Talimogene Laherparepvec for the Treatment of Advanced Melanoma

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Running Title: Talimogene Laherparepvec in Melanoma

Disclosure of Potential Conflicts of Interest: P.A. Ott is a consultant/advisory board member for Alexion, Amgen, and Bristol-Myers Squibb. F.S. Hodi is a consultant/advisory board member for Amgen, Genentech, Merck, and Novartis. No other potential conflicts of interest were disclosed.
Abstract

Talimogene Laherparepvec (T-VEC) is a first-in-class oncolytic virus that mediates local and systemic anti-tumor activity by direct cancer cell lysis and an “in situ vaccine” effect. Based on an increased durable response rate compared to granulocyte macrophage-colony stimulating factor (GM-CSF) in a randomized phase 3 trial, it was approved by the FDA for the treatment of melanoma metastatic to skin or lymph nodes. The drug is currently in clinical trials as monotherapy and in combination with immune-checkpoint inhibitors and radiation in melanoma and other cancers. The mechanism of action, toxicity, and efficacy as well as its role in current clinical practice and potential future applications will be reviewed.
Introduction

Novel systemic treatment modalities such as inhibition of the immune checkpoints Cytotoxic T-Lymphocyte Antigen 4 (CTLA-4) and Programmed Death-1 (PD-1)/PD-L1 as well as BRAF and MEK inhibition have expanded the range of therapeutic modalities for advanced melanoma (1-11). The anti-tumor activity of both MAPK pathway targeted therapy (for BRAF$^{V600}$ mutant melanoma) and immune checkpoint inhibition (independent of a BRAF mutation) with response rates of 60% and higher is striking and has improved the prognosis for many patients. Both CTLA-4 and/or PD1/PD-L1 blockade with monoclonal antibodies can achieve durable clinical benefit suggesting that endogenous tumor directed T cell responses, suppressed by inhibitory pathways such as CTLA-4 and/or PD-1/PD-L1, can be invigorated resulting in affective tumor control(1, 2, 10-13). Many patients experience primary or secondary resistance to PD-1 and/or CTLA-4 inhibition. Alternative treatments for these patients are therefore still urgently needed. Talimogene laherparepvec (T-VEC), an agent with a different and potentially complementary mechanism of action to immune checkpoint blockade is a recent addition to the therapeutic armamentarium for patients with advanced melanoma.

Mechanism of Action

T-VEC is an intralesionally delivered oncolytic immunotherapy comprised of a genetically engineered attenuated herpes simplex virus type 1 (HSV-1) of the JS-1 strain. TVEC invades both cancerous and healthy cells, but can only replicate in cancer cells, where it secretes granulocyte macrophage- colony stimulating factor (GM-CSF) in the process. The genes
encoding neurovirulence infected cell protein 34.5 (ICP34.5) and the infected cell protein 47 (ICP47) are functionally deleted in the virus, while the gene for human GM-CSF is inserted. ICP 34.5 is required for viral replication in normal cells, which is mediated by interaction with proliferating cell nuclear antigen (PCNA)(14) whereas cancer cells proliferate independently of ICP34.5 expression. ICP47 is critical for the evasion of HSV-infected cells from cytotoxic T cells by interfering with peptide processing and presentation on MHC-I(15). Deletion of ICP47 in TVEC prevents potentially limited viral antigen presentation, which could compromise its function as an in situ vaccine. ICP47 deletion also leads to increased expression of the US11 gene, resulting in increased virus replication in cancer cells without decreasing tumor selectivity. GM-CSF is a pro-inflammatory cytokine that promotes the recruitment and maturation of dendritic cells (DCs) as well as macrophages into potent antigen presenting cells, leading to priming of tumor specific T cells(16). It has been used successfully as an immune adjuvant in many cancer vaccines.

TVEC has 2 distinct mechanisms of action: the lytic function of the virus destroys tumor cells directly, whereas the lysis of the cancer cells leads to release of tumor antigens, virus, and GM-CSF, attracting DCs thereby creating an in situ vaccine (Fig. 1). In a subcutaneous murine melanoma model, tumor growth inhibition on the contralateral, uninjected site was only seen when TVEC contained GM-CSF, establishing a systemic effect of the lytic virus that is likely mediated by a host immune response(17, 18).

Clinical Development
Phase 1

In a phase 1 study, 30 patients with previously treated melanoma, breast cancer, gastric adenocarcinoma, or head and neck cancer who had cutaneous or subcutaneous lesions accessible for injections were treated with different doses and schedules of T-VEC (19). The most common adverse events were grade 1 fever, constitutional symptoms, nausea, anorexia, and injection site reactions. One patient was reported to experience grade 2 fever, rigor, hypotension, tachycardia, and constitutional symptoms. Overall, the toxicities were more intense in HSV-seronegative patients; an initial low-dose of T-VEC, leading to HSV seroconversion, followed by a series of higher dose injections was better tolerated. There were no partial or complete responses, however flattening of both injected and non-injected metastases was seen in 6 of 26 evaluable patients. Post-treatment biopsies of injected lesions demonstrated inflammation and necrosis.

