



## Potent Tumor-Directed T cell Activation and Tumor Inhibition Induced by a 4-1BB x 5T4 ADAPTIR<sup>™</sup> Bispecific Antibody

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### Introduction

- 4-1BB (CD137) is an activation-induced costimulatory immune receptor expressed on tumor-infiltrating T cells and NK cells
- Stimulation of 4-1BB leads to enhanced proliferation, increased survival, intensified cytolytic activity, and induced IFN- $\gamma$  production of T and NK cells
- 4-1BB-targeting immunotherapies have shown promising anti-tumor effects clinically but one monospecific 4-1BB agonist has induced dose-limiting hepatic toxicities

ALG.APV-527 augments CD8<sup>+</sup> T cell proliferation and IFN-γ production in the presence of 5T4+ cells





ALG.APV-527 augments CD8<sup>+</sup>T cell proliferation. Primary PBMC were stimulated with α-CD3 Ab in

solution and serial dilutions of ALG.APV-527 in the presence of 5T4-expressing CHO-K1 cells. (A)

Representation of the proliferation of CD8<sup>+</sup> T cells. The number of CD8<sup>+</sup> T cells (**B**) and CD4<sup>+</sup> T cells (**C**)

ALG.APV-527 enhances CD8+ T cells' ability

to secrete IFN-γ. (D) Primary CD8<sup>+</sup> T cells and

anti-CD3 abs coated on beads at a 1:1 T cell/

beads ratio were incubated with ALG.APV-527 in

the presence of Mitomycin C treated HCT116

tumor cells expressing endogenous levels of 5T4

(6.2 x 10<sup>4</sup>/ 5T4 receptors/ cell). IFN- $\gamma$  production

was measured in the supernatant after 72h

using ELISA. Normalized IFN- $\gamma$  levels (mean of

12 donors) and nonlinear curve fit log (agonist)

vs. normalized response variable slope) is

plotted here using GraphPad Prism.

were calculated at 96 hours via flow cytometry. Three healthy donors are represented here.

EC50 = 0.1 (0.14-0.21) nM

ALG.APV-527 conc (nM)

20



• 5T4 is a tumor-associated antigen expressed in patients in a variety of malignancies, including NSCLC, head and neck, mesothelioma, renal, pancreas, bladder, breast, colorectal, gastric, ovarian and cervical cancers

### About ALG.APV-527

- ALG.APV-527 is a bispecific therapeutic in the ADAPTIR<sup>™</sup> format containing two sets of binding domains, scFv, targeting 5T4 and 4-1BB which are linked to a silent Ig Fc domain, providing an antibody-like in vivo half-life
- The scFv originate from the Alligator Gold<sup>®</sup> human scFv library (Alligator Bioscience)
- Each scFv has then been optimized and developed for use in the bispecific ADAPTIR<sup>™</sup> format (Aptevo Therapeutics)
- ALG.APV-527 features target-driven T cell activation, optimized stability, good manufacturing properties with potential for improved risk-benefit in humans than other monospecific 4-1BB antibodies
- ALG.APV-527 is cross-reactive to 4-1BB and 5T4 of cynomolgus monkey. It binds to human and cynomolgus 5T4 and 4-1BB expressing cells and enhances activation of CD3-stimulated human and cynomolgus T cells
- Demonstrated an extended antibody-like serum half-life of 9 days

### **ALG.APV-527** Mode of Action

# Anti-4-1BB scFv **Modified**



Anti-5T4 scFv

ALG.APV-527 enhances NK cell effector function in presence of 5T4+ cells



ALG.APV-527 promotes the proliferation and upregulation of NKD2G on NK cells. 10<sup>3</sup> 10<sup>4</sup> 1 Cell Trace (A-C) Primary NK cells were stimulated with IL-2 and serial dilutions of ALG.APV-527 in the presence of 5T4-expressing CHO-K1 cells. (A) Representation of the proliferation of NK cells. (B) The number of CD335<sup>+</sup> NK cells were assessed in 2 healthy samples on day 6 by flow cytometry. (C) Representative expression of NKG2D on treated NK cells



5T4 expression in human normal and tumor tissue. Formalin-fixed paraffin-embedded tissue microarrays (TMAs) were acquired from US Biomax Inc, and stained for 5T4 detection (clone MAB4975, R&D Systems). 5T4 expression was detected in tumors from NSCLC, Head and Neck, mesothelioma, pancreatic, bladder, renal and ovarian cancer with variable frequency and intensity, as exemplified above. Occasional membranous staining of 5T4 was detected in specific cell populations in normal tissue, specifically on the smooth myocytes of the esophagus, osteocytes in bone marrow and epithelial cells in the adenohypophysis. There was no 5T4 staining on cells from any major organ system such as cardiovascular, respiratory or hepatic systems.

### ALG.APV-527 localizes to 5T4<sup>+</sup> tumors



#### 5T4-dependent localization of ALG.APV-527 in a B16 twin tumor model

Day 0: each mouse received one 5T4 negative and one 5T4 positive B16 tumor injected subcutaneously (SQ, 1x10<sup>5</sup> cells in 100 µL) at each side of the hind flank/back. Intraperitoneal (IP) treatment of ALG.APV-527 (100 µg) was given on days 6 and 13 and mice were sacrificed on day 14 (24 h after the final treatment). Tumors were collected and the levels of hIgG positive cells were assessed either by IHC or flow cytometry using an antibody detecting human IgG. IHC of ALG.APV-527 binding to either **A** 5T4-negative tumors or **B** 5T4-positive tumors. **C** Flow cytometry of ALG.APV-527 binding to dissociated 514-positive and -negative tumor cells (percentage of hlgG+ cells out of live CD45- cells)





ALG.APV-527 enhances the expression of cytolytic markers CD25, IFN-γ and granzyme B. (D-F) Primary NK cell assays and Mitomycin C-treated HCT116 tumor cells expressing endogenous levels of 5T4 (6.2 x 10<sup>4</sup>/ 5T4 receptors/ cell). IL-2 pre-stimulated NK cells were incubated with a dilution of ALG.APV-527 and surface expression of (D) CD25 was measured via flow cytometry. The secretion of (E) IFN- $\gamma$  and (F) Granzyme B was measured in the supernatant after 72h of culture using ELISA. ALG.APV-527 (+ IL-2 pre-stim) ALG.APV-527 (no IL-2 pre-stim) Isotype control

ALG.APV-527 conc (nM)

### ALG.APV-527 inhibits tumor growth of a human HCT116 colon carcinoma



### Summary and Conclusions

0.01

> ALG.APV-527:

- Augments CD8<sup>+</sup> T cell proliferation and IFN-  $\gamma$  production but only in the presence of 5T4<sup>+</sup> expressing cells
- Enhances the cytotoxic profile of NK cells via an increase in CD25<sup>high</sup> & NKD2G expression and the production of IFN- $\gamma$  and Granzyme B
- > 5T4 is expressed in a wide range of tumor indications, but not in any vital organs such as the heart or the liver

>The  $\alpha$ -4-1BB x  $\alpha$ -5T4 ADAPTIR molecule, ALG.APV-527, has the potential to be a unique  $\alpha$ -



