894 A NOVEL ONCOLYTIC IMMUNOTHERAPY, VET3-TGI, OVERCOMES TGFB1 MEDIATED IMMUNOSUPPRESSION, AUGMENTS TYPE-1 IMMUNE RESPONSE, AND DISPLAYS POTENT THERAPEUTIC ACTIVITY IN MULTIPLE MOUSE TUMOR MODELS

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Background TGFB1 mediated immune resistance is one of the major mechanisms of immune suppression utilized across multiple tumor types. Immune resistance imparted by TGFB1 is mediated through its pleiotropic effects on vasculature, fibrogenesis and regulatory/effector immune cells within the tumor microenvironment. Blockade of TGFB1 (TGFBi) will likely improve response to immunotherapy. IL-12 is a cytokine that through IFNg induction, promotes type 1 inflammatory response, M1 macrophages and effector CD8 T cell response. Combining TGFB1 blockade with IL-12 may maximize therapeutic benefits through simultaneously reducing immunosuppression and enhancing anti-tumor immune response. Current studies have developed a vaccinia-based immunotherapy, combining enhanced systemic virus delivery to CXCR3 ligand rich tumors and locally expressed IL-12 and TGFBi within the tumor microenvironment, for efficient control of multiple tumor models.

Methods An oncolytic vaccinia virus expressing CXCR3, IL-12 and a TGFB1 antagonizing mini-monomer was constructed (VET3-TGI) and the expression and function of the transgenes were confirmed. Using *in vivo* mouse RENCA, EMT-6 and MC38 tumor models, the functionality and therapeutic efficacy of VET-TGI were tested with comparison to control virus. Post mortem analysis was used to analyze the impact of VET3-TGI on immune/stromal/endothelial milieu of the tumors and to determine toxicity profile.

Results VET3-TGI infected cells expressed CXCR3 and showed enhanced migration to CXCR3 ligands *in vitro* and improved systemic delivery to tumors expressing CXCR3 ligands *in vivo*, even in the face of pre-existing anti-viral immunity. IL-12 expression and TGFBi blockade of TGFB1 mediated suppression of CD8 T cell proliferation were confirmed *in vitro*. *In vivo* mouse studies using EMT6, RENCA and MC38 tumor models demonstrated potent therapeutic activity, including 100% CRs, even at doses several logs below equivalent clinical doses and in multiple models. Mechanism of activity studies suggested that the therapeutic efficacy of VET3-TGI is associated with considerable modification of the tumor microenvironment. In addition, preliminary toxicity studies demonstrated the safety of VET3-TGI in mouse models.

Conclusions VET3-TGI demonstrated an ability to reduce immunosuppression and dramatically enhance antitumor immune response leading to safe and potent therapeutic activity in multiple mouse tumor models. This data led to the selection of VET3-TGI as our lead clinical candidate. A human version of the virus is currently undergoing clinical manufacture and toxicology testing.

Ethics Approval Animal studies were approved by IACUC, Hilltop animal center

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