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PRIME-BOOST VACCINATION FOR THE TREATMENT OF TRIPLE NEGATIVE BREAST CANCERSeyedeh-Raheleh Niavarani*, Christine Lawson, Lee-Hwa Tai. *Sherbrooke University, Sherbrooke, Canada*

Background Triple negative breast cancer (TNBC) is currently only treated with surgery and chemotherapy.¹ TNBC has higher rates of genetic mutations and contains more tumor infiltrating lymphocytes.² These characteristics provide a strong rationale to use novel immunotherapies such as immunogenic autologous tumor cell vaccines to therapeutically target TNBC. We have demonstrated that an infected cell vaccine (ICV) that is made from irradiated and oncolytic virus tumor infected cells induces beneficial innate and adaptive immune responses in a syngeneic mouse model of TNBC. Moreover, the efficacy of ICV is improved when combined with checkpoint blockade (anti-PD-1).³ Our goal is to further improve ICV by applying a prime-boost cancer vaccination strategy to further enhance anti-tumor immune responses in preclinical and translational studies.^{4, 5}

Methods We will choose the best 'prime vaccine' based on the immunogenicity of TNBC cell lines after treatment with immunomodulators such as chemotherapeutic agents, irradiation, toll-like receptor agonists and anti-viral vaccines. We will measure the release of damage-associated molecular patterns (DAMPs), which act as danger signals to initiate tumor-targeted immune responses,⁶ after the treatment of TNBC cell lines. We will test the polarization of human monocytes when co-cultured with conditioned media (CM) from treated TNBC cells. We will also analyze the migration of human immune cells (CD56+NK cells and CD8+T cells) toward the CM of treated human TNBC cells. Furthermore, we will evaluate the maturation markers on CD11C+ dendritic (DC) cells differentiated from mouse bone marrow cells when co-cultured with the cell lysate of the mouse TNBC cell line treated with 'prime vaccine' candidates. For in vivo studies, we will test our best prime vaccine followed by the ICV as a boost vaccine in our BALB/c-4T1 mouse model. We will analyze the cytotoxicity of T lymphocytes and the secretion of cytokines, and overall survival.

Results From measuring DAMP levels and analyzing immune functions, our preliminary results suggest that oxaliplatin and the seasonal influenza vaccine are the best candidates as strong 'prime vaccine' candidates compared to other treatments. DCs differentiated from isolated bone marrow cells exhibited a higher percentage of markers of maturation when co-cultured ex vivo with cell lysate of 4T1 cells were treated with oxaliplatin compared to control groups. In vivo studies in the BALB/c-4T1 model have begun to test the best prime-boost vaccine combinations.

Conclusions These results demonstrate the therapeutic potential of oncolytic virus-based immunogenic tumor vaccines could be improved by applying the 'prime-boost' cancer vaccination approach to treat TNBC.

Ethics Approval 'The study was approved by the CRCHUS Human Ethics Board, approval number 2018-2414.'

Consent 'Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.'

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OPTIMIZATION OF A GM3-CONTAINING LIPOSOMAL VACCINE THAT DELIVERS ANTIGEN TO CD169+ SPLENIC MACROPHAGES

¹Maarten Nijen Twilhaar*, ²Lucas Czentner Colomo, ¹Joanna Grabowska, ¹Alysa Affandi, ¹Katarina Olesek, ¹Hakan Kalay, ²Rene van Nostrum, ³Paul Crocker, ¹Yvette van Kooyk, ²Gert Storm, ¹Joke den Haan. ¹Amsterdam UMC, Amsterdam, Netherlands; ²Utrecht University, Utrecht, Netherlands; ³University of Dundee, Dundee, UK

Background Although promising developments in cancer vaccination have been made, therapeutic effectiveness is often insufficient. Liposomal vaccine effectiveness could be enhanced by antigen encapsulation and incorporation of molecules that actively target to antigen presenting cells to enhance T cell activation. CD169-expressing splenic macrophages are located in the marginal zone and efficiently capture particulate antigens such as viruses and exosomes from the blood circulation. Upon antigen capture CD169+ macrophages transfer antigen to cross-presenting dendritic cells that are responsible for the activation of CD8+ T cells.

