APVO603: A dual 4-1BB and OX40 bispecific approach utilizing ADAPTIR™ platform technology designed to deliver a conditional T cell / NK response against solid tumors

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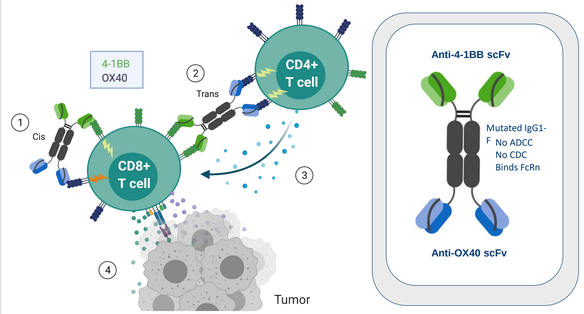
APVO603 (α4-1BB x αOX40), a Dual Costimulatory Molecule Designed to Treat Multiple Solid Tumors

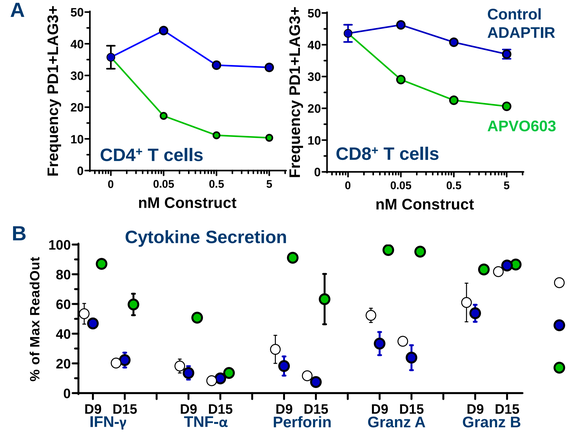
Therapeutic Candidate	• Next generation ADAPTIR for T-cell and NK-cell costimulation • Mutated IgG1 Fc; No ADCC or CDC; retains FcRn binding
Mechanism of Action Benefits	• Activity non-dependent on direct engagement of a tumor antigen • Potential to enhance the tumor microenvironment (TME) responses: Reduction/reversal of suppressive environment; Limitation or reversal of T cell exhaustion • Designed for enhanced effector function and survival of pre-existing TIL and NK cells
Potential Safety Benefits	• Requires engagement of both 4-1BB and OX40 in cis or trans to induce downstream signaling (tumor-dependent response) • 4-1BB and OX40 are expressed on activated lymphocytes and relatively few peripheral lymphocytes. Increased potential to target tumor infiltrating lymphocytes (TIL)
Indications	• Multiple inflamed solid tumor types with resident tumor infiltrating T cells (such as NSCLC, RCC) • Potential to combine with checkpoint inhibitors
Half-life	• 5.5 days in mice; up to 4.8 days in NHP • Fully cross-reactive with cynomolgus macaque
Development Stage	• Preclinical; IND-enabling studies underway • CMC activities in progress to support IND filing

APVO603 is Designed to Potentiate Memory Generation & Tumor Lysis in Recently Activated TIL



1. Cis engagement to maximize unique 4-1BB and OX40 combined co-stimulatory benefit
2. Trans engagement to promote cell communication and drive multi-cellular responses
3. APVO603 stimulation designed to enhance CD8 and NK control of tumors
   - Enhance T / NK tumor cytolytic response
   - Promote a balanced Teff:Treg TME environment
   - Enhance protective memory
   - Restore functionality to exhausted compartment
4. Opportunity for direct solid tumor and heme intervention and in combination with adoptive transfer, TIL, CAR-T/NK, checkpoint, addition immune modulators

Fig.1 APVO603 Reduces T Cell Exhaustion Markers & Prolongs Cytokine Secretion

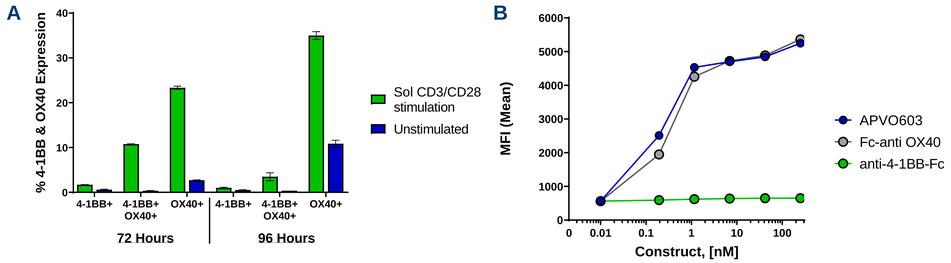


In vitro human T cell cultures were activated with αCD3/αCD28 on days 0, 2, 6, 9, 12 and 15 along with a titration of APVO603 or a control ADAPTIR and evaluated for surface markers of exhaustion (**A**) or cytokine secretion (**B**). **APVO603 reduces surface marker expression associated with T cell exhaustion.** For surface marker expression, activated CD4⁺ or CD8⁺ T cells were analyzed on day 17 for co-expression of LAG3 and PD-1 by flow cytometry.

