Talimogene laherparepvec: First in class oncolytic virotherapy

Robert M. Conry\textsuperscript{a}, Brian Westbrook\textsuperscript{b}, Svetlana McKee\textsuperscript{a}, and Timothy Graham Norwood\textsuperscript{b}

\textsuperscript{a}Medicine/Division of Hematology and Oncology, University of Alabama at Birmingham, Birmingham, AL, USA; \textsuperscript{b}Medicine, University of Alabama at Birmingham, Birmingham, AL, USA

\section*{ABSTRACT}
Oncolytic viruses represent a novel drug class in which native or modified viruses mediate tumor regression through selective replication within and lysis of tumor cells as well as induction of systemic antitumor immunity capable of eradicating tumor at distant, uninjected sites. Talimogene laherparepvec (TVEC) is a type I herpes simplex virus genetically modified to preferentially replicate in tumor cells, enhance antigen loading of MHC class I molecules and express granulocyte-macrophage colony-stimulating factor to increase tumor-antigen presentation by dendritic cells. It is presently the only oncolytic virus approved by the FDA with an indication for advanced melanoma based upon improved durable response rate in a randomized, phase III trial. Clinical trials are underway in melanoma investigating TVEC as neoadjuvant monotherapy and in combination with checkpoint inhibitors for unresectable disease as well as in an array of other malignancies. It is appropriate to review TVEC’s biology mechanism of action, clinical indication and future directions as a prototype of the burgeoning class of oncolytic viruses.

\section*{Introduction}
Oncolytic viruses represent a novel drug class in which native or modified viruses are used for the treatment of cancer. Oncolytic viruses mediate tumor regression through two distinct mechanisms. First, many viruses possess an innate ability to selectively replicate within and lyse tumor cells where antiviral pathways have been inactivated as part of the malignant phenotype. The release of new viral particles allows continued infection which amplifies the locoregional lytic effect. Secondly, locoregional activation of the innate immune system by the virus coupled with antigen release by dying tumor cells creates a favorable microenvironment for priming of adaptive systemic antitumor immunity capable of regressing tumor at distant, uninjected sites. The prototypical drug in this class is an attenuated herpes simplex virus, type 1 (HSV-1) engineered to express granulocyte-macrophage colony-stimulating factor (GM-CSF), designated talimogene laherparepvec (TVEC) marketed under the trade name Imlygic. In a prospective, randomized phase III trial, TVEC significantly improved durable and objective response rates in patients with advanced melanoma. On the basis of these results, TVEC became the first oncolytic virus to receive regulatory approval in the United States in October, 2015. Numerous other oncolytic viruses with varying tropism and lytic activity against an array of tumor histologies have been reviewed elsewhere,\textsuperscript{1,2} but this review focuses on TVEC. We will discuss the basic biology of TVEC, mechanisms whereby TVEC induces antitumor immunity, practical guidelines for administration, and clinical trials in melanoma and other malignancies both as monotherapy and combined with checkpoint inhibitors.

\section*{Biology of TVEC}
TVEC is a genetically modified herpes simplex virus, type 1 (HSV-1) which causes fever blisters and was derived from the JS-1 strain originally isolated from a cold sore.\textsuperscript{3} HSV-1 is a double-stranded DNA virus which is highly lytic. It can infect skin and peripheral nerves, where HSV-1 enters a latent state and may cause recurrent fever blisters during times of stress. TVEC has been engineered by deleting a gene that blocks antigen presentation and the neurovirulence genes to prevent development of fever blisters. TVEC uses surface nectins to enter tumor cells and propagates by exploiting disrupted oncogenic and antiviral pathways, primarily the protein kinase R (PKR) and type I interferon (IFN) pathways.\textsuperscript{3}

The PKR pathway is critical for regulating aberrant cell proliferation and intrinsic cellular antiviral responses.\textsuperscript{4} In normal cells, PKR can be activated by dsRNA, a byproduct of viral replication, resulting in inhibition of protein synthesis through phosphorylation of eukaryotic translation initiation factor 2 (eIF2) with blockade of cellular proliferation and viral propagation. Cancer cells disrupt the PKR-eIF2 pathway to support uncontrolled proliferation with the bystander effect of becoming increasingly permissive of viral replication. BRAF or NRAS driver mutations collectively present in approximately 70% of melanoma cells constitutively activate the MAP kinase pathway which suppresses PKR activation, preventing cells from detecting the stress of aberrant proliferation and limiting protein synthesis.\textsuperscript{5,6} Thus, inherent PKR suppression is a key mechanism for selective TVEC replication and lysis within cancer cells compared to normal cells, especially in tumors driven by MAP kinase pathway activation. This has implications for...
combination treatment strategies in melanoma where BRAF and MEK kinase inhibitors such as dabrafenib and trametinib may make melanoma cells less permissive of TVEC viral replication and cellular lysis.

Type I IFNs have antiviral and antitumor activity through limiting cellular proliferation and promoting viral eradication partly through PKR activation. To permit unrestrained proliferation, cancer cells commonly down regulate expression of type I IFN receptors and inactivate downstream signaling through the JAK-STAT pathway.7,8 Disruption of the type I IFN pathway in many cancers provides another means for selective TVEC replications and lysis of tumor compared to normal cells (Table 1).

