1060: Leveraging multispecific antibody-like molecules to localize CD40 agonism to the tumor to overcome the immunosuppressive microenvironment and potentiate anti-tumor immunity

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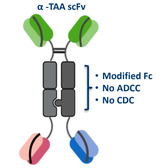
Aptevo™ Therapeutics

Immunotherapies in Solid Tumors Have Hit a Wall - Interest in Targeting CD40

- The **immunosuppressive tumor micro-environment (TME)** remains a major barrier for solid tumor immunotherapy and therefore new therapeutics are needed. T-cell-focused immunotherapies for solid tumors have hit a wall, whether it be immune checkpoint blockade combinations, CAR-Ts or TCEs despite success preclinically. While each modality has unique issues, the overarching challenge is the immunosuppressive nature of the tumor microenvironment
- There is consensus in the field that so-called “cold” tumors need to be made “hot” and that engagement of innate immunity is needed to realize the full potential of anti-tumor immunity. However, what’s needed in the tumor milieu to optimally engage innate immunity is typically not well-tolerated systemically. E.g., CD40 agonism has been a coveted target hampered by systemic toxicity
- The intended design of Aptevo’s CD40-targeting molecules is to reprogram the TME while simultaneously engaging T cells. In the case of APVO451 and APVO452, CD40 aims to polarize macrophages and license dendritic cells while simultaneously activating T cells via CD3. For APVO711, the PD-L1 arm is utilized as both a checkpoint and a tumor targeting arm, to simultaneously enhance tumor localization and reinvigorate T cells.

About APVO451 & APVO452

- **APVO451 (nectin-4) and APVO452 (PSMA) are novel ADAPTIR-FLEX™ trispecific therapeutics** comprising three scFv domains that target a tumor-associated antigen (TAA), CD40, and CD3 to simultaneously enhance T cell killing and overcome TME-mediated immunosuppression.
- Each scFv domain is humanized and engineered to reduce proteolytic cleavage and limit post-translational modifications. They are linked to an effector-null IgG1 Fc to provide an antibody-like *in vivo* half-life. All binding domains show cross-reactivity with both human and non-human primate (NHP) targets.



- **Nectin-4** (APVO451) is overexpressed in multiple cancers, including bladder, head & neck, lung, breast, colon, and ovarian, making it an attractive TAA.
- **PSMA** (APVO452) is primarily targeted in prostate cancer and has long track record of clinical validation, but is also overexpressed in solid tumors including renal, pancreatic and thyroid.
- **CD3** engagement drives targeted T cell cytotoxicity and previously shown efficacy in TCE. The CD3 domain used in APVO451 has demonstrated safety and efficacy as mipletamig in the RAINIER AML clinical trial, with limited cytokine release syndrome (CRS) and 87% clinical benefit (CBR) rate.
- **CD40** is primarily expressed on antigen-presenting cells (APCs), where it promotes IL-12 production and upregulates CD80/CD86-key drivers of T cell activation, differentiation, and effector function.

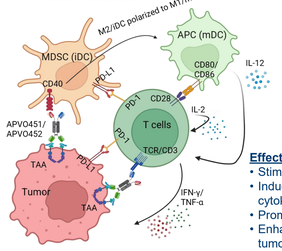
APVO451 & APVO452: Mode of Action

TME-mediated immunosupression

Causes:
- PD-L1 tumor expression
- MDSCs; TAMs

Results:
- Abnormal differention & functionality of myeloid cells
- Exhausted T cells
- Tumor metastases

Effects on MDSCs and APCs
- Induces the upregulation of activation-induced co-stimulatory molecules
- Promotes polarization and cytokine production



Effects on Effector T cells
- Stimulates activation
- Induces inflammatory cytokine production
- Promotes expansion
- Enhances T cell-mediated tumor killing

As designed, APVO452 activity is PSMA-dependent; Unable to induce signaling through CD3-CD40 crosslinking

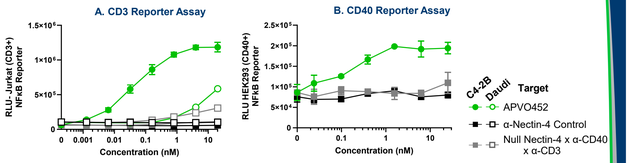


Figure 4. Examining downstream signaling activity in NFκB luciferase reporter assays. (**A**) Jurkat NFκB reporter cells (CD3+) or (**B**) HEK293 NFκB reporter cells (CD40+) were cocultured with target cells and serially diluted APVO452 or control proteins. Crosslinking with PSMA-expressing C4-2B target cells via CD3 stimulation or CD40 stimulation (Solid shapes). Daudi (CD40+) are shown to demonstrate alternative crosslinking does not induce luciferase activity (Open shapes).

