**Potential Tumor-Directed T cell Activation and Tumor Inhibition Induced by 4-1BB x 5T4 ADAPTIR™ 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-γ 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.
- 5T4 is a tumor-associated antigen expressed in patients of 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™ format containing two sets of binding domains, scFv, targeting 5T4 and 4-1BB which are linked to a silent IgFc domain, providing an antibody-like in vivo half-life.
- The scFv originate from the Alligator Gold® human scFv library (Alligator Bioscience).
- Each scFv has then been optimized and developed for use in the bispecific ADAPTIR™ 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**

**ALG.APV-527**

- ALG.APV-527 promotes the proliferation and upregulation of NK2D on NK cells. (A-C) Primary NK cells were cultured with IL-2 and serial dilutions of ALG.APV-527
- ALG.APV-527 enhances the expression of cytolytic markers CD52, IFN-γ and granzyme B. (D-F) Primary NK cell assay and Malignant C-treated HCT116 tumor expressing levels of 5T4 (6.2 x 10⁹ / 5T4 receptors / cell) IFNγ production was measured in the supernatant after 72h using ELISA. Normalized IFNγ levels were plotted here using GraphPad Prism.

**Summary and Conclusions**

- **ALG.APV-527:**
  - Augments CD8+ T cell proliferation and IFN-γ production but only in the presence of 5T4+ expressing cells.
  - Enhances the cytotoxic profile of NK cells via an increase in CD25high & NKDG2 expression and the production of IFN-γ and Granzyme B.
  - Effectively localizes to 5T4+ tumor in vivo.
  - Inhibits colon carcinoma HCT116 tumor growth in a xenograft murine model.

- ST4 is expressed in a wide range of tumor indications, but not in any vital organs such as the heart or the liver.

- The α-4-1BB x α-ST4 ADAPTIR molecule, ALG.APV-527, has the potential to be a unique α-cancer therapeutic agent with an improved safety profile for the treatment of numerous 5T4-expressing solid tumors with high unmet medical need.