

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

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

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

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