Trispecific APVO452 is able to ablate tumor better than a bispecific in an immunosuppressive TME

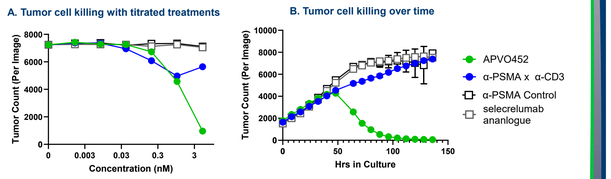
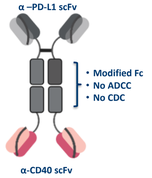


Figure 5. Redirected T cell cytotoxicity targeting PSMA-expressing C4-2B target cells by trispecific and bispecific constructs over time. C4-2B target cell lysis was induced by anti-PSMA x anti-CD40 x anti-CD3ε trispecific constructs at 80 hours (**A**) and at 5nM over 5 days (**B**). The C4-2B target cells were transduced to express NLO and PD-L1 and seeded one day prior to the assay setup. Titrated test proteins or controls, immature DC, and PBL were then added culture on day 0 of the assay. The assay plate was imaged every 8 hours in an Incucyte reader (Sartorius) over the course of 5 days.

About APVO711

- **APVO711 is a novel ADAPTIR™ bispecific therapeutic** comprising scFv domains that target the checkpoint molecule PD-L1 and the innate immune stimulatory receptor CD40 to simultaneously enhance APC polarization and overcome TME-mediated immunosuppression. APVO711 targets PD-L1 which is more broadly expressed in tumors than other bona fide tumor associated antigens, thus allowing for broad applicability.



- Each scFv domain is humanized and engineered to reduce proteolytic cleavage and limit post-translational modifications. They are linked to an effector-null IgG1 Fc to provide an antibody-like *in vivo* half-life. All binding domains show cross-reactivity with both human and non-human primate (NHP) targets.
- In addition to **PD-L1** blocking the exhaustive effects of the PD-1/PD-L1 pathway on T cells, the PD-L1 binding domain can also be utilized as a tumor-targeting arm to enrich APVO711 into the tumor microenvironment.
- **CD40** is primarily expressed on antigen-presenting cells (APCs), where it promotes IL-12 production and upregulates CD80/CD86-key drivers of T cell activation, differentiation, and effector function. Because APVO711 contains an Fc that does not interact with Fc gamma receptors, it has the potential to be less toxic than monospecific CD40 antibodies, while still enhancing antigen presentation, cytokine secretion etc., which in turn enhances NK and T cell cytotoxicity.

As designed, APVO711 CD40 activity is PD-L1 crosslinking-dependent; PD-L1/PD-1 blockade is

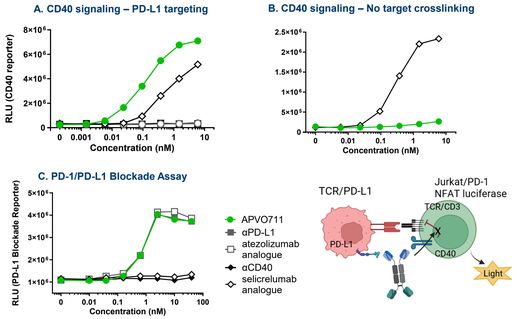


Figure 6. Examining downstream signaling activity in luciferase reporter assays to assess proper functionality/safety. To evaluate CD40 signaling, serially diluted APVO711 or control proteins were added to CD40+/NFkB/HEK293 reporter cells (BPS) cocultured with (**A**) PD-L1/CHO or (**B**) parental CHOK1sv target cells. (**C**) TCR activator/PD-L1 CHO cells were cocultured with PD-1/NFAT/Jurkat reporter cells (BPS) and serially diluted APVO711 or control proteins. (**A**) Crosslinking with PD-L1-expressing target cells via CD40 stimulation. (**B**) Lack of crosslinking does not induce luciferase activity. Biorender Image.

T-cell engagers combined with PD-L1 blockade and CD40 stimulation drive enhanced antitumor responses in the suppressive TME