The HSV-1 neurovirulence protein, infected cell protein 34.5 (ICP 34.5) is necessary for productive infection of neurons and other healthy cells as it dephosphorylates eIF-2, preventing PKR induced blockade of protein synthesis.9 In TVEC, both copies of the ICP 34.5 gene have been deleted allowing viral replication in cancer cells which inhibit PKR by other mechanisms described above. This reduces toxicity by favoring abortive infection in normal cells with an intact PKR pathway.3 TVEC has been further engineered to enhance antigen presentation and T-cell priming by deleting the viral infected cell protein 47 (ICP47) which normally reduces immune destruction of HSV-1 infected cells by binding to transport associated protein to prevent antigen loading of MHC class I molecules.10 This gene deletion in TVEC enhances cell surface MHC class I expression and tumor antigen presentation by infected cancer cells and improves safety by impairing the ability of the virus to evade immune recognition in normal host cells. ICP47 gene deletion also brings the downstream herpes unique short (US 11) gene whose product blocks the shutdown of host cell protein synthesis under an early/intermediate promoter, instead of its native late promoter. This translocation partially de-attenuates the virus and enhances the lytic activity of TVEC in an array of cancer cell lines through impacts on the PKR-eIF2 viral suppression pathway.2,3

To further enhance TVEC immunogenicity, two copies of the human GM-CSF gene were inserted into the deleted ICP 34.5 genomic site. GM-CSF promotes dendritic cell accumulation at sites of inflammation and enhances antigen presenting cell function.11 In murine models, defective HSV-1 vectors with ICP 34.5 deletions regressed only injected tumors. When GM-CSF was incorporated into the virus, regression of both injected and uninjected tumors occurred, consistent with a systemic antitumor immune response.12 Furthermore, mice were protected from rechallenge with the same tumor cells, indicating induction of durable anti-tumor memory responses. In summary, genomic modifications incorporated within TVEC include: 1) deletion of ICP 34.5 to reduce neurovirulence and lytic infection of healthy cells, 2) deletion of ICP47 to enhance antigen presentation by infected cells, 3) translocation of US11 to improve viral lytic activity in tumor cells, and 4) local expression of GM-CSF to enhance T-cell priming by dendritic cells. The relative selectivity of T-VEC for lysis of tumor cells compared to normal cells is primarily related to inherent suppression of type I IFN signaling and the PKR antiviral pathway within most cancer cells.

**Induction of antitumor immune response by TVEC**

TVEC has a dual mechanism of action, an oncolytic effect whereby it directly infects and kills local tumor cells at the injection site as well as an immunotherapeutic effect through induction of local and systemic immune responses.13 TVEC is administered by local injection into cutaneous, subcutaneous or nodal melanoma sites according to the FDA-approved label. TVEC preferentially replicates in tumor cells leading to lysis and release of soluble tumor-associated antigens as well as danger signals from host cell debris and viral pathogen components. Local GM-CSF expression enhances migration and maturation of dendritic cells which ingest soluble tumor antigens and apoptotic tumor cells. The dendritic cells then travel to regional lymph nodes where they present antigens to specific CD4+ helper and CD8+ cytotoxic T-cells, initiating a systemic immune response (Table 2). The response rate of distant metastases is lower than in injected tumor, potentially reflecting insufficient effector T-cell expansion and/or inability of circulating effectors to overcome the immunosuppressive tumor microenvironment at distant sites. Combination therapy with TVEC and immune checkpoint blockade may help overcome these limitations.

Most established cancers exist in an immunosuppressive tumor microenvironment. Lytic TVEC infection of tumor cells results in local release of interferons, chemokines, Toll-like receptor agonists, and danger-associated molecular pattern (DAMP) and pathogen-associate molecular pattern (PAMP)
factors which together promote a more favorable milieu for priming of anti-tumor immune responses. Viral nucleic acid variants (PAMP) and host cell derived heat shock proteins, calreticulin, ATP and uric acid (DAMP) bind Toll-like receptors to promote innate immune responses. Cancer cell lysis releases tumor-associated neoantigens for processing and presentation by dendritic cells activated by TVEC-encoded GM-CSF. This can lead to priming of anti-tumor CD8+ T-cell responses against previously unrecognized antigens. The induction of tumor specific immune responses by TVEC has been clinically demonstrated by the presence of MART-1-specific CD8+ T-cells within injected melanoma nodules.14

Preclinical studies

An ICP34.5- deleted HSV-1 vector forerunner of TVEC was engineered to express the marker gene beta-galactosidase.15 Following intracranial or foot-pad injection in mice, strong expression of the marker gene was observed in the brain and dorsal rood ganglia with little toxicity. These findings indicate that deletion of the HSV-1 neurovirulence gene blocks neurotoxicity but preserves sufficient infectivity to allow potent transgene expression even within neural tissue. A subsequent HSV-1 vector forerunner of TVEC with deletions of ICP 34.5 and ICP47 preferentially lysed human breast cancer cells compared to autologous hematopoietic cells in mixed culture.16 TVEC itself demonstrated strong lytic activity against a variety of human tumor cell lines in vitro, including melanoma cells.17 Murine models demonstrated tumor infiltration by CD8+ T-cells in both injected and noninjected metastases.18 Melanoma patients treated with TVEC demonstrated accumulation of melanoma antigen specific CD8+ T-cells within injected lesions associated with a reduction in CD4+ FoxP3+ regulatory T-cells and CD14+ myeloid-derived suppressor cells.14 Collectively, these observations demonstrate the ability of TVEC to infect and drive transgene expression in a variety of cell types, preferentially lyse an array of human tumor cells, and induce antigen-specific CD8+ adaptive immune responses in both injected and un.injected tumors.

