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

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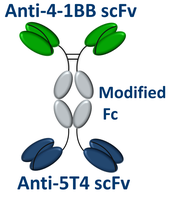
Introduction

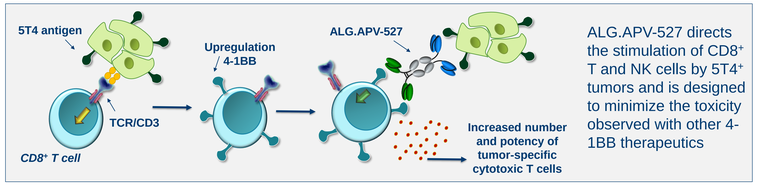
- 4-1BB (CD137) is an activation-induced costimulatory immune receptor expressed on tumor-infiltrating T cells and NK cells
- Stimulation of 4-1BB leads to enhanced proliferation, increased survival, intensified cytolytic activity, and induced IFN-γ production of T and NK cells
- 4-1BB-targeting immunotherapies have shown promising anti-tumor effects clinically 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, pancreas, bladder, breast, colorectal, gastric, ovarian and cervical cancers

About ALG.APV-527

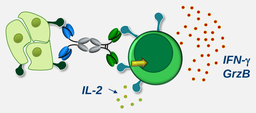
- ALG.APV-527 is an ADAPTIR™ bispecific therapeutic containing two sets of scFv binding domains targeting 5T4 and 4-1BB, linked to an effector-null Ig Fc domain
- The scFvs originate from the Alligator Gold® human scFv library (Alligator Bioscience) and have been optimized for use in the bispecific ADAPTIR™ 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 has an antibody-like *in vivo* half-life



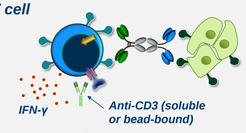
ALG.APV-527 Mode of Action



ALG.APV-527 directs the stimulation of CD8⁺ T and NK cells by 5T4⁺ tumors and is designed to minimize the toxicity observed with other 4-1BB therapeutics

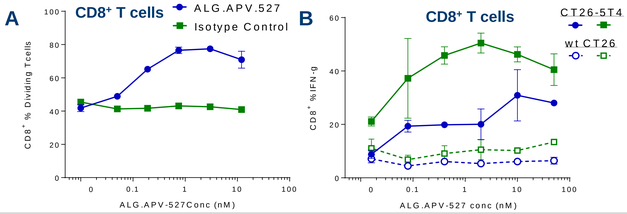


IL-2 upregulates 4-1BB expression on NK cells. Titration of ALG.APV-527 in the presence of 5T4-expressing tumor cells enhances secretion of cytolytic molecules such as IFN-γ and Granzyme B (GrzB) and promotes proliferation.

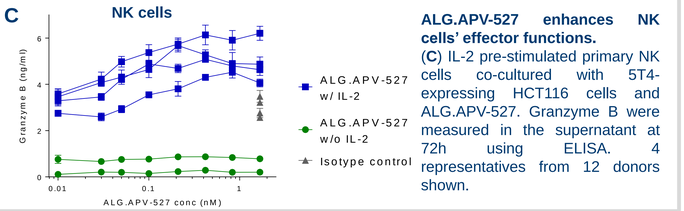


Stimulation of T cells with anti-CD3 induces the upregulation of 4-1BB. Addition of ALG.APV-527 and 5T4⁺ tumors augments primarily CD8⁺ T cells' proliferation and secretion of IFN-γ.

Figure 1. ALG.APV-527 augments CD8⁺ T cells and NK cells

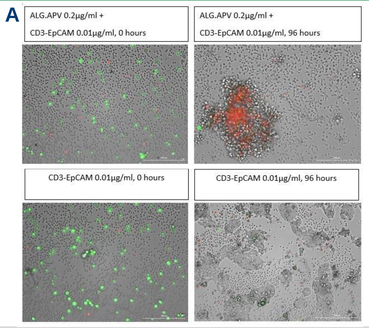


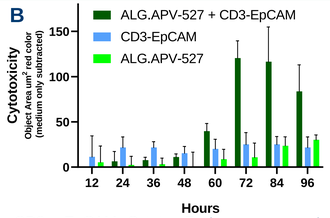
ALG.APV-527 augments T cell proliferation. PBMC were stimulated with anti-CD3 Ab in solution and serial dilutions of ALG.APV-527 in the presence of human 5T4-expressing CT26 cells. Representation of the percentage of proliferating.
(**A**) The percentage of proliferating CD8⁺ T cells were calculated on day 5 via flow cytometry. One representative donor from 4 donors.
(**B**) CD8⁺ T cells producing IFN-γ were analyzed at 48 hours via flow cytometry following treatment with Brefeldin A. Two representatives from 4 donors shown.



ALG.APV-527 enhances NK cells' effector functions.
(**C**) IL-2 pre-stimulated primary NK cells co-cultured with 5T4-expressing HCT116 cells and ALG.APV-527. Granzyme B were measured in the supernatant at 72h using ELISA. 4 representatives from 12 donors shown.