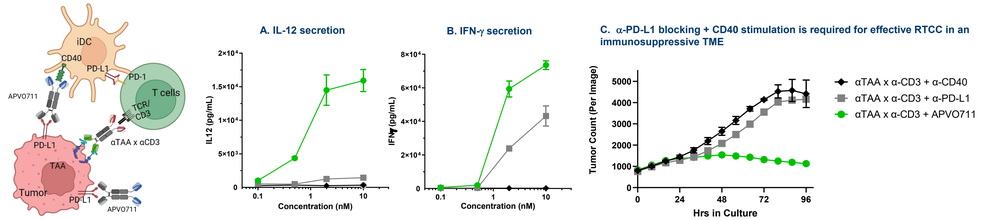


Figure 7. Cocultures of PD-L1/TAA-expressing tumor, iDCs, and PBLs were combined to mimic an immunosuppressive TME and assess the cytokine production and tumor cell killing. Fluorescently labeled PD-L1/TAA-expressing H2122 tumor were cocultured with peripheral blood lymphocytes (PBLs) ± immature DCs (iDCs) in the presence of 1nM TCE and titrated test antibodies. (**A–B**) IL-12 and IFN-γ levels in supernatants after 48 hours. (**C**) Cultures were imaged over 6 days to evaluate tumor cell killing using an Incucyte SX5 imager (Sartorius). Images generated using Biorender.

Trispecific APVO451 is able to ablate tumor better than bispecifics in an immunosuppressive TME

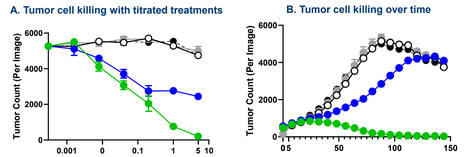


Figure 1. Redirected T cell cytotoxicity targeting nectin-4-expressing H2122 target cells by trispecific and bispecific constructs over time. Nectin-4+ H2122 cells were transduced to express NucLight Orange and PD-L1 and seeded 1 day prior to assay setup. Titrated test proteins or controls, iDCs, and PBLs were added on day 0. Plates were photographed every 8 hours. RTCC assay showing treatment-induced H2122 target cell lysis (**A**) at 80 hours with titrated proteins and (**B**) at 5nM over 6 days.

Trispecific APVO451 stimulates exhausted T cells for targeted tumor cell ablation in an immunosuppressive TME

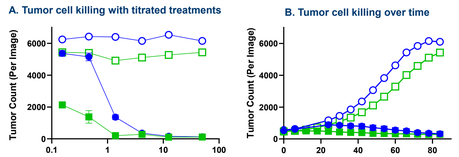


Figure 2. Exhausted T cell cytotoxicity targeting nectin-4-expressing H2122 target cells. H2122 cells were transduced to express NucLight Orange and PD-L1 and seeded 1 day prior to assay setup. Titrated test proteins or controls, iDCs, and either unstimulated T cells (blue) or exhausted T cells were added on day 0. Exhausted T cells were stimulated with CD3/CD28 Dynabeads at a 1:1 ratio every 2 days, for 3 rounds. Plates were photographed every 6 hours. RTCC assay showing treatment-induced H2122 target cell lysis (**A**) at 78 hours with titrated proteins and (**B**) at 4nM over 4 days.

APVO451 induces reduced signaling at lower antigen densities

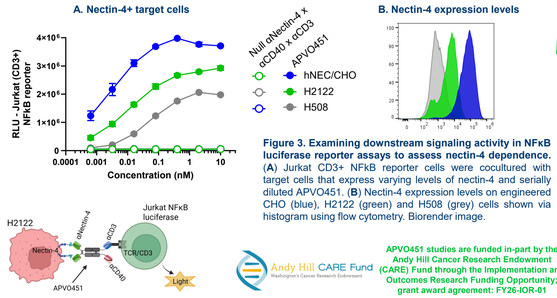


Figure 3. Examining downstream signaling activity in NFκB luciferase reporter assays to assess nectin-4 dependence. (**A**) Jurkat CD3+ NFkB reporter cells were cocultured with target cells that express varying levels of nectin-4 and serially diluted APVO451. (**B**) Nectin-4 expression levels on engineered CHO (blue), H2122 (green) and H508 (grey) cells shown via histogram using flow cytometry. Biorender image.

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OVERALL CONCLUSIONS

- **APVO451 and APVO452 are novel trispecific antibody-like molecules** designed to overcome tumor immunosuppression via dual activation of APCs and T cells through the CD40 and CD3 binding domains, simultaneously engaging both innate and adaptive immunity. Herein, the data supports the potential of APVO451 and APVO452 as potent solid tumor therapeutics as they are demonstrated to ablate human NSCLC H2122 or PSMA tumor cells, respectively, despite the immunosuppressive TME
- **APVO711, a novel bispecific with a unique dual MOA**, designed to overcome tumor immunosuppression via CD40 costimulation, while blocking the PD-1/PD-L1 pathway
- The intended design of these molecules resulted in both CD40- and/or CD3-mediated functionality requiring tumor-dependent crosslinking for immune cell activation. Within an immunosuppressive TME, these CD40 molecules drive cytokine production and enhance cytotoxicity more effectively than bispecific control molecules

PCT applications have been filed to confirm the foreign-filing position; filing dates are prior to November 2026

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