Clinical trials of TVEC monotherapy in advanced melanoma

The initial human study of TVEC was a phase I trial in 30 patients with refractory cutaneous or subcutaneous metastases from melanoma, breast cancer, gastrointestinal adenocarcinoma, or squamous cell carcinoma of the head and neck.16 The virus was administered by local intratumoral injection instead of systemic delivery to reduce toxicity and improve tumor cell infectivity. TVEC was well tolerated, and the most common adverse events were local inflammation and flu-like symptoms. Local reactions were dose limiting at 106 pfu/mL in HSV-seronegative patients, and baseline HSV serologic status did not influence TVEC efficacy. Thus, the dosing regimen established was a priming dose of 106 pfu/mL regardless of baseline serology, followed 3 weeks later by multiple treatment doses of 108 pfu/mL every 2 weeks until confirmed disease progression or unacceptable toxicity (Table 3).16 This approach allowing HSV-seronegative patients to seroconvert prior to high-dose therapy was well tolerated. No objective responses were seen in the trial, but viral replication, GM-CSF expression and HSV-antigen-associated necrosis was observed in melanoma, breast cancer, and head and neck cancer. Areas of tumor necrosis were associated with positive staining for HSV particles and surrounding normal tissues showed no necrosis and rarely stained positive for HSV particles.16 Six patients demonstrated flattening of injected and/or nearby un injected tumors, and systemic immune response were observed in four patients.

The initial phase II trial enrolled 50 patients with unresectable or metastatic melanoma with stage IIIIC or IV disease.19 Most patients (74%) had received prior systemic treatment for advanced melanoma. Up to 10 tumors were injected with TVEC on each treatment day, preferentially delivered to any new or progressing lesions. The maximum total injection volume was 4mL per treatment day with the volume injected into each lesion varying from 0.1mL for tumors < 0.5cm to 4mL for lesions > 5 cm in greatest diameter. Patients initially received a priming dose of 106 pfu/mL followed 3 weeks later by treatment doses of 108 pfu/mL every 2 weeks for a maximum of 24 treatments, per the current label.19 TVEC was well tolerated with toxicity primarily limited to transient flu-like symptoms, nausea, and local injection site inflammation. The objective response rate (ORR) by RECIST 1.0 criteria was 26% with 8 of 13 responding patients experiencing complete response and 12 responses lasted > 6 months. Regression of both injected and distant tumors occurred, including visceral metastases. On an extension protocol, two additional patients had late conversion to CR by 24 months. Only 3 of 20 patients (15%) with visceral metastases had an objective response. The overall survival was 58% at one year and 52% at two years in an era predating commercial availability of BRAF inhibitors or immune checkpoint blockade. Several patients experienced pseudo-progression with tumor enlargement and/or appearance of new small tumors before a response, as described with other forms of immunotherapy.20,21

Based upon the encouraging durability of responses and landmark survival with low toxicity in the phase II study, a phase III trial (OPTiM) was conducted in 436 patients with stage IIIIB to IV melanoma (Anditbacka RH 2015)22. Patients were randomized 2:1 to intraslesional TVEC or GM-CSF administered subcutaneously at 125 mcg/m2 daily for 14 days in 28-day cycles. Due to the frequency of pseudo-progression with TVEC, treatment

| Therapy | Phase (Enrollment) | Primary Outcomes |
|---------|-------------------|-----------------|
| TVEC Monotherapy | I (n = 30) | Established dosing regimen |
| TVEC Monotherapy | II (n = 50) | ORR = 26%, CR = 16% |
| TVEC v. GM-CSF | III (n = 436) | TVEC improved DRR v. GM-CSF (16% v. 2%, p < 0.001) |
| Neoadjuvant TVEC v. Immediate Resection | II (n = 150) | Enrollment completed May 2017, results pending |
| Ipilimumab +/- TVEC | lb/lII (n = 198) | Addition of TVEC to Ipilimumab improved ORR (39% v. 18%, p = 0.002) |
| Pembrolizumab +/- TVEC | lb/lII (n = 660) | lb (n = 21) demonstrated tolerability and ORR = 57% with combo; phase III enrolling |
| TVEC + Dabrafenib and Trametinib | lb (n = 20) | Enrolling |
discontinuation because of progressive disease by response assessment criteria was not required before 24 weeks unless alternate therapy was clinically indicated. After 24 weeks, treatment continued until clinically relevant melanoma progression associated with reduced performance status, intolerable toxicity, complete remission, lack of response by 12 months, or disappearance of all injectable tumors in the case of TVEC. After 12 months, patients with stable or responding melanoma could continue treatment for six additional months. The primary endpoint was durable response rate (DRR), defined as the percentage of patients experiencing a response lasting ≥ 6 months and beginning within the first 12 months of treatment. DRR was higher in the TVEC arm (16% v. 2%, p < 0.001), as was ORR (26% v. 6%) with CR in 11% of the TVEC group. Median OS was 23.3 months with TVEC and 18.9 months with GM-CSF (p = 0.051), and 5-year survival in the TVEC arm was 33%. The most common adverse events with TVEC were fatigue, chills, pyrexia, nausea, and local injection reactions. Cellulitis occurred in 2% of patients. In exploratory analyses, OS was superior in the TVEC arm for patients with stage III or stage IV M1a (skin or lymph node only metastases) and for treatment naïve patients with p < 0.001 in both comparisons. Based on these encouraging results, TVEC became the first oncolytic viral therapy to demonstrate significant clinical benefit in a phase III randomized trial.13 This led to TVEC approval as monotherapy for unresectable cutaneous, subcutaneous, or nodal melanoma lesions following initial surgery by the U.S. Food and Drug Administration in October, 2015.