Methods Here we prepared liposomes that contain a physiological ligand for CD169, the ganglioside GM3, to facilitate uptake by CD169+ macrophages. We assessed how various amounts of targeting molecule GM3, decoration with PEG and liposomal size affected binding and uptake by CD169+ macrophages in vitro and in vivo. In addition, we evaluated the stability of liposomal preparations in plasma. As a proof of concept, we prepared GM3-liposomes with a long ovalbumin peptide and tested the capacity of these liposomes to induce CD8+ and CD4+ T cell activation and compared it to control liposomes and soluble peptide.

Results These data indicate that targeting of splenic CD169 macrophages can be optimized by careful selection of constituents of the liposomal delivery vehicle. Moreover, optimized GM3-mediated liposomal targeting to CD169 macrophages results in potent immune responses.

Conclusions GM3-mediated liposomal targeting to CD169 macrophages presents as a promising strategy for cancer vaccines

Ethics Approval All animal experiments were approved by the local animal welfare body.

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IMMUNOLOGICAL CONSIDERATIONS FOR DEVELOPING OPTIMAL WHOLE TUMOR CELL-DERIVED CANCER VACCINES

Flavio Salazar Onfray*. *Universidad de Chile, Santiago, VT, Chile*

Background Immunotherapies based on checkpoint blockers (ICB), targeting inhibitory immune pathways such as cytotoxic T

lymphocyte-associated protein 4 (CTLA-4) or programmed cell death protein 1 (PD-1), have shown significant success in promoting tumor regression and prolonging survival in cancer patients, particularly in melanoma and other solid tumors. However, many patients do not respond or develop resistance to these interventions, bringing the scientific communities to focus their efforts in combinatorial therapies. A major factor involved in initial resistance to ICB is lack or weak T cell tumor infiltration, characterizing the so-called ‘cold tumors’. In fact, high lymphocyte infiltration and interferon (IFN)- γ status related to a T cell inflamed phenotype (‘hot tumors’) constitute key factors for effective anti-PD-1/PD-L1 therapies. For this reason, immunological treatments that induce adaptive cellular responses in cold tumor-patients may be a desirable goal. In this context, tumor vaccines become once again an attractive alternative and/or complement for cancer treatment.

Methods Here, a prototype for a generic melanoma vaccine, named TRIMELVax, was tested using B16F10 mouse melanoma model. This vaccine is made of heat shock-treated tumor cell lysates named TRIMEL combined with the *Concholepas concholepas* hemocyanin as adjuvant. TRIMEL is derived from a mix of equal amounts of Mel1, Mel2 and Mel3 cells, which were taken to a final concentration of 8×10^6 cells/mL, HS-treated at 42°C for 1 hour plus 2 hours at 37°C and then lysed through three cycles of freeze/thaw in liquid nitrogen.

Results While B16F10 lysate provides appropriate melanoma-associated antigens, both a generic human melanoma cell lysate and hemocyanin adjuvant contributes with danger signals promoting conventional dendritic cells type 1 (cDC1), activation, phagocytosis and effective antigen cross-presentation. TRIMELVax inhibited tumor growth and increased mice survival, inducing cellular and humoral immune responses. Furthermore, this vaccine generated an increased frequency of intratumor cDC1s but not cDC2s. Augmented infiltration of CD3+, CD4+ and CD8+ T cells was also observed, compared with anti-PD-1 monotherapy, while TRIMELVax/anti-PD-1 combination generated higher tumor infiltration of CD4+ T cells. Moreover, TRIMELVax promoted an augmented proportion of PD-1^{lo} CD8+ T cells in tumors, a phenotype associated to prototypic effector cells required for tumor growth control, preventing dysfunctional T cell accumulation.

Conclusions The therapeutic vaccine TRIMELVax efficiently controls the weakly immunogenic and aggressive B16F10 melanoma tumor growth, prolonging tumor-bearing mice survival even in the absence of ICB. The strong immunogenicity shown by TRIMELVax encourages clinical studies in melanoma patients.

