Ethics Approval IRB exempted for case report with no patient-identifiable information

Consent N/A

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594

BT-001, AN ONCOLYTIC VACCINIA VIRUS ARMED WITH A TREG-DEPLETING HUMAN RECOMBINANT ANTI-CTLA4 ANTIBODY AND GM-CSF TO TARGET THE TUMOR MICROENVIRONMENT

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Background Checkpoint inhibitor antibodies have improved survival in a variety of cancers, however, a great unmet need remains since only a small fraction of patients responds. Reasons for lack of efficacy are believed to include lack of tumor infiltrating immune cells, a notion supported by improved efficacy observed following combined checkpoint blockade with tumor oncolytic virotherapy which promotes intratumoral T cell infiltration. Oncolytic vaccinia viruses (oVV) also allow genetic encoding of transgenes. This is of special interest for therapeutic proteins exhibiting toxicological limitation or pharmacokinetic issues. Here, BioInvent and Transgene present a potentially safe and more efficacious strategy to combine checkpoint inhibition in the context of oncolytic virotherapy.

Methods Using the F.I.R.S.T™ discovery platform we have isolated a human recombinant Treg-depleting antibody that has been vectorized alongside GM-CSF into the Invir.IO® oVV. This product named BT-001 consists of a Copenhagen double deleted vaccinia virus encoding the human CTLA4-specific antibody 4-E03 IgG1, which shows improved Treg-depletion compared with ipilimumab in a human PBMC-based NOG/SCID-transfer model. BT-001 also encodes GM-CSF, the cytokine expressed in clinically approved products. A surrogate murine mAb was vectorized into the same oVV (mBT-1) allowing for functional and mechanistic in vivo studies.

Results Our studies demonstrate that 4-E03 and GM-CSF were expressed as functional molecules after infection by BT-001 of human tumor cell lines in vitro. Moreover, following intratumoral administration in immune competent and immune deficient mice transplanted with mouse or human tumors, transgene expression was sustained at levels associated with receptor saturation for days to weeks. In contrast, and supporting the tumor-selective nature of oVV, blood concentrations of anti-CTLA4 mAb were lower compared to those observed following i.v. administration of therapeutic doses of mAb. The in vivo anti-tumor activity of mBT-1 was assessed in multiple syngeneic mouse tumor models including CT26, EMT6, A20 and C38. Murine surrogate mBT-1 conferred cures in the majority of challenged mice irrespective of tumor origin. The excellent anti-tumoral profile depends on anti-CTLA4 expression and could be boosted by co-administration

of anti-PD-1 mAb. Intratumoral treatment with mBT-1 also induces abscopal anti-tumor responses and protects against tumor rechallenge demonstrating a long-lasting systemic anti-tumor activity.

Conclusions A clinical batch of BT-001 has been produced and toxicological evaluation is ongoing. Transgene and BioInvent have applied for a clinical trial targeting injectable superficial tumors. Here, the tumor-localized delivery of anti-CTLA4 may allow a better tolerated and more effective combination therapy with antibodies targeting the PD-1/PDL1 axis.

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595

ARMED MYXOMA VIRUS DEMONSTRATES EFFICACY IN SYNGENEIC TUMOR MODELS ALONE AND IN COMBINATION WITH IMMUNE CHECKPOINT INHIBITORS

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Background Oncolytic Viruses (OV) selectively replicate in and lyse tumor cells and provide stimulation to the immune system. This represents a promising therapeutic option for cancer patients that do not respond well to treatment with immune checkpoint inhibitors. Myxoma virus (MYXV) is a member of the Poxv family of double stranded DNA viruses. The natural host of MYXV is a subset of rabbits and hares, but MYXV can infect cancer cell lines of humans and other species. The genome of MYXV is relatively large and is amenable to engineering for expression of transgenes making it an excellent oncolytic virus for introduction of immunomodulatory proteins.

Methods Armed MYXV were tested for oncolytic activity and transgene production in syngeneic mouse cancer models in vitro and in vivo. In vivo models were further assessed for activity when in combination with immune checkpoint inhibitors and for immune mechanisms of action contributing to the efficacy of armed MYXV.

Results Armed MYXV demonstrated oncolytic activity, transgene production capability and in vivo activity following intratumoral and intravenous administration of armed myxoma viruses in murine cancer models. Additional combination therapy with clinically relevant immune checkpoint inhibitors is demonstrated.

Conclusions Armed Myxoma viruses present an efficacious novel oncolytic viral therapy with the ability to modulate immune responses in murine cancer models.

Ethics Approval Animal studies we approved by OncoMyx and the TD2 IACUC.

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596

ARMED MYXOMA VIRUS DEMONSTRATES THERAPEUTIC ACTIVITY IN XENOGRFT MODELS

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Background Oncolytic Viruses (OV) selectively replicate in and lyse tumor cells and provide stimulation to the immune system. This represents a promising therapeutic option for cancer

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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597

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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598

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