APVO442: A bispecific T cell-engaging candidate utilizing the ADAPTIR-FLEX™ platform technology with unique properties designed for optimal tumor distribution and cytotoxic response against PSMA-expressing solid tumors

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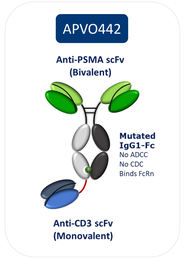
AACR Annual Meeting 2021
Abstract number LB172

APVO442 is Designed for Optimal Safety and Activity Against Prostate Cancer

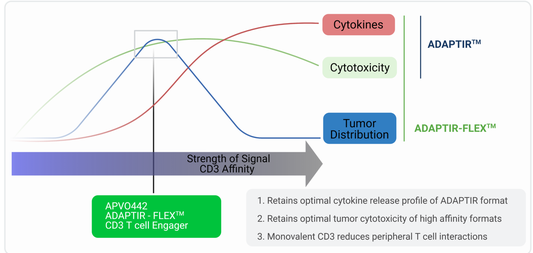
Prostate-Specific Membrane Antigen (PSMA), is a tumor-associated antigen (TAA) that is expressed on prostate cancers, including metastatic castration-resistant prostate cancer (mCRPC).
Current chemotherapeutic approaches for mCRPC are challenged by development of resistance resulting in limited clinical benefit.

APVO442 is Aptevo's bispecific candidate targeting PSMA and CD3. This candidate was designed in Aptevo's ADAPTIR-FLEX™ platform to create a unique bivalent PSMA and low affinity monovalent CD3 molecule with the potential to maximize PSMA engagement while limiting peripheral CD3 activity to deliver a safer and more potent tumor-directed bispecific approach against mCRPC.



THERAPEUTIC CANDIDATE	• ADAPTIR-FLEX (αCD3 x αPSMA) T-Cell Engager • Mutated IgG1 Fc; No ADCC, CDC; retains FcRn binding
FUNCTION/ MOA	• Engages T cells via CD3 epsilon to lyse PSMA+ tumor cells • Low-affinity monovalent CD3 binding • High avidity PSMA binding • Reduces potential binding to circulating T cells • Enables potential for better tumor biodistribution • Low level of cytokines in absence of targets (pre-clinical)
INDICATIONS	• Metastatic castration – resistant prostate cancer • Other PSMA+ tumors
HALF-LIFE	• 9.3 days in murine models
Manufacturability	• Heterologous chain pairing "knob – in – hole" • Manufacturability comparable to ADAPTIR format • Utilizes standard mAb production processes
DEVELOPMENT STAGE	• Lead candidate selected • CMC activities initiated • Pre-clinical lead studies ongoing

APVO442's Unique Format is Designed to Overcome Limitations of High Affinity CD3 T Cell Engagers



1. Retains optimal cytokine release profile of ADAPTIR format
2. Retains optimal tumor cytotoxicity of high affinity formats
3. Monovalent CD3 reduces peripheral T cell interactions

Characteristic	APVO442	High Affinity Monovalent CD3	Benefit
PSMA binding	++	Variable	Enriched tumor Targeting
CD3 binding	+	+++	Reduced T cell sink/activation
T cell activation	++	++	Retained optimal T cell profile
T cell proliferation	++	++	
T cell mediated tumor killing	++	++	
Cytokine production	+ (tumor specific)	+++	Reduced toxicity profile
PK	++	+	Enriched Tumor distribution and potency
Tumor regression	++	++	

APVO442 is designed with Aptevo's ADAPTIR-FLEX technology to generate a low-affinity monovalent CD3 engagement/high-affinity bivalent PSMA targeting

- Retains improved safety profile observed with ADAPTIR CD3 format
- Retains potency T cell activation and cytotoxicity profile seen with high affinity T cell engagers
- Has the potential for improved tumor biodistribution based on low affinity CD3 binding

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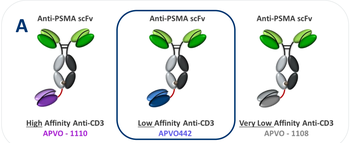
Figure 1: ADAPTIR-FLEX™ Molecules Targeting PSMA with a Range of CD3 Affinity Show Distinct Target Binding Properties