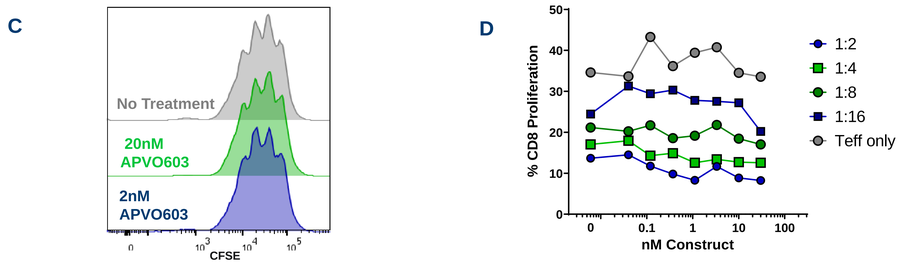
APVO603 costimulation treatment augments cytokine secretion in restimulated cells. Sups from D9 or D15 reactivated cells were analyzed for cytokine secretion via multiplex- based assay (Milliplex). 0.5 nM of control or APVO603 were compared to untreated. All wells were treated with αCD3/ αCD28 treatment.

These data suggest that the costimulatory effects of APVO603 may diminish the detrimental & exhaustive effects of repeated stimulation within the TME.

Fig. 2 APVO603 has Limited Impact on iTreg



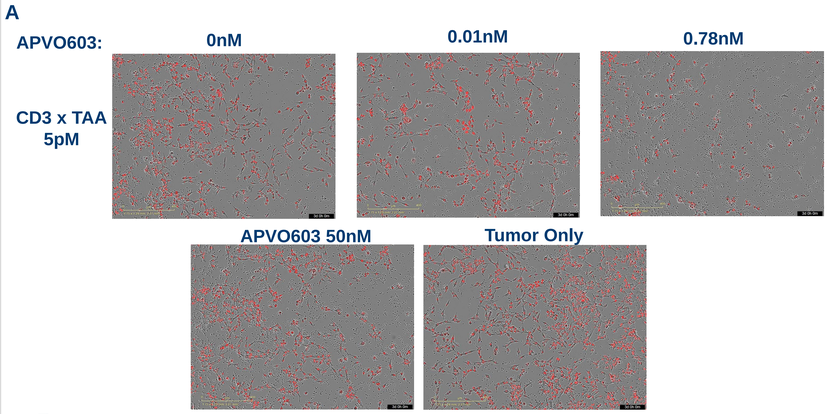
iTreg cells preferentially bind the anti-OX40 scFv end of APVO603. (A) Inducible Tregs (iTreg, Cellero) were stimulated with Immunocult CD3/CD28 T cell activator (25uL/ml, StemCell) and examined for the expression of 4-1BB and OX40. Using commercial mAb, the expression of OX40 is much higher than 4-1BB. (**B**) APVO603 or the scFv binding domains from APVO603 were titrated on activated iTreg to examine binding and detected using a fluroescenated anti-human Fc Ab.



iTreg proliferation remained unaltered in the presence of APVO603. (C) iTreg were cultured with or without titrated APVO603, ImmunoCult CD3/CD28 activator and IL-2. CFSE dilution was assessed at 96 hours. Representative histogram overlays of iTreg cells shown.

APVO603 has minimal impact on iTreg suppression of CD8⁺ T cell proliferation *in vitro*. (D) iTreg were cultured with titrated APVO603, ImmunoCult CD3/CD28 activator , IL-2 and matched donor CFSE-labeled CD8⁺ T cells. At 96 hours, CFSE dilution as assessed with various CD8:iTreg cell ratios.

Fig. 3 APVO603 Augments *In Vitro* Cell Killing



APVO603 promotes increased tumor lysis in an *in vitro* PBMC cytotoxicity assay. Primary T cells were co-cultured with human tumor cells with a known tumor associated antigen (TAA) at a 10:1 ratio. Purified T cells were treated with a titration of APVO603 in combination with a T-cell engaging bispecific antibody targeting the TAA (CD3 x TAA), used to mimic TCR/MHC: peptide signaling that is essential for T cell activation and co-stimulatory receptor expression. Incucyte images were taken over time of NucLight Orange (Sartorius) stably transfected tumor cells cultured with purified T cells, titrated APVO603 and CD3 x TAA. (**A**) Representative images of tumor plus T cells at 72 hours with APVO603 and CD3 x TAA (5pM, suboptimal stimulatory dose). (**B**) Cytotoxicity was quantified over time by measuring the total orange object area of tumor cells with test reagents and comparing them to no treatment and staurosporine (positive control, 0.5ug/ml, Fisher). APVO603-treated T cells do not induce tumor cell killing.

Summary and Potential Advantages of APVO603

- APVO603 may reduce or reverse negative effects of T-cell exhaustion and suppressive immune responses by augmenting cytokine production and reducing markers of T cell exhaustion following repeat stimulation *in vitro*
- APVO603 therapy induces a dose-dependent *in vivo* antitumor responses and significantly increases the survival in MB49-inoculated mice
- APVO603 was well tolerated in NHP with a favorable safety profile (up to 50 mg/kg) without liver toxicity
- APVO603 has limited impact on iTreg as demonstrated by proliferation or its suppression on CD8⁺ T cells.

	4-1BB mAb	OX40 mAb	APVO603 Bispecific
Safety	Liver toxicity (4-1BB superagonist)	Good safety profile in NHP and Humans	Bispecific targeting requires dual 4-1BB and OX40 expression and limits on target toxicity
Efficacy	Potent agonist, limited by therapeutic index	Lack of clinical response as single agent	Bispecific targeting allows for cis / trans engagement of T cells for enhance potency

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