IN SITU VACCINATION WITH ONCOLYTIC VESICULAR STOMATITIS VIRUS IMPROVES ANTI-TUMOR IMMUNE RESPONSE AND OUTCOME IN BLADDER CANCER

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Background: The majority of nonmuscle invasive bladder cancer (NMIBC) cases progress towards muscle invasive disease. Transurethral resection followed by chemotherapy and/or BCG immunotherapy can stall progression in the minority of NMIBC cases. Cystectomy prior to muscle invasion provides the best option for survival. However, bladder removal significantly affects morbidity and quality of life. There are no effective treatment options for patients with chemo/BCG-resistant and late stage disease. Compared to other solid cancer types, the urinary bladder is an ideal organ to evaluate oncolytic virotherapies due to the urgent medical need for alternate bladder-sparing therapies and its established immunosensitivity to BCG therapy. The current work describes the in vitro oncolytic activity and transgene production capability in human cancer cell lines, and in vivo activity of armed myxoma viruses in xenograft human cancer models.

Methods: The current work describes the in vitro oncolytic activity and transgene production capability in human cancer cell lines, and in vivo activity of armed myxoma viruses in xenograft human cancer models.

Results: Armed Myxoma viruses demonstrate transgene production and oncolytic activity in multiple human cancer cell lines in vitro and in vivo.

Conclusions: Armed Myxoma viruses present a novel oncolytic viral therapy with ability to modulate immune responses in human cancer models.

Ethics Approval: This study was approved by OncoMyx Therapeutics and the TD2 IACUC.

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REVERSAL OF EPICENETIC SILENCING OF CGAS AND STING IN MELANOMA ENHANCES THE ACTIVITY OF TUMOR INFILTRATING LYMPHOCYTES

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Background: It is becoming more evident that STING activity in tumor cells can have a functional role in mediating antitumor immune responses. We have recently shown that activation of STING signaling in human melanoma cell lines enhances their antigenicity and susceptibility to lysis by human melanoma tumor infiltrating lymphocytes (TIL) through the augmentation of MHC class I molecules. However, the frequent impairment of this pathway through loss of cGAS and/or STING expression in melanoma cell lines limits their antigen presentation and subsequently their sensitivity to cytotoxic T cell mediated killing. In this study, we asked if this suppression is, in part, epigenetically regulated and if it is indeed a driver of melanoma resistance to T cell-based immunotherapies.

Methods: To determine the role of DNA methylation in melanoma STING and cGAS silencing, we performed genome-wide DNA methylation profiling across a panel of 16 human melanoma cell lines. We subjected melanoma cell lines that indicated STING and/or cGAS promoter hypermethylation to treatment with 5-aza-2’-deoxycytidine (5AZADC) and evaluated their protein expression by immunoblot. We next assessed phosphorylation of IRF3 and induction of IFN-beta by STING-dependent phosphorylation of IRF3, induction of CXCL10 (~300 pg/ml, P < 0.0001) and IFN-beta (~900 pg/ml, P < 0.0001) and upregulation of MHC class I. We also observed up to a 8-fold increase in TIL production of IFN-gamma in co-culture studies using 5AZADC-pretreated melanoma cellskiller (NK) and cytotoxic CD8+ T cells. The increased functionality of NK and CD8+ T cells was associated with improved survival as determined through depletion studies. Moreover, improved survival and reduced bladder tumor volume was observed in recombinant VSVd51 treated mice who failed BCG therapy. In parallel, VSVd51-induced inflammation of the tumor microenvironment was recapitulated in human BC cell lines, spheroids and patient tissue exposed to recombinant VSVd51 infection.

Conclusions: These translational results suggest that a recombinant VSVd51 is a promising immunotherapy that could provide a bladder-sparing therapeutic benefit in individuals diagnosed with NMIBC each year.

Ethics Approval: The study was approved by the CIIUSSS de l’Estrie CHUS Ethics Board, approval number 2018-2465.

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NEW CHECKPOINTS CONTROLLING FUNCTION OF CYTOTOXIC LYMPHOCYTES INFLITRATING HUMAN CARCINOMA

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Abstracts

Background Although present in high numbers, T and NK cells appear functionally impaired in the renal cell carcinoma (RCC) tumor milieu, as they cannot be stimulated to degranulate and IFN-γ production. This is in part due to altered regulation of signaling downstream of the T cell receptor (TCR). Increased diacylglycerol kinase alpha (DGK-α) has been observed in T and NK cells from the RCC tumor microenvironment (TME). Ex vivo inhibition of DGK-α by the commercially available inhibitor R59022 was able to restore responsiveness to stimulation. 2 Inhibition of DGK-α is reported to also block tumor cell growth and survival. 3, 4 Many T cells from RCC additionally express the immune checkpoint Programmed cell Death-1 (PD-1). Interaction of PD-1 with PD-L1 on tumor cells blocks Akt signaling and inhibits T cell function. In the clinic, blocking the PD-1/PD-L1 interaction allows tumor control in some patients; however, the majority of patients do not respond long-term. Since DGK-α acts downstream of PD-1 it may, if overactive, curb T cell function despite PD-1/PD-L1 blockade. Thus, we hypothesize that dual inhibition of PD-1 and DGK-α might be required to fully unleash the T cell’s potential in the TME. Current DGK-α inhibitors are not suitable for clinical application. Therefore, we investigate alternative means using RNA interference (RNAi) to target DGK-α alone as well as in combination with PD-1.

Methods Knockdown was achieved by RNAi using INTASYL™ compounds, developed by Phio Pharmaceuticals. These compounds incorporate drug-like properties into siRNA, resulting in enhanced uptake with no need for transfection reagents. Efficacy was analyzed on mRNA and protein level by rt-qPCR, flow cytomtery and Western Blot. Functional assays include cytotoxicity and cytokine production in tumor-mimicking environments.

Results Using INTASYL™ compounds, silencing of DGK-α was observed in human U2OS osteosarcoma as well as K562 erythroleukemic cells. PD-1 knockdown was achieved in human T cells isolated from peripheral blood mononuclear cells (PBMC). Synergy of DGK-α and PD-1 knockdown is tested in tumor-mimicking in vitro systems using T cell/tumor cell co-cultures at high tumor cell density where T and NK cells become functionally suppressed as observed in the TME.

Conclusions Strong activity of specific T and NK cells is necessary for tumor control. Dual targeting of PD-1 and DGK-α may be required to fully enable T and NK cell reactivity in the TME. Self-delivering RNAi technology represents a promising approach to targeting intracellular immune checkpoints such as DGK-α, in addition to PD-1 inhibition.

REFERENCES