ADAPTIR-FLEX Constructs with Variable CD3 Affinity

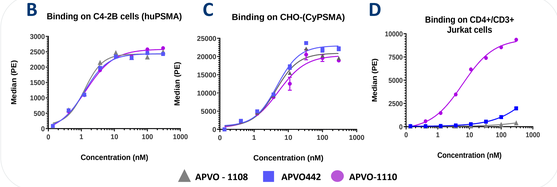


Design of ADAPTIR-FLEX constructs with Distinct binding profiles
ADAPTIR-FLEX constructs were designed with high affinity, low affinity, or very low affinity monovalent CD3 binding properties and paired with bivalent PSMA to generate functional bispecific molecules (**A**).

APVO442 retains strong binding to PSMA and reduced affinity to CD3
Binding of ADAPTIR-FLEX molecules to conformational PSMA and CD3 targets was assessed by on-cell binding via flow cytometry on relevant target-expressing cell lines including human PSMA expressing C4-2B cells (**B**) CHO cells stably expressing full length cynomolgus PSMA (**C**) and Jurkat CD4+ T cells expressing CD3 (**D**). The on-cell binding values of EC_{50} and Max binding RLU are listed for each of the three cell lines (**Table**).

APVO442 displays a distinct high affinity PSMA binding (<5 nM) and low affinity CD3 (< 200 nM) binding profile.

Varying CD3 Affinity Molecules Retain Similar PSMA Binding



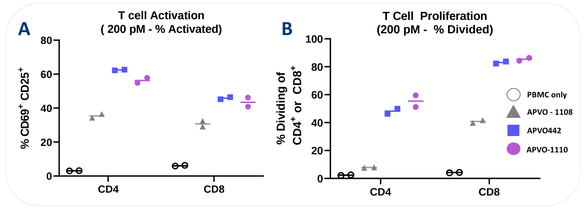
Modified Constructs Display a Range of CD3 Binding

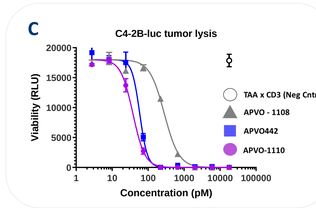
Binding Properties of ADAPTIR-FLEX Molecules

On- Cell Binding Parameters	APVO – 1108		APVO442		APVO-1110	
	EC_{50} (nM)	Max	EC_{50} (nM)	Max	EC_{50} (nM)	Max
Human PSMA binding affinity	1.3	2500	1.2	2400	1.4	2600
Cyno PSMA binding affinity	4.4	19500	4.7	22000	5.1	19000
Human CD3 binding affinity	> 200	409	> 200	2000	6.8	9000

Figure 2: APVO442 Delivers Optimal Activation and Potency Across a Range of PSMA Expressing Tumor Targets

APVO442 Elicits Favorable Activation, Proliferation and Potency Profiles in Comparison to Other ADAPTIR-FLEX Constructs





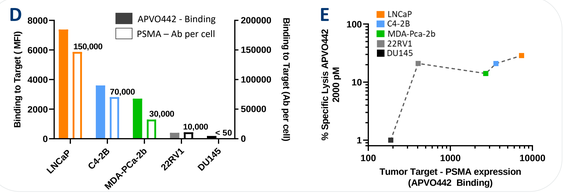
Parameter (C4-2B + PBMC)	APVO – 1108 (EC_{50} – pM)	APVO442 (EC_{50} – pM)	APVO-1110 (EC_{50} – pM)
CD4 Activation	160	33	28
CD4 Proliferation	265	27	17
CD8 Activation	103	49	43
CD8 Proliferation	163	21	14
Cytotoxicity C4-2B	282	59	38

Activity profile of ADAPTIR-FLEX constructs with variable CD3 binding properties – T cell activation, proliferation, and cytotoxicity were assessed by co-culturing human peripheral blood mononuclear cells (PBMCs) with a huPSMA expressing tumor target cell (C4-2B) in the presence of PSMA-targeted ADAPTIR-FLEX constructs or a negative control bispecific (TAA x CD3).