TVEC monotherapy trials clearly demonstrate distant responses in uninjected tumors, presumable through immune activation against tumor-associated antigens. However, responses in lung or visceral sites are uncommon. Subanalysis of the OPTiM trial examining TVEC treatment in 132 patients with stage IV M1b or M1c disease with metastases to lung or visceral sites compared to 163 patients with stage IIIB – IV M1a melanoma revealed DRR of 5% v. 25%, ORR of 9% v. 41% and CR rates of 4% v. 17%, respectively.23 Given the activity of other systemic melanoma therapies across metastatic stages, the role of TVEC monotherapy may be largely limited to stage IIIB – IV M1a disease.24

Retrospective review of data from the OPTiM trial identified patients with cutaneous melanoma arising in the head and neck as a particularly favorable subgroup responding to TVEC. Among 61 such patients, the DRR was 36% with a complete response in 11% of the TVEC group. Median OS was 23.3 months with TVEC and 18.9 months with GM-CSF (p = 0.051), and 5-year survival in the TVEC arm was 33%. The most common adverse events with TVEC were fatigue, chills, pyrexia, nausea, and local injection reactions. Cellulitis occurred in 2% of patients. In exploratory analyses, OS was superior in the TVEC arm for patients with stage III or stage IV M1a (skin or lymph node only metastases) and for treatment naïve patients with p < 0.001 in both comparisons. Based on these encouraging results, TVEC became the first oncolytic viral therapy to demonstrate significant clinical benefit in a phase III randomized trial.13 This led to TVEC approval as monotherapy for unresectable cutaneous, subcutaneous, or nodal melanoma lesions following initial surgery by the U.S. Food and Drug Administration in October, 2015.

TVEC monotherapy trials clearly demonstrate distant responses in uninjected tumors, presumable through immune activation against tumor-associated antigens. However, responses in lung or visceral sites are uncommon. Subanalysis of the OPTiM trial examining TVEC treatment in 132 patients with stage IV M1b or M1c disease with metastases to lung or visceral sites compared to 163 patients with stage IIIB – IV M1a melanoma revealed DRR of 5% v. 25%, ORR of 9% v. 41% and CR rates of 4% v. 17%, respectively.23 Given the activity of other systemic melanoma therapies across metastatic stages, the role of TVEC monotherapy may be largely limited to stage IIIB – IV M1a disease.24

**Current role of TVEC in melanoma treatment**

Subgroup analysis of the OPTiM trial demonstrated a considerably higher durable response rate of 33% in stage III B/C patients and overall survival was significantly improved with TVEC for stage III B/C or IV M1a disease. The phase II trial reported objective response in 67% of injected lesions, 41% of injected non-visceral lesions, and only 13% of visceral lesions unavailable for injection.27 The median time to response in injected tumors, uninjected non-visceral tumors, and visceral tumors was 18, 23 and 51 weeks, respectively. Furthermore, multivariate analysis of the OPTiM trial demonstrated significantly improved DRR and OS among patients with below median baseline tumor burden.28 In the authors’ experience, TVEC is especially active against dermal satellite or in transit metastases. Thus, TVEC is highly effective at controlling locoregional disease, but systemic effects are limited and typically require combination approaches. TVEC monotherapy may be considered for first line treatment of patients with unresectable stage III or IV M1a melanoma or patients with more advanced BRAF wild-type melanoma and relative contraindications to checkpoint inhibition, including advanced age, poor performance status, or significant autoimmune disease. Anti-PD1 monotherapy or combined BRAF and MEK inhibition produce objective responses in 40–60% of patients with 3-year PFS of 23–30% and 3-year OS of approximately 50%.29,30 Thus, these agents are generally preferred over TVEC monotherapy in the first line treatment of visceral melanoma metastases. TVEC is contraindicated in patients with active herpetic infection and those requiring daily antiviral therapy although a history of fever blisters is permissible. Since TVEC is a live, attenuated virus, immunocompromised patients may be at risk for disseminated herpetic infection. TVEC should be avoided in patients with HIV, lymphoma, leukemia, or on significant immunosuppressive therapy. Since there have been no studies in pregnant women, TVEC should not be used in this population.

**Administration of TVEC**

For dermal or very superficial lymph node metastases, TVEC administration can be accurately directed visually and by palpation. In the case of subcutaneous nodules or deeper lymph nodes in the cervical, axillary or inguinal regions, ultrasound guidance in the clinic may be necessary to ensure intratumoral delivery. Use of ultrasound expands the population of patients who are candidates for intratumoral therapy and may be performed by experienced medical oncologists, surgeons or interventional radiologists. Development of proficiency with intralésional injection is becoming increasingly important for melanoma clinicians as multiple oncolytic viruses and Toll-like receptor agonists are in late-stage clinical trials to augment systemic CPB1.31 The needle should be placed in the center of the tumor and the correct volume for a particular lesion delivered through the dermis producing a diffuse malignant plaque affecting almost an entire arm.32

TVEC is classified as a biosafety level 1 agent because it is not known to consistently cause disease in healthy adults. It is stored at −70°C and frozen vials are thawed at room temperature in a sterile biosafety cabinet. Thawed virus can be refrigerated prior to administration for 12 hours at 10⁶ PFU/mL or 48 hours for 10⁸ PFU/mL. Universal precautions should be followed when administrating TVEC, including wearing a gown or lab coat, gloves, and face protection.32 As many lesions as
possible are injected up to 4 mL total volume on each treatment day with priority given to the largest lesions followed by any new lesions. Lesions should be cleaned with alcohol prior to injection and local anesthetics are generally unnecessary. Injection sites should be covered with gauze and an occlusive dressing and should remain covered for a week after each treatment due to potential viral shedding in wound drainage. Pregnant or immunocompromised providers should not have direct contact with storage, handling or administration of TEC, nor come in contact with the injection site.