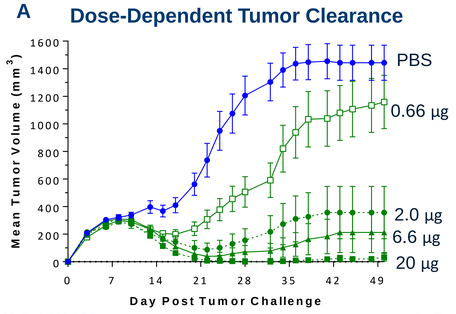
Figure 2. ALG.APV-527 promotes increased *in vitro* tumor lysis

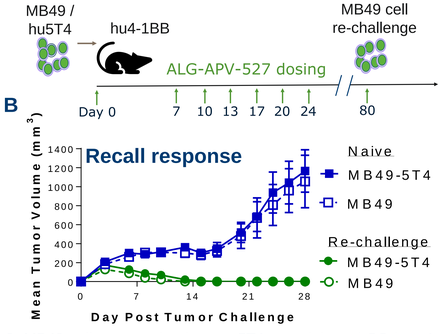




ALG.APV-527 promoted increased tumor lysis in a CD8⁺ T cell cytotoxic killing assay.
(**A**) Representative live cell images of cytotoxic primary CD8⁺ T cells co-cultured with HT29 human tumor cells expressing endogenous levels of 5T4 in combination with an antibody targeting CD3 x EpCAM. Cytotox Red was measured at 0 and 96 hours (Cytation 5). Sub-optimal concentrations of CD3-EpCAM were used to mimic TCR/MHC: peptide signaling, essential for CD8⁺ T cell activation and 4-1BB upregulation. Green viability stain faded as cells divided. (**B**) Cytotoxicity was quantified over time by measuring the total red color object area (background with medium only was subtracted).

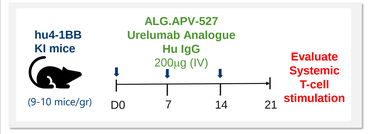
Figure 3. ALG.APV-527 induces rejection of established tumors and promotes anti-tumor memory response

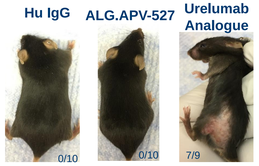


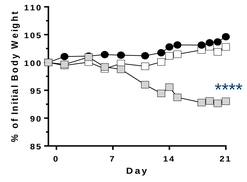


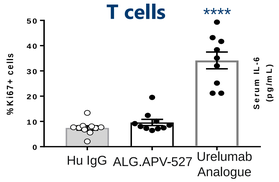
ALG.APV-527 induces the generation of memory cells. (**A**) Day 0, MB49 cells expressing human 5T4 were injected SQ into 4-1BB knock-in mice. Starting on day 7, treatments of ALG.APV-527 were administered IP twice weekly until day 24, (8 mice/treatment). (**B**) Surviving mice that had cleared their primary tumor were re-challenged with MB49 tumor cells on day 80. Naïve mice were used as controls. No further therapy was given.

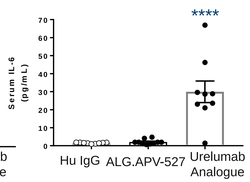
Figure 4. ALG.APV-527 has a favorable safety profile in a murine study

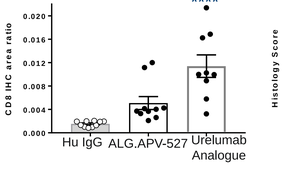


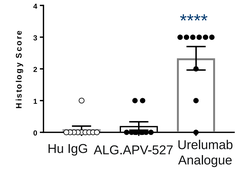












ALG.APV-527 has a favorable safety profile. Surviving human 4-1BB KI mice previously treated with therapy were later treated with 200 µg (IV). Mice were sacrificed on D21. (A) Ulcerative dermatitis severity was scored by a blinded observer at D20 using the published scoring system (Hampton J Am Assoc Lab Anim Sci 2012). (B) Body weight was monitored. (C) Spleens were stained for Ki67+ CD8⁺ T cells. (D) Serum cytokines were collected on D21. (E-G) Livers were processed for H&E and IHC for CD8+ infiltrate expression. IHC sections was quantified using Visiopharm software: results are represented as the Ratio of CD8 (µm²)/Total area scanned (µm²). **** P=<0.0001

Summary and Conclusions

ALG.APV-527:

- Augments CD8⁺ T cell proliferation & IFN-γ production & the cytotoxic profile of NK cells in the presence of 5T4⁺ tumor cells
- Inhibits growth of 5T4⁺ tumor cells in a human 4-1BB KI murine model and induce tumor-specific memory cells
- Induced cytotoxic killing of 5T4-expressing tumor cells when CD8⁺ T cells were stimulated with a sub-optimal concentration of CD3-EpCAM showing that the ALG.APV-527 induced tumor cell killing is dependent on CD3/TCR activation of T cells.

- ALG.APV-527 has a favorable non-clinical safety profile with no indications of systemic activation or liver toxicity in NHP or murine models
- The anti-4-1BB x anti-5T4 targeting ADAPTIR 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 unmet medical need
- CTA documents are prepared for filing of a phase 1 clinical trial