AACR Annual Meeting April 2019 – Abstract Number LB-199

APVO436, a Bispecific anti-CD123 x anti-CD3 ADAPTIR™ Molecule for Redirected T-cell Cytotoxicity with Limited Cytokine Release, is Well Tolerated in Repeat Dose Toxicology Studies in Cynomolgus Macaques

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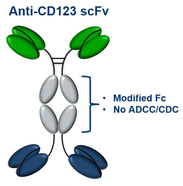
Introduction

Depletion of CD123 over-expressing tumor cells may improve patient outcomes in several hematological malignancies including AML, MDS, ALL, CML, HCL and BPDCN. CD123 is infrequently expressed by normal cells, making it an attractive tumor target currently being pursued using several different approaches including T-cell engaging immunotherapies. Cytokine release syndrome (CRS) is a concern with T-cell engaging immunotherapies that may require complex clinical development strategies to manage safely. We have developed APVO436, a bispecific anti-CD123 x anti-CD3 ADAPTIR molecule for redirecting T-cell cytotoxicity to CD123-expressing tumor cells. APVO436 is currently in phase I clinical testing in AML and MDS. A potential advantage of the ADAPTIR platform is reduced cytokine release compared to other formats (Mol Cancer Ther; 15(9):2155-65). We previously compared APVO436 activity to another CD123 x CD3 bispecific antibody containing the amino acid sequence of MGD006. We also demonstrated that APVO436 induces activation of AML patient T cells to kill endogenous tumor cells. Here we extend these studies to test the capacity of APVO436 to induce memory T-cell generation and describe the results of repeat dose toxicology studies in cynomolgus macaques.

ADAPTIR Molecule Targeting CD123 and CD3

ADAPTIR molecules are bispecific antibody-like therapeutics containing two sets of binding domains linked to an immunoglobulin Fc domain to extend the half-life of the molecule *in vivo*. The anti-CD123 x anti-CD3 ADAPTIR molecule binds both CD123 and CD3 to redirect T-cell cytotoxicity against CD123 expressing tumor cells. The anti-CD123 binding domain is a fully human single chain variable fragment (scFv) that binds human and non-human primate (NHP) CD123. The anti-CD3 binding domain is a humanized scFv derived from a murine antibody that binds human and NHP CD3. In order to avoid interactions with other components of the immune system that could lead to CD3 clustering and non-specific T cell activation, the Fc region has been engineered to minimize complement fixation and interaction with Fcγ receptors.



Experimental Design of 28-Day Study of APVO436 in Cynomolgus Monkeys

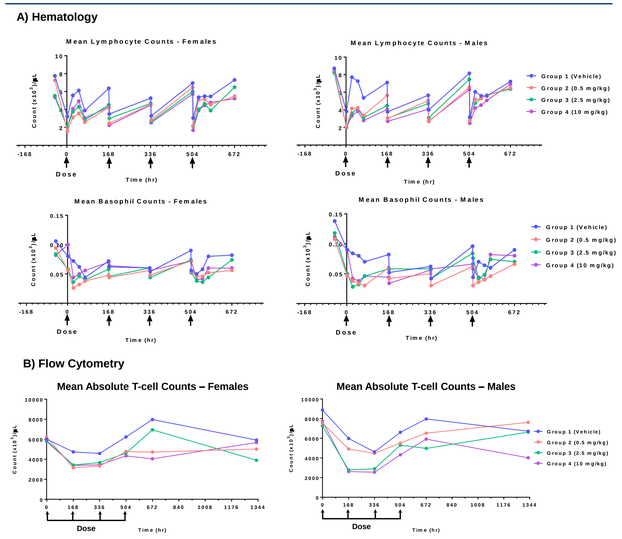
Repeat dose GLP study with 4 week dosing (QW) and 5 week recovery period

Group Number	Test Material	Dose Level (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/kg)	Number of Males/Females	
					Main	Recovery
					Day 29	Day 57
1	Control	0	0	5.0	3/3	2/2
2	APVO436	0.5	0.1	5.0	3/3	2/2
3	APVO436	2.5	0.5	5.0	3/3	2/2
4	APVO436	10.0	2.0	5.0	3/3	2/2

The study was conducted at a contract laboratory according to GLP guidelines to determine the potential toxicity of APVO436 when given once weekly by intravenous (IV) bolus injection for 4 weeks to male and female cynomolgus monkeys. The IV route of exposure was selected because this is the intended route for clinical studies. The study animals were divided into 4 groups of 10 animals each (5 male and 5 female per group), and dosed with either a vehicle control or APVO436 (at 1 of 3 dose levels) once weekly for 4 weeks. The doses chosen for this study represent a dose range with a mass excess up to ~750-fold over the anticipated effective dose in human patients, and in excess of over the amount needed to saturate T cells.