Acetaminophen or indomethacin can be used for prevention and treatment of fever, chills, or injection site reactions consisting of pain, erythema or swelling. Herpes cellulitis is typically self-limited and clears within 24 hours. Persistent cellulitis or associated fever and leukocytosis should prompt suspicion of superimposed bacterial infection. In the extremely unlikely event that viremia or encephalitis is suspected, standard clinical polymerase chain reaction testing for HSV DNA is reliable in blood and cerebrospinal fluid as it detects epitopes conserved within TVEC.33 If clinically significant disseminated herpetic infection occurs, acyclovir is recommended as TVEC is susceptible to anti-viral therapy.34 Patients on low dose corticosteroids equivalent to ≤ 10 mg of daily prednisone are eligible for TVEC.

**Chronic granulomatous dermatitis at TVEC injection sites**

TVEC produces objective response in 41% of stage IIIB-IV M1a melanoma. However, clinical response assessment can be misleading due to immune-related inflammation at established tumor sites. Our group has reported five cases of granulomatous dermatitis developing at sites of TVEC injection associated with pathologic complete response in 4 of 5 patients.35 TVEC was injected into a median of 20 tumors for a median of 9 doses over approximately 5 months before biopsy of persistent, indurated nodules with variable residual melanin pigment. Granulomatous dermatitis with melanophages and free melanin pigment was observed in all samples with no evidence of melanoma cells in four patients. The fifth patient was rendered melanoma-free by resection of the one nodule out of four containing residual tumor. It is logical that repetitive administration of TVEC into cutaneous melanoma metastases can lead to chronic granulomatous dermatitis locoregionally by mimicking unresolved herpes viral infection with the added stimulus of GM-CSF production promoting macrophage accumulation. Granuloma formation with residual melanosis creates confusion over the degree of tumor response and need for further therapy. Tumor biopsies should be strongly considered following 4–6 months of TVEC administration to differentiate viable melanoma nodules from granulomatous inflammation. Most patients with confirmed benign granulomatous dermatitis remain relapse-free following treatment discontinuation and inflammatory nodules typically regress spontaneously over several months.35 Multiple marker immunohistochemistry of tumor biopsies obtained prior to TVEC and at the time of pathologic complete response demonstrated a marked increase in quiescent tumor infiltrating lymphocytes post-treatment that were Ki67 low, granzyme B low, and PD1 high consistent with maturation of an effective adaptive immune response.35 Both CD4+ and CD8+ T-cells were enriched following TVEC therapy with no significant change in regulatory T-cells.

**Neoadjuvant TVEC for high risk resectable melanoma**

A phase II trial in resectable stage III B/C or IV melanoma randomized 150 patients to immediate surgical resection versus 12 weeks of neoadjuvant intratumoral TVEC followed by surgery. Enrollment was completed in May 2017 with results pending (NCT02211131).

**TVEC plus ipilimumab**

The unique mechanism of action and tolerability of TVEC make it an attractive candidate for combination therapies with other immune activating agents. Although some studies indicate that GM-CSF can stimulate local myeloid-derived suppressor cells,36,37 combination of oncolytic viral therapy with ICB is supported by murine melanoma models.18,38 Furthermore, a randomized phase II trial of ipilimumab with or without subcutaneous GM-CSF (NCT01134614) demonstrated significantly improved OS with the combination and a trend toward lower toxicity.39 Based upon these data a phase III trial of ipilimumab plus nivolumab with or without GM-CSF is ongoing (NCT0239571).

A phase I/II trial of ipilimumab + TVEC (NCT01740297) in 19 patients with advanced melanoma demonstrated tolerability of standard dose TVEC according to the current label beginning on week 1 with ipilimumab added on week 6 at the FDA approved dose of 3 mg/kg intravenously every 3 weeks for four doses.40 A randomized, open-label phase II continuation of this trial comparing TVEC + ipilimumab to ipilimumab alone in 198 patients with advanced melanoma met its primary endpoint of improved ORR with the combination (39% v. 18%, p = 0.002).41 Responses to the combination were durable with 89% ongoing at a median follow up of 16 months. Perhaps most important, visceral lesion decreases were observed in 52% of patients with combination therapy compared to 23% of patients with ipilimumab alone, suggesting that TVEC substantially enhanced anti-tumor immunity at distance, uninjected sites in the setting of CTLA-4 blockade. Grade 3 or 4 adverse events occurred in 45% of patients treated with the combination and 35% receiving ipilimumab alone. TVEC + ipilimumab primarily increased flu-like symptoms with no unexpected adverse events or treatment-related deaths.41