T - cell activation was assessed at 24 hrs. and proliferation and 96 hrs. post co-culture and measured by surface expression of CD25 and CD69 or dilution of a cell tracker dye on $CD4^+$ and $CD8^+$ T cells by flow cytometry – displayed is % activated at 200 nM (**A,B**). For cytotoxicity assays, luciferase expressing C4-2B cells were used and the fraction of live C4-2B cells was quantified by bioluminescence and is represented in RLU (relative light units) (**C**). Potencies of activation, proliferation, and cytotoxicity for $CD4^+$ and $CD8^+$ T cells are shown as EC_{50} in pM (**Table**).

APVO442 retains T cell activation, proliferation and cytotoxicity profiles comparable to the high affinity APVO-1110.

APVO442 Elicits Potent and Target – Dependent Cytotoxicity Across a Range of PSMA+ Tumor Targets

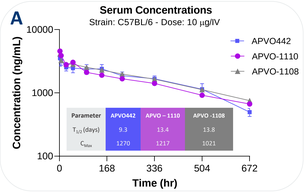


APVO442 delivers a potent T cell anti-tumor response across a range of PSMA expressing targets – The total number of PSMA receptors per cell were determined by quantitating the total number of anti-PSMA antibody bound to cells (ABC) on LNCap, C4-2B, , MDA-Pca-2b, 22RV1, or DU145 by flow cytometry (**D – open bar; # of receptors displayed**). Binding of APVO442 to target cell lines was evaluated by flow cytometry and compared to the ABC for target tumor cell lines (**D – closed bar**). T cell mediated cytotoxicity was plotted against APVO442 binding to target cells to evaluate the correlation between PSMA expression and functional activity by APVO442. Data is presented as fold cytotoxicity over no – tumor target control (**E**). For cytotoxicity studies, targets were loaded with chromium-51 (51Cr) and incubated with constructs or controls. The percentage of target cell lysis was measured by specific 51Cr release into the supernatant and displayed as the fold over no target in the assay.

APVO442 binds to PSMA+ tumor cells and delivers consistent cytotoxicity across a range of PSMA expressing tumor targets.

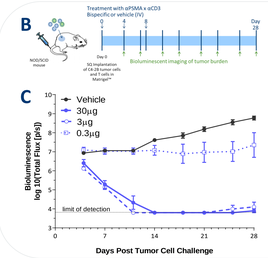
Figure 3: APVO442 Delivers an Optimal CD3 Dependent Anti-Tumor Response *In vivo*

APVO442 Displays a Favorable *In Vivo* PK Profile in Mice



APVO442 displays favorable *in vivo* PK in C57Bl/6 mice– 10 µg of APVO442, APVO-1108, or APVO-1110 were injected intravenously (IV) to female C57BL/6 animals and blood was collected at 2, 6, 24, 48, 96, 150, 222, 336, 504, and 672 hrs. Serum was evaluated for the presence of bispecific molecules by electrochemiluminescent assay (ECLA) and serum concentrations over time were used to determine PK parameter estimates by non-compartmental analysis (NCA)(**A**). APVO442 displayed comparable PK profiles to APVO-1108 and APVO-1110 with properties comparable to similar ADAPTIRs and other antibody-like molecules (**Inset**). **APVO442 displays a PK profile with antibody like properties in mice.**

APVO442 Augments a Favorable In *Vivo* Efficacy Profile in Mice

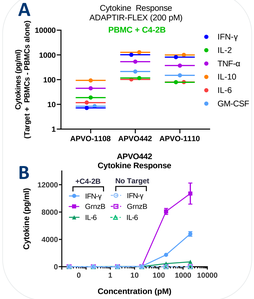


Parameter	APVO442	APVO – 1110	APVO -1108
Log tumor - % reduction (30, 3, 0.3 µg) - Day 28	98, 94, 29	93, 98, 72	92, 97, 9
% Tumor - Free Incidence 3 µg Max - Day 14	100	100	87.5%