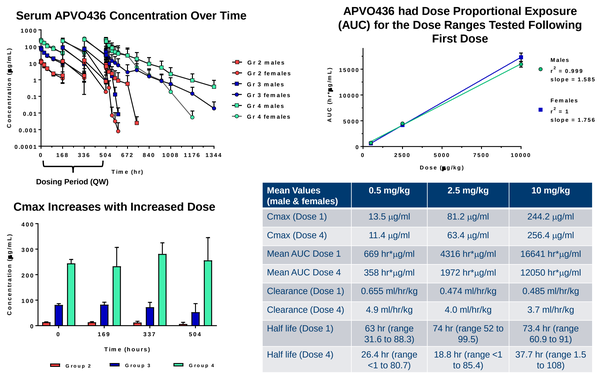
Parameters Measured
- Safety Pharmacology
  - Body weight, clinical observations post-dose for adverse events, body temperature, blood pressure, ECG and heart rate, neurology and ophthamalogy examination, respiratory rate
- Laboratory evaluations
  - Clinical pathology, bioanalytical evaluation, including PK and ADA, Cytokines, Flow cytometry on peripheral blood throughout study and bone marrow at necropsy.
- Necropsy, including: urinalysis, macroscopic observations, organ weights and full histopathology
  - Day 29 for main study; Day 57 for recovery

APVO436 Induced Transient Changes in Peripheral Blood Populations in Cynomolgus Monkeys



Peripheral blood cell populations were monitored for APVO436 induced changes by standard hematology assessments (A), and by flow cytometry measurements (B) to assess changes in lymphocyte subsets (T cells, B cells, NK cells), and monocytes, and to evaluate changes in CD123+ cell populations (including basophils, plasmacytoid DC (pDC), and granulocytes). Peripheral blood samples were analyzed on a weekly basis, prior to each dose, to look at changes over time.

Exposure of APVO436 in NHP Supports Clinical Dosing



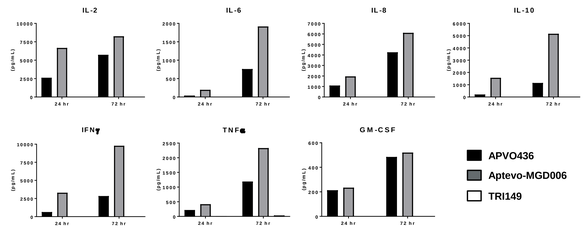
Mean Values (male & females)	0.5 mg/kg	2.5 mg/kg	10 mg/kg
Cmax (Dose 1)	13.5 µg/ml	81.2 µg/ml	244.2 µg/ml
Cmax (Dose 4)	11.4 µg/ml	63.4 µg/ml	256.4 µg/ml
Mean AUC Dose 1	669 hr*µg/ml	4316 hr*µg/ml	16641 hr*µg/ml
Mean AUC Dose 4	358 hr*µg/ml	1972 hr*µg/ml	12050 hr*µg/ml
Clearance (Dose 1)	0.655 ml/hr/kg	0.474 ml/hr/kg	0.485 ml/hr/kg
Clearance (Dose 4)	4.9 ml/hr/kg	4.0 ml/hr/kg	3.7 ml/hr/kg
Half life (Dose 1)	63 hr (range 31.6 to 88.3)	74 hr (range 52 to 99.5)	73.4 hr (range 60.9 to 91)
Half life (Dose 4)	26.4 hr (range <1 to 80.7)	18.8 hr (range <1 to 85.4)	37.7 hr (range 1.5 to 108)

The toxicokinetics (TK) of APVO436 were evaluated in male and female cynomolgus monkey serum following once weekly IV bolus injections at dose levels of 0.5, 2.5, and 10 mg/kg for 4 weeks with a 35-day recovery period. While ADA did impact the mean PK curves, some individual animals maintained high AUC (up to 27344 hr*µg/kg), low clearance values (0.21 ml/hr/kg) and longer T ½ (up to 108 hrs, 4.5 days) following the last dose.

Anti-CD123 x Anti-CD3 Bispecific Antibody Construct Comparison

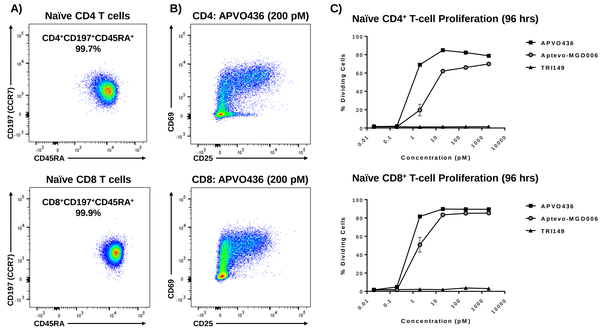
- **APVO436**: anti-CD123 x anti-CD3 ADAPTIR (161 kDa)
- **Aptevo-MGD006**: The CD123 and CD3 binding domain sequences for flotetuzumab (MGD006) were obtained from patent W02015026892 and engineered in Macrogenic's dual-affinity re-targeting format as reported in *Sci Transl Med. 2015 May 27;7(289):289ra82* (59 kDa)
- **TRI149**: A negative control ADAPTIR protein that contains a mutated version of the APVO436 CD123 binding domain that does not bind CD123 (161 kDa)

