

Triple threat to cancer: rationale for combining oncolytic viruses, MEK inhibitors, and immune checkpoint blockade

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ABSTRACT

In a recent edition of *Science Translational Medicine*, we identified an enhanced therapeutic activity when talimogene laherparepvec (T-VEC) was combined with MEK inhibition in murine melanoma tumor models. MEK inhibition increased viral replication independent of mutation status. Combination therapy increased PD-1/PD-L1 expression and PD-1 blockade further enhanced tumor regression. Further clinical development of this strategy for treating melanomas warranted.

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Advances in the treatment of melanoma over the past decade have served as a paradigm for new anti-neoplastic drug classes, including molecularly targeted therapy, immune checkpoint blockade, and oncolytic viruses.¹ Further therapeutic benefit has been seen with combination treatment within drug classes, as for example with BRAF and MEK inhibitors in patients with melanoma harboring BRAF V600E/K mutations and with ipilimumab and nivolumab.^{2,3} Combination therapy, however, has been associated with increased toxicity for immune checkpoint inhibitors and emergence of drug resistance for targeted therapy. To date, few studies have explored combinations across different drug classes. In a recent issue of *Science Translational Medicine*, we sought to evaluate the impact of combining inhibition of the MAPK pathway and oncolytic virus treatment in melanoma.⁴ We utilized talimogene laherparepvec (T-VEC), an oncolytic herpes simplex virus, type 1 (HSV-1) encoding granulocyte-macrophage colony-stimulating factor (GM-CSF) and trametinib, a selective MEK inhibitor (MEKi) using human melanoma cell lines, and a murine melanoma model using D4M tumor cells derived from a BRAF-mutated spontaneous melanoma model and permissive to HSV-1 infection.

Oncolytic viruses and MEK inhibitors induce immunogenic cell death through different pathways. Thus, we initially explored whether combination T-VEC and BRAF inhibitors could enhance human melanoma cell killing *in vitro*. While moderate enhancement in melanoma cell killing was observed in BRAF V600E mutated human melanoma cell lines, no improvement was seen in BRAF wild-type cell lines regardless of NRAS mutation status. We also evaluated the selective MEKi, trametinib, and found a significant increase in cytotoxic activity when combined with T-VEC treatment, and this effect was independent of BRAF or NRAS mutation status. The effect

was also evident with other MEK inhibitors, and combined treatment was associated with an increase in T-VEC replication with an increase of viral protein production. Furthermore, trametinib-mediated apoptosis was also increased in melanoma cells co-infected with T-VEC. Using a human melanoma xenograft tumor model, we also confirmed that the T-VEC/MEKi combination resulted in reduced tumor cell proliferation, increased viral replication, and melanoma cell apoptosis. While treatment with T-VEC and MEKi alone induced tumor regression, leading to complete eradication of tumors in 30% of the treated mice, and 60% of these mice rejected subsequent tumor challenge. Evaluation of the tumor microenvironment showed an influx of proliferating CD8+ T cells expressing interferon- γ and Granzyme B. T-VEC alone and combination T-VEC/MEKi were also associated with a decrease in regulatory CD4+ FoxP3+ T cells (Tregs) and an increase in the CD8/Treg ratio. Using immune cell depletion and *Batf3*^{-/-} mouse models, we confirmed that treatment was dependent on CD8+ T cells and *Batf3*+ dendritic cells, which have been identified as important for antigen presentation for viral clearance and tumor eradication.⁵ Further interrogation of the CD8+ T cells demonstrated that initial responders were HSV-1 glycoprotein B-specific effector CD8+ T cells with later antigen spreading to gp100- and TRP2-specific CD8+ T cell responses. These data collectively show that T-VEC and MEKi treatment mediates tumor regression through *Batf3*+ dendritic cells with early priming of viral-specific CD8+ T cells and later antigen spreading to induce melanoma-specific T cell responses.