Ethics Approval All animal experiments were performed in accordance with institutional guidelines for animal care and were approved by the Ethical Review Committee at the Universidad de Chile, Ethical Number CBA0885 (approval date: May 2016).

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Immuno-conjugates and chimeric molecules

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POTENT TUMOR-DIRECTED T CELL ACTIVATION AND IN VIVO TUMOR INHIBITION INDUCED BY A 4-1BB X 5T4 ADAPTIR™ BISPECIFIC ANTIBODY

¹Michelle Nelson*, ¹Robert Miller, ²Anneli Nilsson, ²Lill Ljung, ¹Allison Chunyk, ¹Catherine McMahan, ¹David Bienvenue, ²Maria Askmyr, ¹Gabriela Hernandez-Hoyos, ²Sara Fritzell. ¹Aptevo Therapeutics, Seattle, WA, USA; ²Alligator Bioscience, Lund, Sweden

Background 4-1BB (CD137) is an activation-induced co-stimulatory receptor that regulates immune responses of activated CD8+ T cells and NK cells, by enhancing proliferation, survival, cytolytic activity and IFN- γ production. Its ability to induce potent anti-tumor CD8+ and NK cell activity makes 4-1BB an attractive target for designing novel therapeutics for immuno-oncology. However, clinical development of a mono-specific 4-1BB agonistic antibody has been hampered by dose-limiting hepatic toxicities. To minimize systemic immune toxicities and enhance activity at the tumor site, we have developed a novel 4-1BB x 5T4 bispecific antibody that stimulates 4-1BB function only when co-engaged with 5T4, a tumor-associated antigen. The combined preclinical dataset presented here provides an overview of the mechanism of action and the efficacy and safety profile of ALG.APV-527, supporting its advancement into the clinic.

Methods ALG. APV-527 was built based the ADAPTIR™ platform with binding domains to 4-1BB and 5T4 generated using the ALLIGATOR-GOLD® human scFv library. ALG.APV-527 was tested using primary cells in the presence or absence of cells expressing 5T4. Cell Trace-labelled PBMC sub-optimally stimulated with anti-CD3, to induce 4-1BB expression, cells were gated using flow cytometry. T cell cytotoxicity was assessed by quantifying cell death in CD8+ T cell/tumor cell co-cultures, and images were obtained using a cell live imaging system (Cytation 5). For tumor inhibition studies, human 4-1BB knock-in mice were injected subcutaneously with MB49 cells transfected with human 5T4. Cured mice were subsequently used in a toxicity study and liver pathology was evaluated.

Results In vitro, ALG.APV-527 enhances primary CD8+ T cell and NK cell function and proliferation in the presence of 5T4-expressing cells. Using imaging, ALG.APV-527 in combination with a bispecific T cell engager caused increased cell death in T cell/tumor cell co-cultures. ALG.APV-527 inhibited growth of established tumors at doses as low as 2 μ g/mouse in a syngeneic bladder cancer model. Following recovery, mice exhibited a memory response when rechallenged with tumor. In a high dose safety study in human 4-1BB knock-in mice, ALG.APV-527 did not cause significant systemic immune activation, whereas urelumab analogue treated mice induced dermatitis, elevated serum cytokines, CD8+ T-cell liver infiltration and systemic T-cell proliferation.

Conclusions ALG. APV-527 induces potent CD8+ T cell and NK cell co-stimulation and T-cell cytotoxicity and has potent in vivo anti-tumor activity, without inducing systemic toxicity. Based on preclinical data, ALG.APV-527 is a promising anti-cancer therapeutic for the treatment of a variety of 5T4-expressing solid tumors.

Ethics Approval All studies were review and approved by the Internal Animal Care and Use Committee (IACUC) of Aptevo Therapeutics

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DIFFERENTIAL EXPRESSION OF SURFACE PROTEIN-ENCODING GENES HIGHLIGHTS THERAPEUTIC VULNERABILITIES OF FOUR SCLC SUBTYPES

Elizabeth Park*, Carl Gay, C Allison Stewart, Kasey Cargill, Lixia Diao, Qi Wang, Robert Cardnell, Jing Wang, John Heymach, Lauren Byers. *The University of Texas MD Anderson Cancer Center, Houston, TX, USA*