Reduced Cytokine Secretion Induced by APVO436 in Naive T Cell Cultures in Presence of CD123+ Tumors



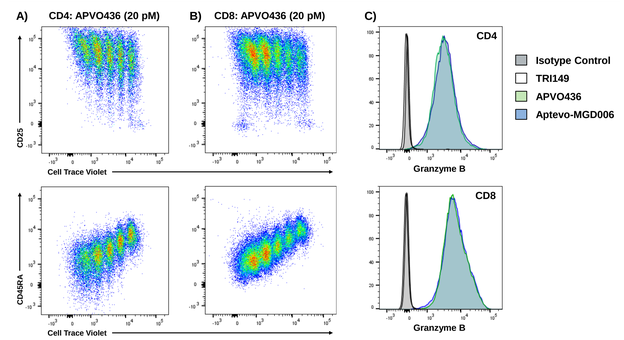
Purified naïve T cells isolated from normal donor peripheral blood were cultured with CD123+ Molm-13 tumor cells and 1 nM APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149 for 24 or 72 hours. Levels of several cytokines commonly produced by activated T cells were measured in the culture supernatants using multiplexed analyte assays. The negative control ADAPTIR TRI149 did not induce detectable cytokine release.

APVO436 Induces Activation and Proliferation of Naïve CD4+ and CD8+ T Cells



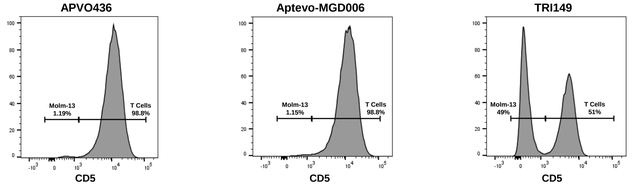
Naïve CD4+ and CD8+ T cells were isolated from normal donor peripheral blood and assessed for CD197 (CCR7) and CD45RA expression by flow cytometry (A) then cultured with Molm-13 tumor cells and APVO436 or the negative control ADAPTIR TRI149. To measure T-cell activation, upregulation of CD69 and CD25 on T cells was monitored at 24 hours using multi-color flow cytometry, after gating on live CD4+ and CD8+ T cells (B). To monitor proliferation naïve T cells were labeled with Cell Trace Violet at the start of the culture and after 6 days T-cell proliferation was measured by calculating the percentages of CD4+ (CD8-) or CD8+ T-cells that had undergone at least one cell division, according to their Cell Trace Violet profile (C).

APVO436 Induces the Generation and Expansion of Activated Memory T cells with Cytotoxic Potential



Naïve T cells were isolated from normal donor peripheral blood and cultured with CD123+ Molm-13 tumor cells and APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149. To monitor cell division and transition to a memory phenotype naïve T cells were labeled with Cell Trace Violet at the start of the culture and after 6 days were assessed via multi-color flow cytometry (A,B). To assess cytotoxic potential intracellular staining for Granzyme-B was performed at day 6 of the culture (C).

APVO436 Induced Memory T Cells Demonstrate Cytotoxic Activity Against CD123+ Tumor Targets



Naïve T cells were isolated from normal donor peripheral blood and cultured with CD123+ Molm-13 tumor cells and APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149 for 7 days. T-cell cytotoxic activity against Molm-13 target cells in the cultures was assessed using multi-color flow cytometry. After 7 days, live Molm-13 target cell numbers were counted by gating on 7AAD negative CD5 negative cells.

Summary and Conclusions

Summary of Repeat Dose NHP Toxicity Study Results
- NOAEL determined to be 10 mg/kg with no adverse events due to repeated dosing of APVO436
- Minimal cytokines detected in animals dosed with APVO436, most levels were below the limit of quantitation
- Variable decreases in peripheral CD123+ target cells suggesting pharmacologic activity of APVO436 in NHP
- Redistribution of NHP T cells as expected for APVO436 mechanism of action
- Elimination half-life ranged up to 108 hours with antibody-like clearance and volume of distribution of APVO436
- Dose proportional exposure as measured by AUC and Cmax after the first and last dose
- Decrease in exposure and faster clearance after last dose compared to first dose in individual animals due to variable ADA at later time points

Summary of Memory T Cell Experiments
- APVO436 induces activation and proliferation of naïve CD4 and CD8 T cells in the presence of CD123+ tumor target cells with lower levels of several T-cell cytokines induced compared to another CD123 x CD3 bispecific antibody
- APVO436 induces the generation of functional memory T cells with cytolytic function from naïve T cells

Conclusions
- These data support clinical studies with APVO436 as a potential treatment for AML and other hematological malignancies with possible safety advantages over other CD123 targeting therapies. APVO436 is currently being tested in a Phase I trial of AML and MDS