**TVEC plus pembrolizumab**

Pembrolizumab is an IgG4 monoclonal antibody directed against PD-1 and is indicated for the treatment of unrectable or metastatic melanoma and multiple other malignancies.42 In the randomized phase III KEYNOTE-006 trial in advanced melanoma, pembrolizumab improved ORR, PFS and OS compared to ipilimumab (anti-CTLA4) with fewer serious adverse events.43 The addition of pembrolizumab to TVEC would theoretically allow efficient T-cell priming by TVEC at sites of injected tumor and enhanced killing of uninjected tumors by effector T-cells freed from the
immunological braking mechanism of the PD1 pathway. Tolerability of this combination has been evaluated in the phase Ib portion of the phase Ib/III MASTERKEY-265 trial (NCT02263508). Twenty one patients with stage IIB-IV M1c melanoma with injectable tumors and no prior systemic therapy received TVEC monotherapy at an initial dose of $10^6$ PFU/mL followed by $10^8$ PFU/mL every 2 weeks from week 3. Pembrolizumab was given intravenously at 200 mg every 2 weeks beginning with the third TVEC dose and both drugs were continued for up to 2 years. No dose limiting toxicity was observed and no additional toxicity was seen with the combination compared with that expected with the monotherapies. The small phase IB portion of the trial reported a confirmed ORR of 62% and a CR rate of 33% per immune response criteria, which was greater than the ORR seen with pembrolizumab in a phase III trial (34%) and with TVEC seen in the OPTiM trial (26%). These results must be interpreted with caution as melanoma patients with injectable skin metastases often follow a more favorable clinical course. Patients responding to combination therapy had increased CD8+ T cells, elevated PD-L1 protein expression and interferon-gamma gene expression in tumors following TVEC treatment. Response to combination treatment was not associated with baseline CD8+ T cell infiltration or interferon-gamma signature. These findings indicate that oncolytic virotherapy may enhance the efficacy of anti-PD1 therapy by modifying the tumor microenvironment. The phase III portion of the trial is ongoing and will randomize 660 patients with advanced melanoma to receive pembrolizumab with either TVEC or placebo.

**TVEC plus kinases inhibitors**

A phase Ib trial is ongoing in BRAF mutated advanced melanoma combining TVEC with dabrafenib and trametinib, BRAF and MEK kinase inhibitors, respectively (NCT 03088176). However, inhibition of the MAP kinase pathway may theoretically make melanoma cells less permissive of TVEC replication and lysis as described earlier in this review.

**Clinical trials with TVEC in non-melanoma malignancies**

A phase I/II trial of TVEC combined with concurrent cisplatin and radiotherapy was conducted in 17 patients with newly diagnosed stage III or IV head and neck squamous cell carcinoma. There were no delays to chemoradiation therapy or dose-limiting toxicity. At 29 months median follow-up, locoregional control was 100% and RFS was 76% (NCT01161498). In an ongoing phase Ib/III trial in recurrent or metastatic squamous cell carcinoma of the head and neck, patients are randomized to pembrolizumab with or without TVEC delivered to involved cervical nodes (NCT 2626000). A phase I trial examined one to three doses of TVEC monotherapy delivered endoscopically to 17 patients with advanced pancreatic adenocarcinoma. Despite suboptimal dosing, responses were seen in both primary tumors and some uninjected lesions. However, most patients had end-stage disease with rapid progression and the trial was terminated for strategic reasons other than toxicity. Additional clinical trials are ongoing for patients with sarcoma in combination with radiotherapy neoadjuvantly (NCT 02453191) and combined with pembrolizumab for advanced disease (NCT 03069378), as monotherapy for hepatocellular carcinoma or other liver metastases (NCT 02509507), as monotherapy for inoperable locally recurrent breast cancer (NCT 02658812), and combined with neoadjuvant chemotherapy for breast cancer (NCT 02779855).

**TVEC for regional advanced merkel cell carcinoma**

Merkel cell carcinoma (MCC) is an uncommon, aggressive cutaneous malignancy with propensity for locoregional recurrence and hematogenous spread. Approximately 80% of cases are caused by clonal integration of Merkel cell polyomavirus (MCPyV), and the remainder carry an extremely high burden of ultraviolet signature mutations. Thus, MCC is an attractive target for immunotherapy because MCPyV positive tumors express viral oncoproteins, and virus-negative tumors carry a large number of neoantigens created by ultraviolet signature mutations providing non-self-epitopes for immune recognition. Our group has reported complete and partial response to TVEC monotherapy in two of two patients with regionally advanced MCC that are both ongoing at approximately a year following therapy initiation. Since that report, we have treated two additional consecutive patients with multiple regionally recurrent MCC nodules in the neck with a clinical complete response to TVEC following four months of treatment in one patient and improving partial response after two months of TVEC in the other (unpublished observation). A study of TVEC with or without hypofractionated radiotherapy is currently recruiting patients with MCC (NCT02819843).

