A Breakthrough in Fighting Solid Tumors with a Novel Trispecific, APVO451, Targeting Nectin-4, CD3 and CD40 to Overcome the Immunosuppressive Tumor Microenvironment

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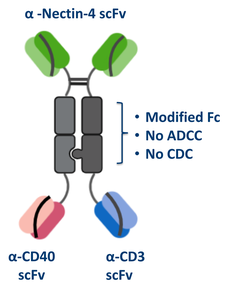
Aptevo™ Therapeutics

About APVO451

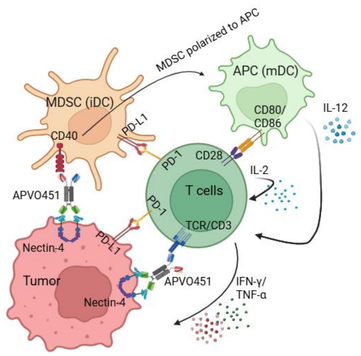
- The immunosuppressive **tumor micro-environment (TME)** remains a major barrier for solid tumor immunotherapy and therefore new therapeutics are needed.
- **APVO451 is a novel ADAPTIR-FLEX™ trispecific therapeutic** comprising three scFv domains that target Nectin-4, CD40, and CD3 to simultaneously 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** is overexpressed in multiple cancers, including lung, breast, colon, and ovarian, making it an attractive tumor-associated antigen (TAA).
- **CD3** engagement drives targeted T cell cytotoxicity and previously shown efficacy in T cell engagers (TCE). The CD3 domain used in APVO451 has demonstrated safety and efficacy as mipletamig in the RAINIER AML clinical trial, with no cytokine release syndrome (CRS) and 85% complete remission (CR) 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: Mode of Action

APVO451 – designed to overcome TME-mediated immunosuppression



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

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

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

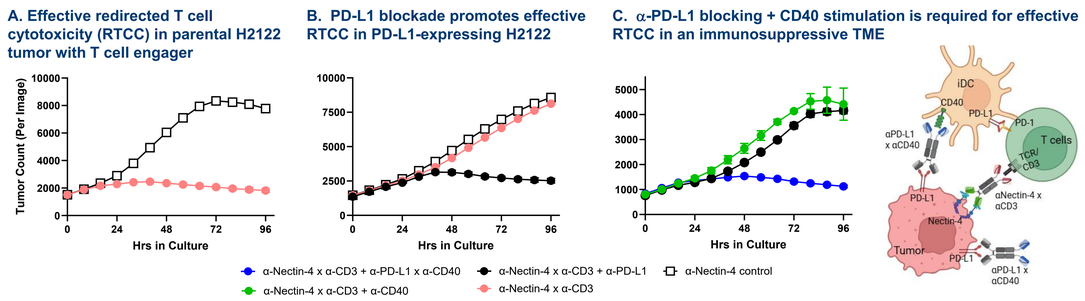


Figure 1. Incucyte killing assays were used to assess whether combining of bispecific antibodies can overcome tumor-induced immunosuppression. Fluorescently labeled nectin-4-expressing H2122 tumor cells (parental or PD-L1$^+$) were cocultured with peripheral blood lymphocytes (PBLs) ± immature DCs (iDCs) in the presence of titrated bispecifics and imaged over 6 days to evaluate tumor cell killing using an Incucyte SX5 imager (Sartorius). (**A**) PBLs cultured with parental H2122 cells were treated with 1 nM of a α-Nectin-4 x α-CD3 bispecific T cell engager (TCE) or control antibody. (**B**) PBLs cocultured with PD-L1-expressing H2122 tumor cells were treated with 1 nM of a α-Nectin-4 TCE ± PD-L1 blocking antibody. (**C**) PBLs were cocultured with PD-L1$^+$ H2122 cells and iDCs treated with 1 nM of a α-nectin-4 TCE ± PD-L1 blocking antibody or α-PD-L1 × α-CD40 bispecific. Images generated using Biorender.

Addition of an α-PD-L1 x α-CD40 bispecific when combined with a TCE enhanced cytokine production and activation of immune cells

