Dual-targeting of 4-1BB and OX40 with an ADAPTIR™ Bispecific Antibody with Potential To Enhance Anti-Tumor Responses to Solid Tumors

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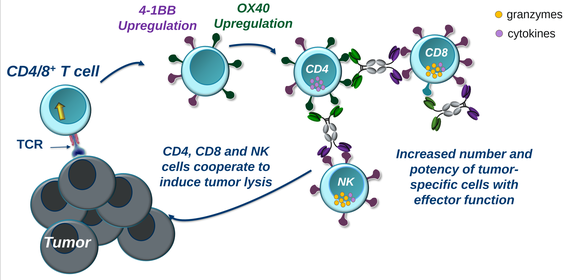
APVO603 (α-4-1BB x α-OX40) Designed for Treatment of Multiple Tumor Targets

THERAPEUTIC CANDIDATE	• Next generation ADAPTIR (α4-1BB x αOX40) T-Cell Engager • Mutated IgG1 Fc; No ADCC, CDC; retains FcRn binding
FUNCTION/ MOA	• Targets co-stimulatory receptors OX40 and 4-1BB • Enhances effector function and survival of pre-existing anti-tumor T cells and NK cells • Requires engagement of both receptors to induce downstream signaling • Activity is not dependent on direct engagement of a tumor antigen • 4-1BB and OX40 are expressed on activated lymphocytes, with limited expression on peripheral lymphocytes therefore potential for drug 'sink' is reduced
INDICATIONS	• Multiple inflamed solid tumor types (such as NSCLC & RCC)
HALF-LIFE	• 5.5 days in mice; non-GLP NHP PK study to start in 2020; fully cross-reactive with cynomolgus macaque
DEVELOPMENT STAGE	• Preclinical; Pre-IND-enabling studies underway • Lead candidate identified; CMC activities in progress
PARTNERSHIP STATUS	• Wholly-owned by Aptevo Therapeutics

APVO603 Mode of Action



Potential Key Advantages

- Enhances pre-existing anti-tumor responses
- Enhances effector lymphocyte populations: CD4, CD8 & NK cells
- Potential to reduce toxicities observed for competitor 4-1BB monospecific antibodies

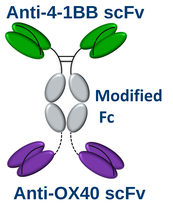


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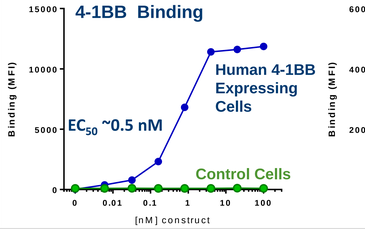
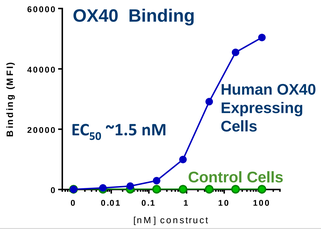
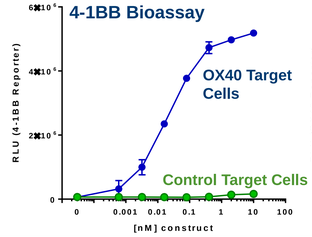
Fig. 1 APVO603 Binds 4-1BB And OX40 At Low nM Concentrations

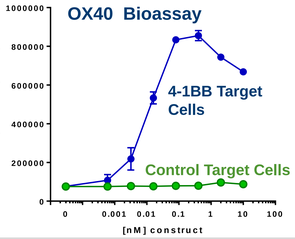




Binding of human 4-1BB- or human OX40-expressing cells. Serial dilutions of APVO603 ADAPTIR was incubated with transfected target cells and subsequently labelled with a fluorescently-conjugated goat-α-human Fc secondary antibody. The y-axis displays the mean fluorescence intensity units (MFI). Similar binding was observed to cynomolgus OX40- and 4-1BB-expressing transfected cells.

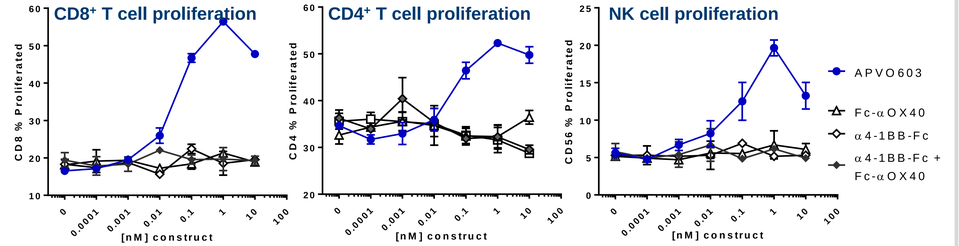
Fig. 2 Both 4-1BB and OX40 Receptors are Required to Induce Activity





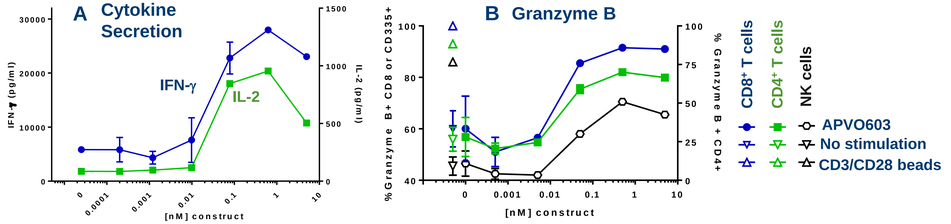
Functionality of APVO603 to induce NFκB signaling. For the 4-1BB bioassay, APVO603 was incubated with OX40 target cells or CHOK1SV control cells and an OX40 NFκB reporter cell line. Alternatively, in the OX40 Bioassay, serially diluted construct was incubated with 4-1BB-expressing target cells or a CHOK1SV control cell line along with a 4-1BB NFκB reporter cell line. The assay was incubated for 5 hours, followed with the addition of Bio-Glo luciferase reagent. The y-axis displays the relative light units (RLU).