Phase 2

Fifty patients with unresectable stage IIIC – IV melanoma with one or more injection accessible tumor lesion were enrolled on a phase 2 study, assessing response rate, survival, and safety of T-VEC(20). Thirty seven (74%) of the patients had received prior systemic therapy and 20 (40%) had M1c visceral disease. Based on the experience from the phase 1 study, patients received intratumoral injections of up to 4 mL of $10^6$ pfu/mL of T-VEC, followed 3 weeks later by up to 4 mL of $10^8$ pfu/mL, and subsequently every 2 weeks for a maximum of 24 treatments. In a small subset of patients, peripheral blood and tumor biopsies were obtained for assessment of effector T cells, CD4+FoxP3+ regulatory T cells (Treg), CD8+FoxP3+ suppressor T cells, and myeloid-
derived suppressive cells (MDSC)(21). Eight complete responses (CRs) and 5 partial responses (PRs) were observed resulting in an overall response rate (ORR) of 26%. Twelve of the 13 responses lasted longer than 6 months. Compared to untreated melanoma lesions, melanoma metastases regressing after treatment with TVEC exhibited an increase of MART-1 specific T cells compared to melanoma lesions from untreated patients, whereas numbers of T regs and MDSC were decreased. Evidence for increased MART-1 specific T cells was seen in TILs and peripheral blood from a patient with a CR after T-TVEC suggesting the induction of a systemic melanoma specific immune response. Consistent with the observations in the phase 1 study, T-VEC was overall well tolerated with low grade constitutional symptoms, injection site reactions, and gastrointestinal symptoms as the most commonly observed adverse events.

Phase 3

In a phase 3 study, 436 patients with unresected stage IIIB to IV melanoma were randomized at a 2:1 ratio to receive TVEC versus subcutaneously administered GM-CSF(22). T-VEC or GM-CSF was administered until CR, clinically significant progressive disease (PD), intolerable side effects, or 12 months of therapy without an objective response. As in the phase 2 study, T-VEC was initially administered at $10^6$ pfu/mL for seroconversion, whereas subsequent doses were given at $10^8$ pfu/mL 3 weeks after the first dose and then every 2 weeks. T-VEC injection was restricted to cutaneous and subcutaneous metastases; different lesions could be prioritized for injection differently at any visit depending on size and emergence of new lesions. GM-CSF was given daily subcutaneously at 125μg/m² during the first 14 days of a 28 day cycle; it was chosen as a comparator arm based on overall survival benefit compared to historical control observed in
a previous study in melanoma patients at high risk for recurrence(23). The primary endpoint was durable response rate (DRR) defined as PR or CR with an onset during the first 12 months of treatment and lasting for at least 6 months. Secondary endpoints included overall survival, best overall response, and duration of response. Approximately half of the patients in each arm were previously untreated; 45% of patients in the T-VEC arm and 39% of patients in the GM-CSF arm were stage IVM1b/c. The study met its primary endpoint: DRR was significantly higher in the T-VEC arm (16.3%) compared with the GM-CSF arm (2.1%). The overall response rate was also significantly increased in the TVEC arm (26.4%) compared to GM-CSF alone (5.7%) as was the number of CRs (10.8% vs. 1%). Median overall survival (OS) was 23.3 months in the T-VEC arm and 18.9 months in the GM-CSF arm (HR, 0.79; 95%CI, 0.62 – 1.00; p=0.051) and it was therefore unclear, at least from the primary study analysis, whether T-VEC was associated with improved OS.

Subgroup analyses showed higher anti-tumor activity of T-VEC in patients with stage IIIB, IIIC, and IVM1a disease: with T-VEC, DRR was 33% in patients with IIIB or IIIC and 16% in patients with stage IVM1a, respectively compared to 0% and 2% with GM-CSF. In contrast, DRR was 3 and 7% in patients with stage IVM1b and M1c disease who received T-VEC compared to 4% and 3% in patients who received GM-CSF. Furthermore, the improved efficacy of T-VEC over GM-CSF was predominantly seen in treatment-naive patients.

The treatment was overall well tolerated: the most common toxicities included injection site reactions, fatigue, chills, and fever and were in line with previous experience from phase 1 and 2 trials. The only ≥ 3 toxicity that occurred in ≥ 2% of patients was cellulitis; other grade 3 or 4 toxicities included fatigue, extremity pain, vomiting, injection site pain, edema, and extremity
pain. No death was attributed to either of the study drugs. This adverse event profile compares favorably with toxicities after CTLA-4 and/or PD-1 pathway inhibition.

