**Potent Tumor-Directed T cell Activation and Tumor Inhibition Induced by a 4-1BB x 5T4 ADAPTOR™ 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 effect, clinically however, a monospecific 4-1BB agonist induced dose-limiting hepatic toxicities.
- 5T4 is a tumor-associated antigen expressed in a variety of malignancies, including NSCLC, head and neck, mesothelioma, renal, pancreatic, bladder, breast, colorectal, gastric, ovarian and cervical cancers.

**About ALG-APV-527**

- ALG-APV-527 is an ADAPTOR™ bispecific therapeutic containing two sets of scFv binding domains targeting 5T4 and 4-1BB which are linked to an effector-null IgFc domain, providing an antibody-like in vivo half-life.
- The scFvs originate from the Alligator Golt® human scFv library (Alligator Bioscience).
- Each scFv has been optimized for use in the bispecific ADAPTOR™ format (Aptevo Therapeutics).
- ALG-APV-527 features target-driven T cell stimulation, optimized stability, good manufacturing properties with potential for better risk/benefit in humans than other monospecific 4-1BB antibodies.
- ALG-APV-527 is cross-reactive to 4-1BB and 5T4 from cynomolgus monkey. It enhances stimulation of CD3-activated human and cynomolgus T cells in vitro.

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

- ALG-APV-527 directs the stimulation of 5T4+ T and NK cells by 5T4+ tumors and is designed to reconcile the toxicity concerns with 4-1BB therapies.
- IL-2 upregulates 4-1BB expression on NK cells. Titration of ALG-APV-527 in the presence of 5T4-expressing tumor cells enhances secretion of cytokytic molecules such as IFN-γ and granzyme B (GzB) and promotes proliferation.
- Stimulation of T cells with anti-CD3 upregulates the expression of 4-1BB. Addition of ALG-APV-527 and 5T4+ tumors augments primarily CD8+ T cells proliferation and secretion of IFN-γ.

**Summary and Conclusions**

- **Augments CD8+ T cell proliferation and IFN-γ production in the presence of 5T4+ expressing cells**
- **Enhances the cytotoxic profile of NK cells via production of IFN-γ and Granzyme B**
- **Inhibits growth of a bladder cancer expressing human 5T4 in a human 4-1BB knock-in murine model**
- **Displays antibody-like half-life in NHP and is well tolerated with repeated dosing**

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

ALG-APV-527 has a favorable non-clinical safety profile with no indications of systemic activation or liver toxicity in NHP.

**The safety of ALG-APV-527 was evaluated in a GLP toxicity study performed in cynomolgus monkeys. 4 repeated-dose groups were included in the study, one as vehicle control. ALG-APV-527 was given by intravenous infusion (over 1 hr) into the left ear vein. Samples were collected throughout the study for clinical pathology, PK, ADA, and immunophenotyping by flow cytometry. Samples were also collected at necropsy for histology and histopathology.**

**ALG-APV-527 induces rejection of established tumors and promotes anti-tumor memory response**

**ALG-APV-527 has a favorable safety profile in a non-human primate GLP toxicity study**

**ALG-APV-527 has an antibody-like half-life in non-human primates (NHP)**