combinations
Recently, cancer immunotherapies have gathered an unprecedented level of interest owing to newly FDA-批准的immunologic treatments based on targeting immune checkpoint inhibition pathways that can relieve T-cell exhaustion in the tumor microenvironment. Ligands that activate checkpoint pathways and cause T-cell exhaustion are often expressed by tumor cells or within the tumor microenvironment. These ligands include PD-1, PD-L1, PD-L2, and many others. Multiple blocking antibodies against these molecules are now in clinical trials and have shown low toxic effects and durable responses in clinical studies in various solid tumors. In addition, combinations of multiple checkpoint inhibitors appear to improve outcomes in melanoma compared with single checkpoint inhibitors. Phase 3 studies in advanced melanoma show that patients treated with the combination of ipilimumab (anti-CTLA4) and nivolumab (anti-PD1) have an 11.5-month median survival compared with 2.5 months for ipilimumab alone.

There is presently a clear opportunity to investigate the impact of combining immunostimulatory OVs with immune checkpoint blockade in cancers, effectively accelerating the antitumor immune response while removing the barriers that may otherwise impair T-cell-mediated tumor killing. Indeed, talimogene laherparepvec is now in a clinical trial in combination with ipilimumab. Currently, pembrolizumab (anti-PD1) and ipilimumab are being tested in combination with talimogene laherparepvec, Cavaatak, and Reolysin (eTable 2 in the Supplement). These combinations may allow greater response rates in immune-sensitive tumors, such as melanoma, and may render checkpoint inhibitor-resistant tumors more sensitive to treatment. The number of potential combinations of immune therapies is enormous, and an important question is whether preclinical studies can accurately predict which of these combinations will be the most efficacious in humans. Overall, further work is required to evaluate the possible direct effects of each type of OV on immune checkpoint mechanisms and determine the best combinations for future trials.

The creation of novel OVs expressing checkpoint inhibitory antibody molecules is under way in many laboratories. This development would allow in situ expression, avoiding adverse effects from systemic delivery of the antibody, and increase the local immune response against the tumor cells. An engineered oncolytic adenovirus Delta-24,RGDOX (DNAtrix Inc) has been shown to enhance antitumor immune responses through expression of OX40L, an immune checkpoint costimulatory ligand.

Other OVs in Development
Many laboratories are developing improved OVs at the preclinical level. In addition to immune stimulation, efforts are being made to improve virus potency and tumor targeting. For example, in the past few years, seed sequences of differentially expressed microRNAs have been incorporated into OVs to increase tumor cell selectivity. This strategy can be adapted to different viruses and may provide a safer and more efficient way to increase virus efficacy by reducing collateral damage to healthy cells. At present, one clinical trial on adenovirus AdsPSTD (CgA-E1AmiR122) uses miR-122 to control E1A expression to ensure tumor selective replication.

It is also recognized that improved tumor cell lysis may be necessary for therapeutic efficacy. It is thought that this improvement can serve the dual role of shrinking tumors and provoking greater antitumor immune responses via release of tumor antigens and danger signals. A wide range of next-generation OVs are in preclinical development with improved lytic properties. For example, rQNestin34.5 was developed for use in brain tumors in which ICP34.5 expression (deleted in other OHSVs) is restored under transcriptional control of the nestin promoter to drive expression selectively in glioma stem cells. Other combinations have also been reported, including histone deacetylase inhibition, which promotes oncolytic herpesvirus replication in animal models.

Conclusions
Recent clinical trials have shown great promise for immune therapies in cancer treatment. However, the ability of tumor cells to evade the immune system remains a major challenge. After years of development and study, OVs are showing promise as immunotherapeutic
agents. Because these viruses have co-evolved with humans, they provide unique ways to stimulate immune attack, which may overcome the formidable interactions of tumor cells with the immune system. Improved viruses, in terms of tumor selectivity and potency, and optimized combinations with other immune therapies may lead to further advances in patient outcomes. Given the increasing array of OVs in development, as well as other immune stimulatory agents, the challenge in the field will be to successfully identify the OVs and combinations that will be the most effective for patients, particularly those with tumors that are resistant to other therapies.