Limitations of the study, which was designed and initiated prior to the wide-spread availability of immune checkpoint inhibitors and BRAF/MEK inhibitors for advanced melanoma patients, include the choice of the comparator arm (GM-CSF), the complexity of the measurements assessing the primary endpoint involving many modalities (clinical assessments, radiography, and biopsies), and the small number and size of baseline lesions in a subset of the patients. A perceived ineffectiveness of GM-CSF (known to have modest, if any single agent anti-tumor activity in melanoma) may have led to differential decisions about continuation of treatment in the two treatment arms. These concerns seem to be supported by the fact that 58.3% of patients in the T-VAC arm discontinued treatment prior to the protocol-specified 24 weeks as compared to 75.1% in the control arm and only 1.4% never received treatment in the T-VEC arm as compared to 9.9% in the GM-CSF arm. These potential biases were nevertheless deemed unlikely to affect the statistically robust difference in DRR (the primary endpoint) and TVEC was approved by the FDA for the treatment of advanced melanoma in October 2015.

**T-VEC in Current Clinical Practice for Advanced Melanoma**

Substantial improvements have been made in the treatment of advanced melanoma in recent years. BRAF and combined BRAF/MEK inhibition (in BRAF<sup>V600</sup> mutant melanoma) and CTLA-4 and PD-1 inhibition have all shown improved overall survival in advanced melanoma.

Combined PD-1/CTLA-4 inhibition has demonstrated improved PFS and a RR of 60%,
independent of BRAFV600 status. While TVEC was developed and the registration trial
OPTIM-3 designed and largely conducted in a “pre-checkpoint inhibition, pre-MAPK pathway
inhibition era,” the recent FDA approval places the drug in a vastly different therapeutic
landscape for advanced melanoma. Nevertheless, despite the availability of a number of effective
drugs for melanoma, the approval of another drug with an entirely different mechanism of action
is welcome, both for standard of care treatment and current as well as future investigations.

Given TVEC’s benign toxicity profile, the durability of tumor responses, the requirement for the
presence of skin metastases, and the efficacy in stage IIIB,C and IVM1a disease, the drug
appears as an attractive choice for 1) “slow growing” lymph node, in transit or distant skin
metastases, e.g. patients who had several resections in the past and are no longer deemed
resectable 2) BRAF wild-type patients who have unequivocally progressed on PD-1 and/or
CTLA-4 inhibition; 3) BRAF wild type patients with multiple comorbidities or autoimmune
disease who are not deemed good candidates for immune checkpoint inhibition.

Since the drug is a live, genetically engineered virus that actively replicates in the host, special
attention needs to be given with regards to healthcare provider and patient education,
transmission precautions, and environmental safety.

**Current and Future Clinical Development**

Multiple clinical trials are ongoing assessing T-VEC in melanoma in the neoadjuvant setting as
well as in the metastatic setting with a focus on correlative studies such as analysis of talimogene
laherparepvec DNA in blood and urine as well as T cell tumor infiltration prior and after
treatment. T-VEC is also studied as monotherapy in advanced head and neck, inflammatory breast cancer and hepatocellular cancer and in combination with radiation in the neoadjuvant setting of sarcoma (Table 1). From a clinical development perspective, the main attraction of T-VEC may be its potential as a combinatorial agent partnering with checkpoint inhibition. In this regard, its role as an in situ vaccine is particularly attractive. Immune checkpoint inhibition given by itself may be limited by the size and specificity of pre-existent tumor specific T cells that are generated by physiological interaction of the evolving tumor and the host immune system. In non-responders to immune checkpoint inhibition, it is possible that there is insufficient priming of tumor-specific T cell clones – and as a result the critical threshold of T cells necessary to trigger an immune infiltrate is not reached. While traditional peptide vaccines targeting native antigens such as gp100 and MART-1, given with incomplete Freund’s adjuvant (IFA), have not provided synergy with CTLA-4 or PD-1 inhibition in advanced melanoma (13, 24), T-VEC (and other novel vaccines) may provide the necessary stimulation to broaden the repertoire of T cells engaged in the anti-tumor response. In support of this hypothesis, synergy of CTLA-4 and PD-1 inhibition with the whole tumor cell vaccines GVAX (autologous tumor + GM-CSF), FVAX (autologous tumor + Flt3 ligand), and TEGVAX (autologous tumor + GM-CSF + TLR4 and TLR 7/8 agonists) was demonstrated in the mouse B16 melanoma model (25, 26). Both CTLA-4 and PD-1 inhibition also showed synergy with a novel material engineered scaffold vaccine that co-delivers autologous tumor lysate, GM-CSF and the TLR-9 agonist CPG with precise spatial and temporal control (27). Recently reported preliminary efficacy data from an ongoing study combining T-VEC and the anti-CTLA antibody ipilimumab appear to support synergy between the 2 agents: 10 of 17 evaluable patients (56%) had an objective response including 6 CRs and 4 PRs, which is substantially higher than what would be expected from either of the drugs alone
(28). The combination of T-VEC and the PD-1 inhibitor pembrolizumab are underway in melanoma and head and neck cancer (Table 1).