**Conclusion: TVEC in the field of oncolytic virotherapy**

Oncolytic virotherapy uses an array of replication-competent viruses that have been adapted to amplify and spread preferentially at sites of tumor growth. In 1991, a genetically modified HSV-1 virus led to tumor control without associated encephalitis when administered intracranially in a murine glioma model. TVEC was first described in 2003 and approved by the FDA in October 2015 to treat advanced melanoma. Although TVEC remains the only FDA-approved oncolytic virotherapy at the time of this writing, clinical trials are underway with a wide variety of other modified viruses including vaccinia virus, adenovirus, parvovirus, reovirus, coxsackie virus, measles, poliovirus, Newcastle disease virus, Seneca valley virus and vesicular stomatitis virus. Naturally occurring viruses have diverse tissue tropism making some more advantageous than others in different tumor histologies. Since neutralization by antiviral antibodies is a significant impediment to repetitive dosing of oncolytic virotherapy, sequential use of immunologically non-cross-reactive viruses will likely be more efficacious. Oncolytic viruses are in clinical trials treating a broad array of malignancies including melanoma, sarcoma, glioblastoma, myeloma, mesothelioma and carcinomas of
the breast, lung, colon, prostate, kidney, liver pancreas, bladder, ovary, and head and neck.

Systemic delivery of oncolytic virotherapy is another major goal to markedly extend the spectrum of applicable tumor types and stages for this burgeoning drug class. Several oncolytic viruses have been administered intravenously in human clinical trials with occasional encouraging results supporting feasibility. To overcome circulating antiviral antibodies, intravenous delivery of infected carrier cells to transport oncolytic viruses to tumor sites has shown success in murine models. Thus, oncolytic virotherapy is a strategically attractive class of immunotherapeutics that is sure to rapidly expand in the clinic using a variety of viral platforms to treat a wide array of malignancies both locoregionally and systemically as both monotherapies and in combinations with checkpoint inhibitors.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

1. Fountzilas C, Patel S, Mahalingam D. Review: oncolytic virotherapy, updates and future directions. Oncotarget. 2017;8(20):102617–102639. doi:10.18632/oncotarget.18309.
2. Russell SJ, Peng KW. Oncolytic virotherapy: a contest between apples and oranges. Molecular Therapy. 2017;25(5):1107–1116. doi.org/10.1016/j.ymtl.2017.03.026. PMID:28392162.
3. Kohlhapp FJ, Kaufman HL. Molecular pathways: mechanism of action for talimogene laherparepvec, a new oncolytic virus immunotherapy. Clin Cancer Res. 2015;21(22):5048–54. doi.org/10.1158/1078-0432.CCR-15-2667. PMID:26719429.
4. Williams BR. PKR; a sentinel kinase for cellular stress. Oncogene. 1999;18:6112–6120. doi.org/10.1038/sj.onc.1203127.
5. Farassati F, Yang AD, Lee PW. Oncogenes in Ras signaling pathway dictates host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol. 2001;3:745–50. doi:10.1038/35087061. PMID:11483960.
6. Smith KD, Mezhib JJ, Bickenbach K, Veerapong J, Charron J, Posner MC, Roizman B, Weischbeum RB. Activated MEK suppresses activation of PKR and enables efficient replication and in vivo oncolysis by Delta-gamma (1) 34.5 mutants of herpes simplex virus 1. J Virol. 2006;80:1110–20. doi.org/10.1128/JVI.80.3.1110-1120.2006. PMID:16149888.
7. Pansky A, Hildebrand P, Fasler-Kan E, Baselgia L, Ketterer S, Beglinger C, Heim MH. Defective Jak-STAT signal transduction pathway allows latent herpes simplex virus 1 complexes with protein phosphatase 1 alpha to dephosphorylate the alpha subunit of the eukaryotic translation initiation factor 4E. J Biol Chem. 2001;276:75340–7540. doi.org/10.1074/jbc.M101892200. PMID:11483964.
8. Zhang KX, Matsui Y, Hadaschik BA, Lee C, Jia W, Bell JC, Fazli L, So AI, Rennie PS. Down-regulation of type I interferon receptor sensitivity to latent host-cell permissiveness to herpes simplex virus 1. Nat Cell Biol. 2000;2:56–62. doi.org/10.1021/pr0607592. PMID:11483964.
9. Harrington KJ, Andtbacka RH, Collichio F, Downey G, Chen L, Szabo Z, Kaufman HL. Efficacy and safety of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in patients with stage IIIB/IV melanoma. Head Neck. 2016 Dec;38(12):1752–1758. doi.org/10.1002/hed.24522.
10. Blackmon JT, Stratton MS, Kwak Y, Pavlidakey PG, Slominski AT, McKeel SB, Viator TM, Kim YJ, Huang CC, Conry RM.
Inflammatory melanoma in transit metastases with complete response to talimogene laherparepvec. JAAAD Case Rep. 2017;3:280–3. doi.org/10.1016/j.jcerr.2017.02.011. PMID:28653030.

27. Kaufman HL, Amatruda T, Reid T, Gonzalez R, Glaspy J, Whitman E, Harrington K, Nemunaitis J, Zloza A, Wolf M, et al. Systemic versus local responses in melanoma patients treated with talimogene laherparepvec from a multi-institutional phase II study. J Immunother Cancer. 2016;4:46.

28. Kaufman H, Amatruda T, Nemunaitis J, et al. Tumor size and clinical outcomes in melanoma patients (MEL pts) treated with talimogene laherparepvec (T-VEC). ASCO. 2015. Presented June 1, 2015.

29. Robert C, Roussy C, France V. Long-term outcomes in patients (pts) with ipilimumab (ipi)-naive advanced melanoma in the phase 3 KEYNOTE-006 study who completed pembrolizumab (pembro) treatment. J Clin Oncol. 2017;35(suppl; abstr 9504).

30. Long G. Five-year overall survival (OS) update from a phase II, open-label trial of dabrafenib (D) and trametinib (T) in patients (pts) with BRAF V600-mutant unresectable or metastatic melanoma (MM). J Clin Oncol. 2017;35(suppl; abstr 9505).