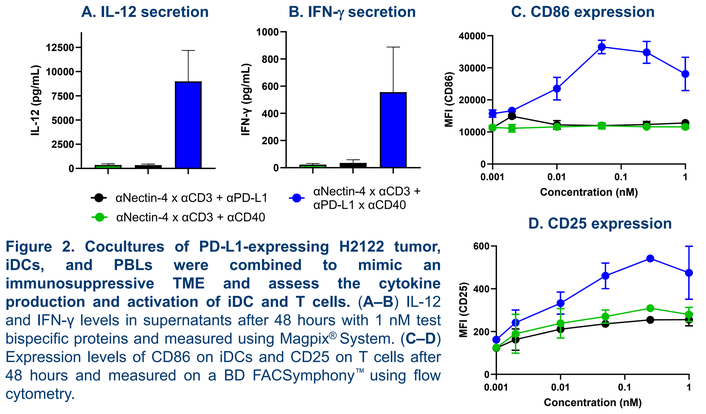
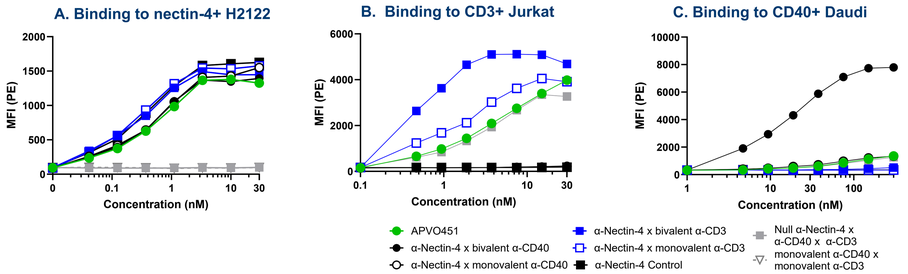


Figure 2. Cocultures of PD-L1-expressing H2122 tumor, iDCs, and PBLs were combined to mimic an immunosuppressive TME and assess the cytokine production and activation of iDC and T cells. (**A–B**) IL-12 and IFN-γ levels in supernatants after 48 hours with 1 nM test bispecific proteins and measured using Magpix® System. (**C–D**) Expression levels of CD86 on iDCs and CD25 on T cells after 48 hours and measured on a BD FACSymphony™ using flow cytometry.

APVO451 was engineered for high-affinity binding to nectin-4, with lower affinity to human CD3 and CD40



D. Binding to nectin-4, CD3, and CD40 antigens

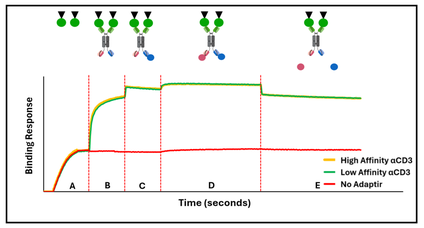


Figure 3. Evaluation of binding affinities based on protein format. (**A-C**) **Cell-based binding (flow cytometry):** Titrations of protein were combined with human target-expressing cell lines: (**A**) nectin-4$^+$ H2122 tumor cells; (**B**) CD3$^+$ Jurkat T cells; (**C**) CD40$^+$ Daudi B cells. (**D**) **Concurrent binding of all binding domains by BLI analysis (Sartorius:** Octet® QKe**):** (**DA**) Nectin-4-biotin was immobilized on a streptavidin sensor. (**DB**) APVO451 was captured via its Nectin-4-binding domain; unloaded sensors (red) served as controls. (**DC**) CD3εγ bound to APVO451 through its αCD3 domain at high- (yellow) and low-affinity (green) sites. (**DD**) CD40 bound to APVO451 through its α-CD40 domain in the presence of CD3εγ. (**DE**) In the absence of CD40 and CD3εγ, both antigens dissociated, returning to the nectin-4-APVO451 baseline.

As designed, APVO451 activity is nectin-4-dependent; Unable to induce signaling through CD3-CD40 crosslinking

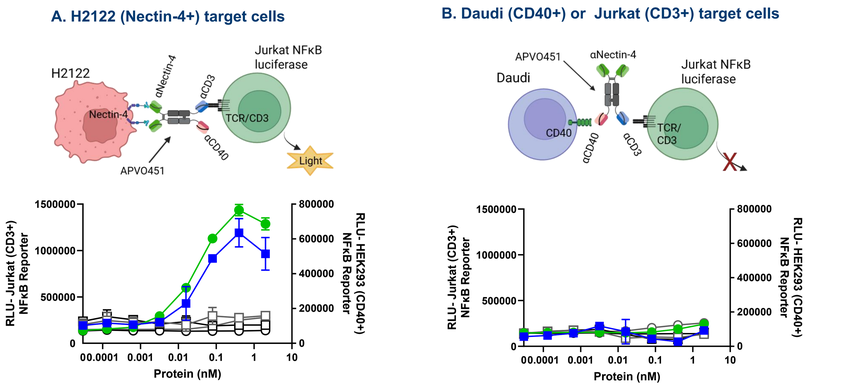


Figure 4. Examining downstream signaling activity in NFκB luciferase reporter assays to assess nectin-4 dependence. Jurkat NFkB reporter cells (CD3+; left axis) or HEK293 NFkB reporter cells (CD40+; right axis) were cocultured with target cells and serially diluted APVO451 or control proteins. (**A**) Crosslinking with nectin-4-expressing H2122 target cells via CD3 stimulation (green circle) or CD40 stimulation (blue square). (**B**) Alternative crosslinking does not induce luciferase activity by using CD40-expressing target cells for downstream signaling via CD3 stimulation (left axis, circles) or by using CD3-expressing target cells for downstream signaling via CD40 stimulation (right axis, squares). Biorender Images.