Fig. 3 APVO603 Promotes Increased *In Vitro* Cell Proliferation



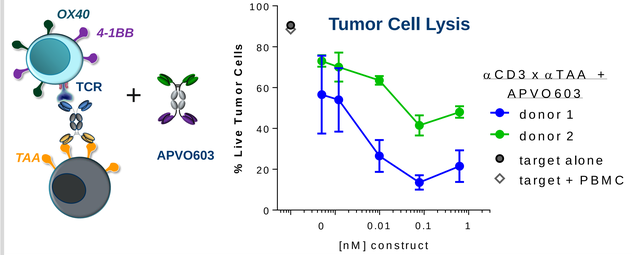
Expansion of *in vitro* PBMC cultures incubated with a dose titration of APVO603. Enriched PBMC were labeled with CellTrace Violet, activated with αCD3 antibody and enhanced with a dilution of therapeutic construct. At 96 hours, cells were stained via FACS staining and analyzed for cell proliferation based on CellTrace Violet dilution.

Fig. 4 APVO603 Enhances Cytokine Secretion and Granzyme B Expression



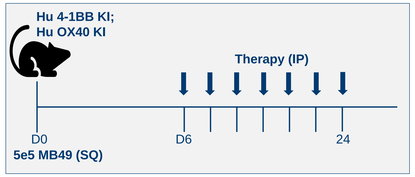
APVO603 enhances the cytolytic potential of lymphocytes. *In vitro* PBMC cultures activated with αCD3 and incubated with APVO603. **A**. At 48 hours, supernatants were analyzed for the levels of cytokines via multiplex-based assay (Milliplex). **B.** 72 hours, cells were harvested and analyzed for intracellular expression of granzyme B and surface markers by flow cytometry.

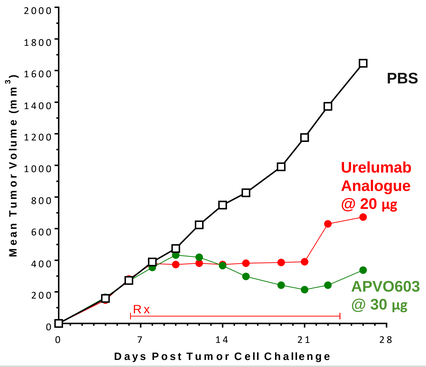
Fig. 5 APVO603 Promotes *In Vitro* Tumor Lysis

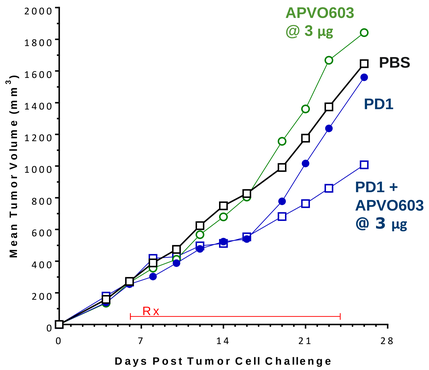


APVO603 enhances the ability of PBMC to kill tumor target cells *in vitro* in a dose-dependent manner with the addition of APVO603 ADAPTIR bispecific protein. Enriched PBMC were cultured with CD3 x Tumor Associated Antigen (TAA; a T cell engager), TAA+ target tumor cells and serial dilutions of APVO603. At 72 hours, cells were harvested, and tumor cells were analyzed for their viability by flow.

Fig. 6 ALG.APV-527 Induces Rejection of Established Tumors and Promotes Anti-tumor Memory Response







APVO603 significantly reduces tumor burden *in vivo*. APVO603 is not murine cross-reactive therefore human 4-1BB and human OX40 double knock-in mice used and were implanted with MB49. Therapy began on day 6. Mean tumor volume for each group is plotted ± SEM. Compared to PBS, only APVO603 at 30 μg was considered significant (p=0.0232). Average of 8 mice per group.

Summary

- APVO603 is a novel ADAPTIR bispecific with a unique mechanism of action that may boost natural anti-tumor responses by activating two different co-stimulatory receptors
- APVO603 has potential application in multiple solid tumor indications with potential to reinvigorate immune responses and enhance tumor rejection
- Preclinical studies demonstrate synergistic activation of CD4⁺ and CD8⁺ T cell and NK cell activation in addition to enhanced tumor cell lysis following APVO603 treatment
- A lead candidate has been identified; CMC activities have been initiated; IND-enabling studies are underway; Non-GLP cynomolgus PK/toxicology to start in 2020
- Current Preclinical and CMC activities support advancing APVO603 towards clinical development for the treatment of solid tumors