Multiple lines of evidence indicate the importance of neoantigens as immunogenic tumor-specific antigens. Neoantigens are encoded by somatic mutations in tumor cells, resulting in peptides with amino acid changes that can be presented to T cells by personal HLA molecules. Because neoantigens are not exposed to central tolerance in the thymus, they should be strongly immunogenic. This assumption is supported by the recently documented association of clinical benefit from immune checkpoint blockade in melanoma, non small cell lung, and colorectal cancer (29-31), the detection of neoantigen specific T cells in cancer patients treated with immune checkpoint blockade and tumor infiltrating lymphocytes (TIL) therapy, and the anti-tumor activity of neoantigen specific TIL (32) amongst other evidence. Tumor antigens released through the cytolytic effect of T-VEC may contain neoantigens. Therefore, the \textit{in situ} vaccination effect of T-VEC may lead to priming of T cells with neoantigens. The availability of neoantigen discovery pipelines using next generation sequencing to identify somatic tumor mutations and neural network/machine learning based algorithms to predict binding of peptides to HLA class I now enable the design of vaccines specifically directed at neoantigens. A neoantigen peptide vaccine has recently shown striking efficacy in a mouse sarcoma model (33, 34). Clinical trials using a personalized neoantigenic peptide vaccine in high risk melanoma and glioblastoma (NCT01970358, NCT02287428) are ongoing. In light of the synergy between vaccines and immune checkpoint blockade in mouse models, combinatorial strategies with CTLA-4 and or PD-1/PD-L1 inhibition are being developed.
Conclusions

T-VEC is a first in class recombinant attenuated oncolytic HSV-1 virus encoding GM-CSF. In phase 1 – 3 trials, the drug has demonstrated a favorable toxicity profile and was associated with durable objective responses in patients with unresectable stage III and IV melanoma, leading to its FDA approval in this disease. The dual mechanism of action (direct cell killing and in situ vaccine effect) is novel and distinct from other agents in the growing landscape of immuno-oncology drugs. Initial data combining T-VEC with CTLA-4 inhibition in advanced melanoma are promising and provide evidence in humans that the combination of a cancer vaccine and immune checkpoint blockade may be synergistic. Given this potential synergy and the relatively modest anti-tumor activity as a single agent (at least in melanoma), T-VEC is a potentially attractive partnering agent for combinatorial immunotherapy approaches; it is currently in development in combination with CTLA-4 and PD-1 blockade as well as radiation. In light of the increasing evidence that immune checkpoint blockade is mainly effective in patients with a pre-existing T cell inflamed tumor microenvironment, T-VEC - along with other approaches that have the potential to mediate tumor directed T cell priming and trafficking into the tumor, such as vaccines - may become an increasingly important tool for cancer immunotherapy.
References

**Table 1:** Ongoing clinical trials of talimogene laherparepvec across tumor types

**Figure 1:** *In situ* vaccination effect of T-VEC, potentially converting a non-T cell inflamed tumor to a T-cell inflamed tumor. Replication of T-VEC in tumor cells leads to their lysis with release of tumor antigens, viral antigens and pathogen-associated molecular patterns (viral DNA, RNA, and proteins), and GM-CSF. This results in recruitment and maturation of antigen presenting cells including dendritic cells, which present tumor antigens to cytotoxic CD8 T cells. GM-CSF: granulocyte macrophage-colony stimulating factor.
<table>
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<th>Disease setting</th>
<th>Design</th>
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DCR: disease control rate (CR+PR+SD); TVEC: Talimogene laherparepvec; ORR: overall response rate; RFS: recurrence-free survival; hepatocellular carcinoma; DLT: dose limiting toxicity; pCR: pathologic response rate; PFS: progression free survival; OS: overall survival; SCCHN: squamous cell carcinoma of the head and neck.
Figure 1:

Noninflamed “cold” tumor

T-VEC

Lysed tumor cells

Inflamed “hot” tumor

Inflammatory cytokines (IFNγ, TNFβ)

GM-CSF

Dendritic cells

T cells
Clinical Cancer Research

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Clin Cancer Res  Published OnlineFirst May 4, 2016.

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