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

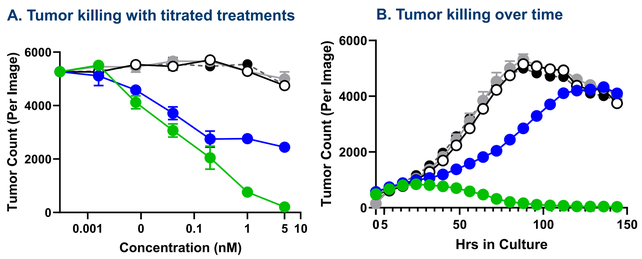


Figure 5. Redirected T cell cytotoxicity targeting nectin-4-expressing H2122 target cells by trispecific and bispecific constructs over time. H2122 cells were transduced to express NucLight Orange and PD-L1 and seeded 1 day prior to assay setup. Titrated test proteins or controls, iDCs, and PBLs were 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.

APVO451 induces activation of iDCs in a suppressive TME

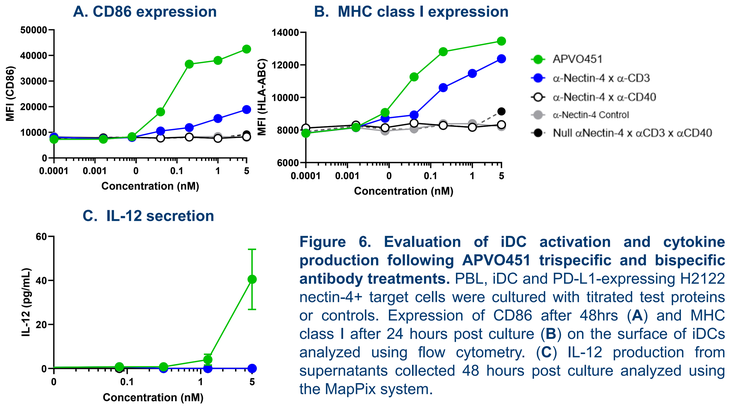


Figure 6. Evaluation of iDC activation and cytokine production following APVO451 trispecific and bispecific antibody treatments. PBL, iDC and PD-L1-expressing H2122 nectin-4+ target cells were cultured with titrated test proteins or controls. Expression of CD86 after 48hrs (**A**) and MHC class I after 24 hours post culture (**B**) on the surface of iDCs analyzed using flow cytometry. (**C**) IL-12 production from supernatants collected 48 hours post culture analyzed using the MapPix system.

APVO451 promotes T cell expansion and cytokine production

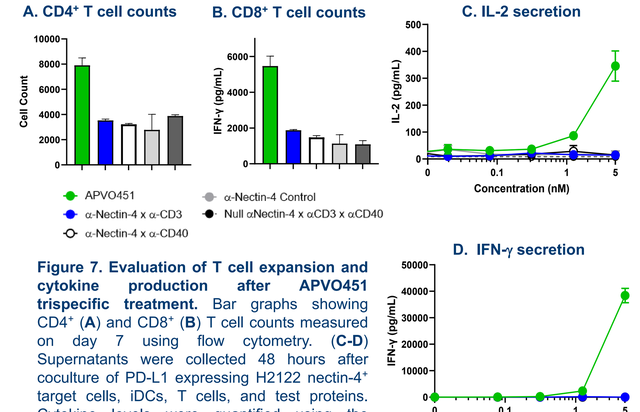


Figure 7. Evaluation of T cell expansion and cytokine production after APVO451 trispecific treatment. Bar graphs showing CD4$^+$ (**A**) and CD8$^+$ (**B**) T cell counts measured on day 7 using flow cytometry. (**C-D**) Supernatants were collected 48 hours after coculture of PD-L1 expressing H2122 nectin-4$^+$ target cells, iDCs, T cells, and test proteins. Cytokine levels were quantified using the Magpix system.

CONCLUSIONS

- ❖ APVO451, a novel trispecific antibody-like molecule, was designed to overcome tumor immunosuppression via dual activation of APCs and T cells through the CD40 and CD3 binding domains
- ❖ Herein, the data supports the potential of APVO451 as a potent solid tumor therapeutic as it is demonstrated to ablate human NSCLC H2122 tumor cells, despite the immunosuppressive TME
- ❖ The intended design of APVO451 resulted in both CD3- and CD40-mediated functionality requiring nectin-4-dependent crosslinking for immune cell activation
- ❖ Within an immunosuppressive TME, APVO451 drives cytokine production and enhances APC and T cell activation and cytotoxicity more effectively than bispecific control molecules

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