31. Lim F, Khalique H, Ventosa M, Baldo A. Biosafety of gene therapy vectors derived from herpes simplex virus type I. Curr Gene Ther. 2013;13(6):478–91. doi.org/10.1186/1566-5232-1306140103224550. PMID:24397529.

32. LeGoff J, Pere H, Belec L. Diagnosis of genital herpes simplex virus infection in the clinical laboratory. Virol J. 2014;11:83. doi.org/10.1186/1743-422X-11-83. PMID:24885431.

33. Martuza RL, Coen DM. Experiments that failed to demonstrate a role for tumor necrosis factor-based antitumor vaccine. J Clin Oncol. 2007;25:2546–52. doi.org/10.1097/01.jco.0000272459.10.1016/j.jco.2007.07.027. PMID:17577033.

34. Tarhini AA, Buttery LH, Shuai Y, Gooding WE, Kalinski P, Kirkwood JM. Differing patterns of circulating regulatory T cells and myeloid-derived suppressor cells in metastatic melanoma patients receiving anti-CTLA4 antibody and interferon-alpha or TLR-9 agonist and GM-CSF with peptide vaccination. J Immunother. 2012;35(6):702–9. doi.org/10.1016/j.jim.2012.03.030. PMID:22309079.

35. Engelard CE, Grossardt C, Veinalde R, Bossow S, Lutz D, Kaufmann Jk, Shevchenko I, Umanisky V, Nettelbeck DM, Weichert W, et al. CTLA-4 and PD-L1 checkpoint blockade enhances oncolytic measles virus therapy. Mol Ther. 2014;22:1949–59. doi.org/10.1038/mt.2014.160. PMID:25156126.

36. Hodi SF, Lee S, McDermott DF, Rao UN, Butterfield LH, Tarhini AA, LeMing P, Puzanov I, Shin D, Kirkwood JM. Sargramostim plus ipilimumab vs ipilimumab alone for treatment of metastatic melanoma: a randomized clinical trial. JAMA. 2014 Nov 5;312(17):1744–53. doi.org/10.1001/jama.2014.13943.

37. Puzanov I, Milhem MM, Andtbacka RH, Minor D, Hamid O, Li A, Chen L, Chastain M, Gorski KS, Anderson A, et al. Primary analysis of a phase Ib multicenter trial to evaluate safety and efficacy of talimogene laherparepvec (T-VEC) and ipilimumab (ipi) in previously untreated, unresected stage IIIB-IV melanoma. J Clin Oncol. 2014;32:9029. abstr.

38. Chesney J, Puzanov I, Collichio F, Singh P, Milhem MM, Grossy J, Hamid O, Ross M, Friedlander P, Garbe C, et al. Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. J Clin Oncol. 2017;35:JCO2017737379. PMID:28981385.

39. Khoja L, Butler MO, Kang SP, et al. Pembrolizumab. J Immunother Cancer. 2015;3:36. doi.org/10.1186/s13045-015-0078-9. PMID:26288737.

40. Puzanov I, Milhem MM, Andtbacka RH, Minor D, Hamid O, Ross M, Friedlander P, Garbe C, et al. Pembrolizumab versus ipilimumab in advanced melanoma. Am J Health Syst Pharm. 2017;73:193–201.

41. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielen O, Olszanski AJ, Malvehy J, Cebon J, Fernandez E, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell. 2017;170:1109–1119. doi.org/10.1016/j.cell.2017.08.027. PMID:28866391.

42. Harrington KJ, Hingorani M, Tanay MA, Hickey J, Bhide SA, Clarke PM, Renouf LC, Thway K, Sibtain A, McNeish IA, et al. Phase I/II study of oncolytic HSV GM-CSF in combination with radiotherapy and cisplatin in untreated stage III/IV squamous cell cancer of the head and neck. Clin Cancer Res. 2010;16(15):4005–15. doi.org/10.1158/1078-0432.CCR-10-0196. PMID:20670951.

43. Chang KJ, Senzer N, Binmoeller K, et al. Phase I dose-escalation study of talimogene laherparepvec (T-VEC) for advanced pancreatic cancer (ca.). J Clin Oncol. 2012;30(suppl; abstr e14546).

44. Blackmon JT, Drahaw R, Viator TM, Terry NL, Conry RM. Talimogene laherparepvec for regionally advanced Merkel cell carcinoma: a report of 2 cases. JAAAD Case Rep. 2017;3(3):185–189. doi.org/10.1016/j.jcerr.2017.02.003. PMID:28443305.

45. Martuza RL, Malick A, Markert JM, Ruffner KL, Cebon J, Fernandez E, et al. Experiments that failed to demonstrate a role for tumor necrosis factor-based antitumor vaccine. J Clin Oncol. 2007;25:2546–53. doi.org/10.1200/JCO.2006.08.5829. PMID:17577033.

46. Tarhini AA, Butterfield LH, Shuai Y, Gooding WE, Kalinski P, Kirkwood JM. Differing patterns of circulating regulatory T cells and myeloid-derived suppressor cells in metastatic melanoma patients receiving anti-CTLA4 antibody and interferon-alpha or TLR-9 agonist and GM-CSF with peptide vaccination. J Immunother. 2012;35:702–710. doi.org/10.1097/JIT.0b013e318272569b. PMID:23090079.