

patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Pox family of double stranded DNA viruses. The natural host of MYXV is a subset of rabbits and hares, but MYXV is able to infect cancer cell lines of humans and other species. The genome of MYXV is relatively large and is amenable to engineering for expression of transgenic proteins making it an excellent oncolytic virus for introduction of immunomodulatory proteins.

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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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 alternative bladder-sparing therapies and its established immunosensitivity to BCG therapy. The current study will determine whether a novel oncolytic Vesicular Stomatitis Virus (VSVd51) containing human immune transgenes can treat NMIBC.

Methods A novel recombinant OV containing a human immune transgene was rescued on the VSVd51 backbone. Features of immunogenic cell death (ICD) on mouse and human bladder cancer cell lines were measured by microscopy, flow cytometry, immunoblot, luminometry, qRT-PCR and ELISA following infection by recombinant VSVd51. The mediating role of immune effector cells was evaluated through pharmacologic in vivo depletion, while combination injection of recombinant VSVd51 following BCG failure was performed in the C57Bl/6-MB49 model. Measurements of ICD was additionally carried out in human BC spheroids and bladder cancer patient tissue following recombinant VSVd51 infection ex vivo.

Results Recombinant VSVd51 liberated danger signals (calreticulin, HMGB1, ATP) and immunogenic cytokines/chemokines were detected from infected mouse and human BC cell lines. Intravesical instillation of recombinant VSVd51 promoted enhanced activation of systemic and bladder infiltrating natural

killer (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 CIUSSS de l'Estrie CHUS Ethics Board, approval number 2018-2465.

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REVERSAL OF EPIGENETIC 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.¹ 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- β and CXCL10 in 5AZADC-treated melanoma cells following their stimulation with dsDNA or 2'3'-cGAMP. We also co-cultured 5AZADC-pretreated melanoma cell lines with their HLA-matched human melanoma TIL in the presence or absence of dsDNA or 2'3'-cGAMP and assessed TIL production of IFN- γ .

Results Using whole genome methylation profiling, we identified a distinct correlation between promoter hypermethylation and loss of STING and cGAS expression in human melanoma cell lines. Reconstitution of STING and cGAS expression through DNA demethylation reinstated functional STING signaling in at least half of the examined cell lines as indicated by STING-dependent phosphorylation of IRF3, induction of CXCL10 (~300 pg/ml, $P < 0.0001$) and IFN- β (~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- γ in co-culture studies using 5AZADC-pretreated melanoma cells

compared to untreated controls in the presence of dsDNA or 2'3'-cGAMP (~2,000 pg/ml, $P < 0.001$).

Conclusions We provide evidence that methylation silencing of cGAS and STING is not only a notable mechanism of STING signaling dysfunction in melanoma, but also plays a role in tumor antigen presentation and recognition by TIL. Collectively, these observations argue that targeting epigenetic loss of STING signaling in melanomas should be considered as a strategy to improve the efficacy of clinical interventions using T cell-based immunotherapies.

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NEW CHECKPOINTS CONTROLLING FUNCTION OF CYTOTOXIC LYMPHOCYTES INFILTRATING HUMAN CARCINOMA

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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 degranulation 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.^{1 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 INTASYLTM 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 cytometry and Western Blot. Functional assays include cytotoxicity and cytokine production in tumor-mimicking environments.

Results Using INTASYLTM 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.

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IN VITRO ANTICANCER AND IMMUNOMODULATORY ACTIVITIES OF NBT-167, A DIMER OF RESVERATROL

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Background Resveratrol (3,4',5-trihydroxystilbene), a stilbenoid isolated from many species of plants, is widely known for its antioxidative, anti-inflammatory, immunomodulatory and anticancer activities. Recently, novel resveratrol oligomers have been isolated from various plants; their diverse structures are characterized by the polymerization of two or more resveratrol units. Little is known regarding the anticancer and immunomodulating activities of these oligomers. In this study, we designed in vitro models to compare resveratrol side by side with its natural dimer NBT-167 for their anticancer and immunological activities.

Methods We isolated resveratrol and its dimer (NBT-167) from plants. The potency of the compounds was compared side by side using cancer cell survival assays and immunological assays with various types of human cells including cancer cell lines, PBMCs and enriched NK, gamma delta T cells, THP-1 monocytic cells, HL-60 promyelocytic leukemia cells as well as mouse RAW264.7 macrophages.

Results NBT-167 was found to be more potent than resveratrol in inhibiting growth of various cancer cells and modulation of cytokine production from anti-IgM, LPS, PHA or SEB stimulated PBMC. Both compounds similarly enhanced IL-2 stimulated NK and gamma delta T cell killing activity against K562 cells and modulated nitric oxide production from LPS/IFN-g induced RAW264.7 macrophages and phagocytotic activity of HL-60 cells. NBT-167 was slightly more potently