Next, we performed gene expression analysis using Nanostring Pan-Cancer immune panel and identified upregulation of genes associated with a pro-inflammatory immune profile in mice treated with the T-VEC/MEKi combination. We also

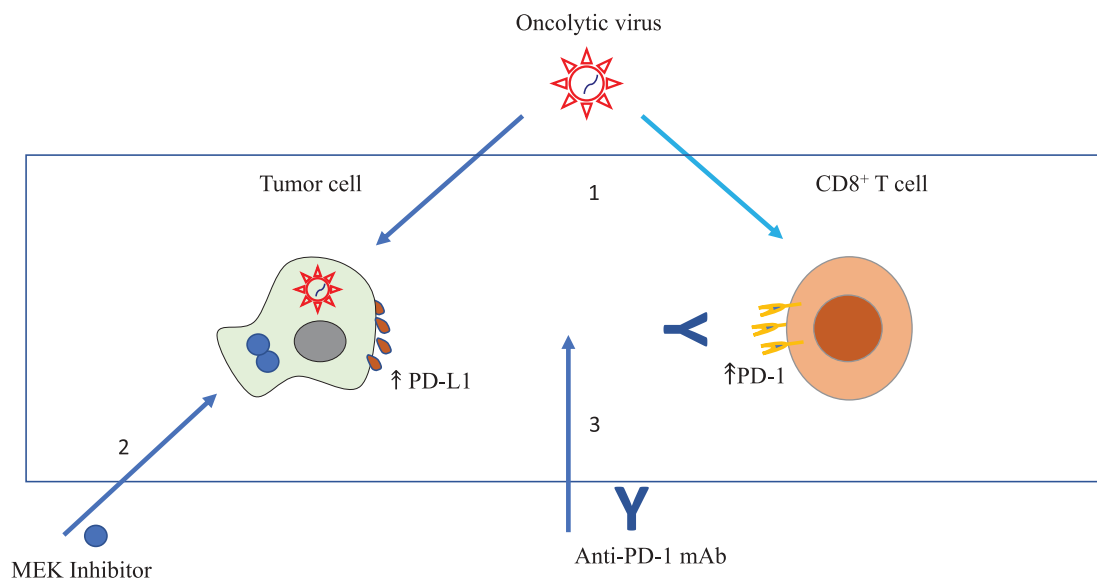


Figure 1. Schematic of how triple therapy using targeted therapy, immune checkpoint blockade, and oncolytic virus immunotherapy can integrate to improve therapeutic antitumor activity. Oncolytic viruses directly infect tumor cells inducing immunogenic cell death and increase PD-1/PD-L1 expression; they also enhance recruitment of T cells, increase PD-1 expression on T cells, and promote a local pro-inflammatory tumor microenvironment.¹ MEK inhibition directly targets oncogenic signaling pathways, induces tumor cell apoptosis, and promotes oncolytic virus replication.² The addition of monoclonal antibodies that block the PD-1/PD-L1 interaction prevents tumor cell suppression of tumor-specific T cell responses.³

observed upregulation of PD-1 and PD-L1 gene expression in the T-VEC/MEKi-treated mice, suggesting that additional therapeutic benefit might be possible with PD-1/PD-L1 blockade. To confirm this, triple combination with T-VEC/MEKi/ α PD-1 was tested in the D4M immune-competent model, and improvement in survival was seen with nearly 80% of the animals completely rejecting tumors. These mice were free from re-challenge and also developed increased numbers of effector CD8+ T cells. We also tested the triple combination in a colorectal cancer model and observed tumor regression in all treated mice. Treatment was not associated with any visible signs of toxicity. These data suggest that triple combination therapy across drug classes is associated with improved therapeutic benefit without a corresponding increase in toxicity in immune-competent murine tumor models.

In summary, our data provide a biologic rationale for combining oncolytic viruses, MEK inhibitors, and PD-1 blockade as a therapeutic strategy for cancer. As shown in **Figure 1**, the combination provides a three-pronged attack on cancer wherein MEKi and T-VEC interact to enhance immunogenic cell death, and interruption of tumor cell suppression of cancer-specific T cells through checkpoint blockade further drives host antitumor immunity. Although clinical validation is needed, all three agents used in our studies are currently approved for the treatment of advanced melanoma and could be rapidly translated into clinical trials to further improve outcomes for patients with melanoma and possibly other cancers as well.

Disclosure of Potential Conflicts of Interest

Dr. Howard L. Kaufman is an employee of Replimune.

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