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 8×106 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-1lo 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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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 monospecific 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 μg/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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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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852 DIFFERENTIAL EXPRESSION OF SURFACE PROTEIN-ENCODING GENES HIGHLIGHTS THERAPEUTIC VULNERABILITIES OF FOUR SCLC SUBTYPES

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Background Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine malignancy that accounts for 15% of lung cancer diagnoses. The severity of this disease is exacerbated by the fact that there are few therapeutic options, which mostly offer limited clinical benefit, culminating in a 5-year survival rate of

Methods To identify transcriptional subtypes, we used non-negative matrix factorization of gene expression data from 81 SCLC tumors and identified four subtypes largely based on differential expression of the transcription factors ASCL1, NEUROD1, and POU2F3. We hypothesized that these subtypes may underlie unique therapeutic vulnerabilities. We examined differential expression of genes that encode surface-expressed proteins that may be targetable by reagents such as therapeutic antibodies or antibody-drug conjugates (ADCs).

Results Our four subtypes are defined either by high expression of ASCL1 (SCLC-A), NEUROD1 (SCLC-N), POU2F3 (SCLC-P), or an absence of those transcription factors and instead a prevalence of immunological factors (SCLC-Inflamed, or SCLC-I). We curated a list of approximately 60 candidate genes encoding surface proteins that are differentially expressed across the four subtypes. Within these 60 candidates, we have identified a few specific to each subtype for which there exist clinically available, targeted ADCs. The most prevalent subtype, SCLC-A, showed high expression of targets such as DLL3 (SCLC-A) and CEACAM5 (SCLC-A). SCLC-N highly expressed SSTR2, a somatostatin receptor that is being actively targeted in SCLC clinical trials. The two non-neuroendocrine subtypes, SCLC-P and SCLC-I shared some common hits such as the NK cell ligand MICA and B7H6. All of the identified and highlighted hits have been or are actively being pursued in clinical trials, highlighting the importance of understanding their expression levels pre- and post-treatment so that novel therapies can be developed that will be effective over the course of disease progression

Conclusions The underlying biology defining our four identified subtypes of SCLC has revealed a striking number of targetable, differentially expressed surface protein encoding genes many of which already have clinically available reagents that could be repurposed for treatment of SCLC on a subtype-specific basis.

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Immunotherapy toxicities

853 PROFILING OF DONOR-SPECIFIC IMMUNE EFFECTOR SIGNATURES IN RESPONSE TO RITUXIMAB IN A HUMAN WHOLE BLOOD LOOP ASSAY USING BLOOD FROM CLL PATIENTS

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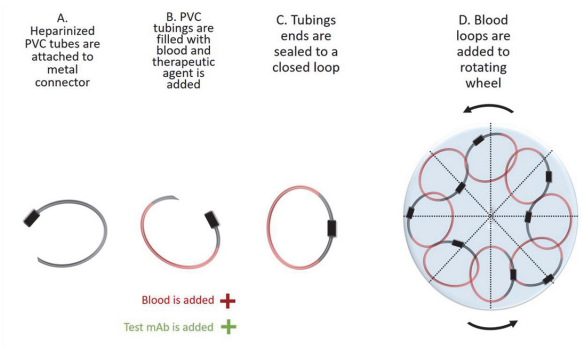
Background Rituximab is widely used in the treatment of haematological malignancies, including chronic lymphoid leukaemia (CLL), the most common leukaemia in adults. However, some patients, especially those with high tumour burden, develop cytokine release syndrome (CRS). It is likely that more patients will develop therapy-linked CRS in the future due to the implementation of other immunotherapies, such as CAR T-cell, for many malignancies. Current methods for CRS risk assessment are limited, hence there is a need to develop new methods.

Methods To better recapitulate an in vivo setting, we implemented the unique human whole blood 'loop' system (figure 1)[1] to study patient-specific immune responses to rituximab in blood derived from CLL patients.

Results Upon rituximab infusion, both complement-dependent cytotoxicity (CDC) and antibody-dependent cellular cytotoxicity (ADCC) profiles were evident in CLL patient blood, coincident with CLL cell depletion. Whereas B cell depletion is induced in healthy persons in the blood loop, only patients display B cell depletion coupled with CRS. With the exception of one donor who lacked NK cells, all other five patients displayed variable B cell depletion along with CRS profile. Additionally, inhibition of CDC or ADCC via either inhibitors or antibody Fc modification resulted in skewing of the immune killing mechanism consistent with published literature

Conclusions Herein we have shown that the human whole blood loop model can be applied using blood from a specific indication to build a disease-specific CRS and immune activation profiling ex vivo system. Other therapeutic antibodies used for other indications may benefit from antibody characterization in a similar setting.



Abstract 853 Figure 1
Schematic of the blood loop assay. A-C preparation of blood loops, D. Loops are kept in motion by attaching to rotating wheel. The air bubble inside the loop drives blood circulation within the loops.

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854 PREDICTION OF SEVERE IMMUNE-RELATED ADVERSE EVENTS IN PATIENTS ON IMMUNE CHECKPOINT INHIBITORS: STUDY OF A POPULATION LEVEL INSURANCE CLAIMS DATABASE FROM THE UNITED STATES

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