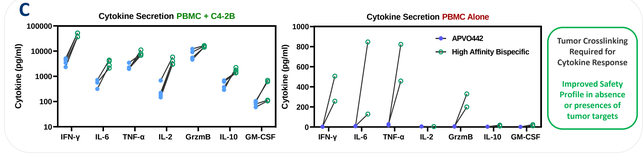
APVO442 displays favorable *in vivo* efficacy in a C57Bl/6 – C4-2B tumor model - Efficacy was evaluated in NOD/SCID mice implanted with C4-2B tumors and administered ADAPTIR-FLEX constructs at 30, 3, or 0.3 µg or vehicle alone at days 0, 4, 8 post implant and tumor growth was monitored by bioluminescent imaging (BLI) at the indicated timepoints (**B**). APVO442 displayed strong anti-tumor control at > 3 µg with maximal and sustained response post day 14 to endpoint. APVO442 activity was comparable to the high affinity APVO-1110 construct in log tumor reduction (day 14 – 28; day 8 shown) (**D; Table**) and % tumor incidence at maximal response (Day 14 max response shown) (**Table**). **APVO442 promotes tumor control comparable with high affinity CD3 molecules**

Figure 4: The Unique CD3 Properties of APVO442 are Designed to Stimulate Optimal Tumor –Specific Cytokine Responses with Reduced Risk of Peripheral Cytokine Release

APVO442 Induces a Favorable Cytokine Profile Dependent on Tumor Crosslinking



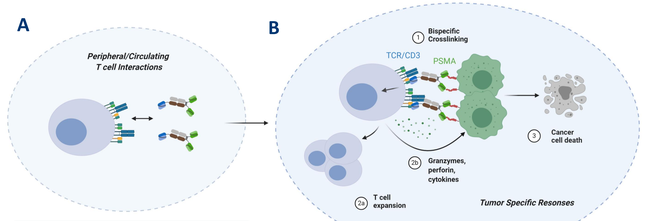
APVO442 Demonstrates a Tumor Selective Cytokine Response that Differentiates it from Standard High Affinity T - cell Engagers



ADAPTIR-FLEX constructs elicit beneficial cytokine responses dependent on tumor PSMA crosslinking – Human PBMCs were treated with a serial dose titration of ADAPTIR-FLEX constructs in the presence or absence of PSMA expressing C4-2B tumor targets. Cytokine responses were measured at 24 hrs. post co-culture by multiplexed analyte assays and response at 200 pM (**A,B**) or 2000 pM (**C**) are displayed. (**A**) APVO442 elicits a cytokine profile similar to high affinity monovalent APVO-1110 that is increased in a PSMA/tumor-specific manner. (**B**) APVO442 elicits key T cell effector cytokines (IFN-γ and Granzyme B) but displays lower IL-6 expression that requires crosslinking by PSMA+ C4-2B targets. (**C**). APVO442 does not trigger cytokine responses from PBMCs directly in contrast to a high affinity CD3 in an alternate bispecific format – similar effector cytokine profiles are observed in the presence of PSMA+ C4-2B targets.

APVO442 elicits a beneficial tumor-specific cytokine profile. Cytokine responses require tumor cross linking and are reduced compared to high affinity CD3 formats offering and improved safety window.

APVO442 is a Unique Approach to Generate a Safe Yet Potent Anti-PSMA Solid Tumor Response



APVO442 is designed to deliver a tumor directed T cell response against mRCP.

The unique design of APVO442 reduces the potential for peripheral T cell engagement to limit on-target CD3 activation (cytokine) and sink effects (**A**).

Low affinity monovalent CD3 cooperates with a high avidity bivalent PSMA to elicit tumor specific T cell activation and cytotoxicity comparable with higher affinity T cell engager approaches (**B**)

Aptevo's unique low affinity CD3 targeting approach positions APVO442 for favorable outcomes compared to traditional high affinity CD3 approaches

APVO442 - Monovalent low affinity CD3 to limit:
- Peripheral T cell activation and cytokine release (Fig 3)
- Avoid a peripheral CD3 sink
- Facilitate improved tumor biodistribution

APVO442 – Bivalent PSMA and Monovalent CD3
- Tumor – specific crosslinking (1) (Fig 2,3)
- T cell expansion (2a) and activation (2b) comparable to high affinity CD3 (Fig 2,3)
- Highly potent tumor specific cytotoxicity (3) (Fig 2, 4)
- Ability to elicit tumor control *in vivo* (Fig 4)