### Table 2. Selected Open Oncolytic Virus Trials

<table>
<thead>
<tr>
<th>Virus</th>
<th>Delivery, Combination</th>
<th>Phase/Sponsor</th>
<th>Tumor Type</th>
<th>Primary Measures</th>
<th>Secondary Measures</th>
<th>Location</th>
</tr>
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<tbody>
<tr>
<td>Herpesvirus</td>
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<tr>
<td>HSV-1716</td>
<td>Intratumoral, single agent</td>
<td>Phase 1/ Pediatric Brain Tumor Consortium</td>
<td>Recurrent pediatric brain/AA, AO, GBM, GC, GS</td>
<td>MTD</td>
<td>Efficacy, imaging, viral PK/PD, immune response</td>
<td>United States</td>
</tr>
<tr>
<td>HF10</td>
<td>Intratumoral, ipilimumab</td>
<td>Phase 2/Takara Bio, Inc</td>
<td>Malignant melanoma</td>
<td>Efficacy</td>
<td>Immune response</td>
<td>United States</td>
</tr>
<tr>
<td>T-VEC</td>
<td>Intratumoral, single agent</td>
<td>Phase 2/Amgen</td>
<td>Melanoma (unresolved)</td>
<td>Efficacy, intratumoral CD8+ T cells</td>
<td>Safety, response measures, correlations with CD8+</td>
<td>Europe</td>
</tr>
<tr>
<td>Ipilimumab</td>
<td>Intratumoral, with/without T-VEC</td>
<td>Phase 2/Amgen</td>
<td>Melanoma (unresolved)</td>
<td>Safety/tolerability, efficacy</td>
<td>Additional safety; additional efficacy</td>
<td>Germany</td>
</tr>
</tbody>
</table>

| Adenovirus     |                                                            |                                 |                              |                                   |                    |                   |
| VCN-01         | Intravenous (single), with/without gemcitabine/abraxane   | Phase 1/ VCN Biosciences, S.L.  | Advanced solid tumors        | Safety/tolerability             | Efficacy, viral PK/PD | Spain            |
| DNX-2401       | Intratumoral, with/without interferon-γ                    | Phase 1b/DNAtrix, Inc.          | Recurrent brain, GBM, GS     | Efficacy (MRI imaging)           | Efficacy, safety, immune response, QoL | United States     |
| Enadenotucirev | Intravenous, pembrolizumab                                 | Phase 1/Psious Therapeutics Ltd | Colorectal cancer, bladder, SCCHN, salivary gland cancer | MTD, safety                  | Viral PK/PD, antitumor activity | United States     |

| Measles Virus  |                                                            |                                 |                              |                                   |                    |                   |
| MV-N15         | Intraperitoneal, mesenchymal stem cell                     | Phase 1/2/Mayo Clinic           | Recurrent ovarian             | MTD, safety, efficacy            | Tumor response, immune response, viral PK/PD | United States     |

| Vaccinia Virus |                                                            |                                 |                              |                                   |                    |                   |
| Sorafenib      | Intratumoral (multiple), with/without PexaVec              | Phase 3/SillaJen, Inc           | Hepatocellular carcinoma     | Efficacy                          | Additional efficacy, safety | United States, New Zealand |
| Prostvac       | Subcutaneous vs no treatment                               | Phase 2/National Cancer Institute | Recurrent prostate cancer   | Decreased PSA rise              |                     | United States     |

| Reovirus       |                                                            |                                 |                              |                                   |                    |                   |
| Reolysin       | Intravenous, pembrolizumab and chemotherapy                | Phase 1b/Oncolytics Biotech     | Pancreatic adenocarcinoma     | Safety                        | Efficacy, immune response | United States     |

| Polio Virus    |                                                            |                                 |                              |                                   |                    |                   |
| PVSRIPO        | Intracerebral (convection-enhanced delivery), single agent | Phase 1/Duke University Medical Center | Recurrent GBM                 | MTD                        | Efficacy          | United States     |

| Coxsackie Virus|                                                            |                                 |                              |                                   |                    |                   |
| Cavatak        | Intratumoral, ipilimumab                                  | Phase 1/Viralytics               | Advanced melanoma             | Safety, tolerability            | Efficacy          | United States     |
| Cavatak        | Intratumoral, pembrolizumab                               | Phase 1/Viralytics               | Advanced melanoma             | Safety, tolerability            | Efficacy          | United States     |

| Other Viruses  |                                                            |                                 |                              |                                   |                    |                   |
| TOCA FC/TOCA 511 | Intratumoral (single dose), single agent                  | Phase 2/3/Tocagen Inc           | Brain, recurrent GBM/AA        | Efficacy (comparison with chemotherapy) |                    | United States     |

Abbreviations: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; GBM, glioblastoma multiforme; GC, gliomatosis cerebri; GS, gliosarcoma; HSV, herpes simplex virus; MRI, magnetic resonance imaging; MTD, maximum tolerated dose; PK/PD, pharmacokinetics/dynamics; PSA, prostate-specific antigen; QoL, quality of life; SCCHN, squamous cell carcinoma of the head and neck; TLS, serum cytokines, intratumoral PD1-expressing cells, neutralizing antibodies.

* Recruiting as of December 30, 2015 (clinicaltrials.gov [oncolytic virus as search term] and EU clinical trials register).

* Efficacy includes overall response rate, progression-free survival, overall survival, durable response rate, time to progression, and radiographic response. Viral PK/PD includes replication, shedding, and replication. Immune response includes antiviral immunity and measurement of circulating immune cells.
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REFERENCES


