AACR Annual Meeting April 2018 – Abstract Number 1786

APVO436, a Bispecific anti-CD123 x anti-CD3 ADAPTIR™ Molecule for Redirected T-cell Cytotoxicity, Induces Potent T-cell Activation, Proliferation and Cytotoxicity with Limited Cytokine Release

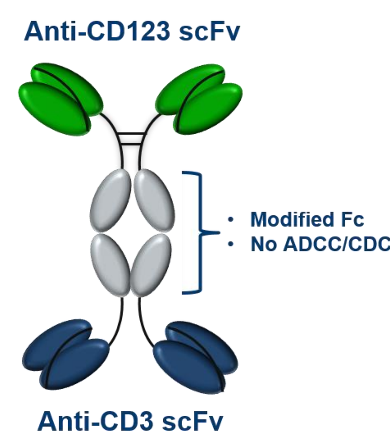
Michael R. Comeau, Robert E. Miller, Robert Bader, Rebecca Gottschalk, Mollie Daugherty, Toddy Sewell, Lynda Misher, Lara Parr, Melissa DeFrancesco, David Bienvenue, Catherine J. McMahan, Gabriela H. Hoyos and Jane A. Gross.

Introduction

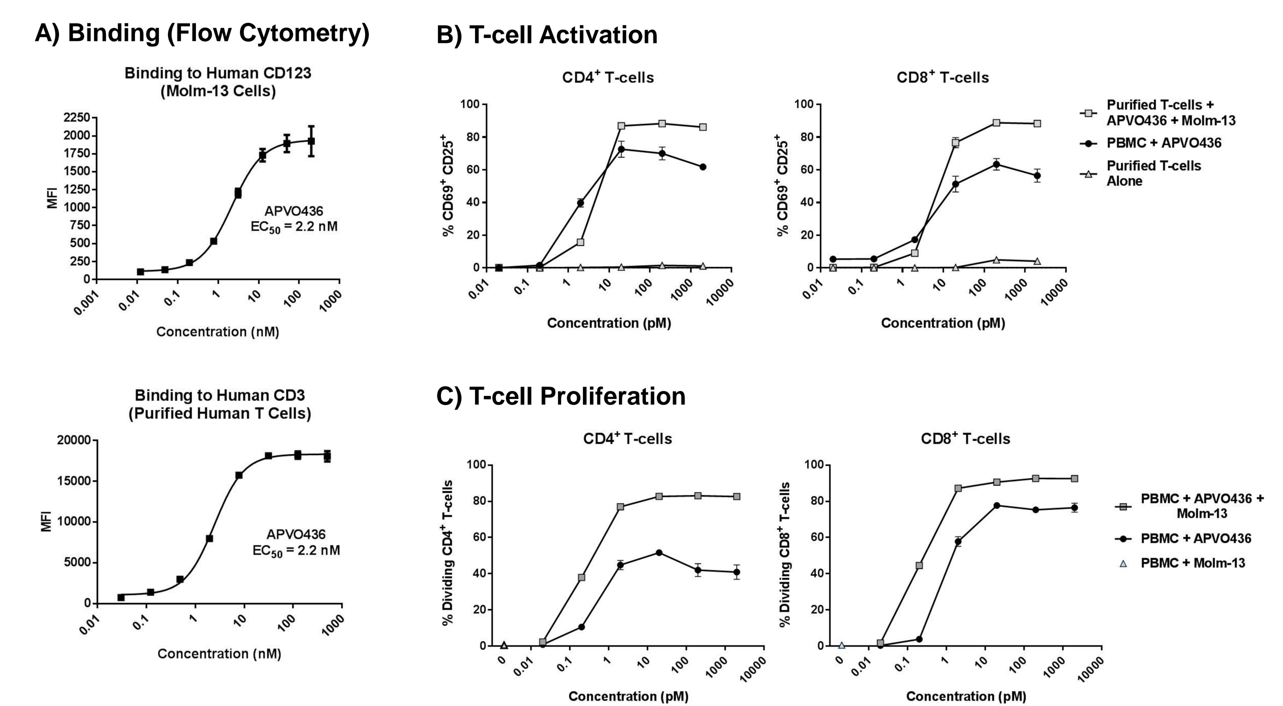
Depletion of CD123 over-expressing malignant cells provides a potential new treatment option that may improve patient outcomes in several hematological malignancies. CD123 is over-expressed in AML, MDS, ALL, CML, HCL and BPDCN and infrequently expressed by normal cells making it an attractive target that is being pursued using a number of different approaches including T-cell engaging immunotherapy. Cytokine release syndrome is a significant concern with T-cell activating therapeutics which has led to severe complications in clinical trials. We have developed APVO436, a bispecific anti-CD123 x anti-CD3 ADAPTIR molecule for redirecting T-cell cytotoxicity to CD123-expressing tumor cells. A potential advantage of the ADAPTIR platform is reduced cytokine release upon T-cell engagement compared to other formats (*Mol Cancer Ther. 2016 Sep;15(9):2155-65*). Here we present in vitro and in vivo activity of APVO436 and compare the activity of APVO436 to another anti-CD123 x anti-CD3 bispecific containing the amino acid sequence of Macrogenics' MGD006.

ADAPTIR Molecules 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 molecules bind 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.

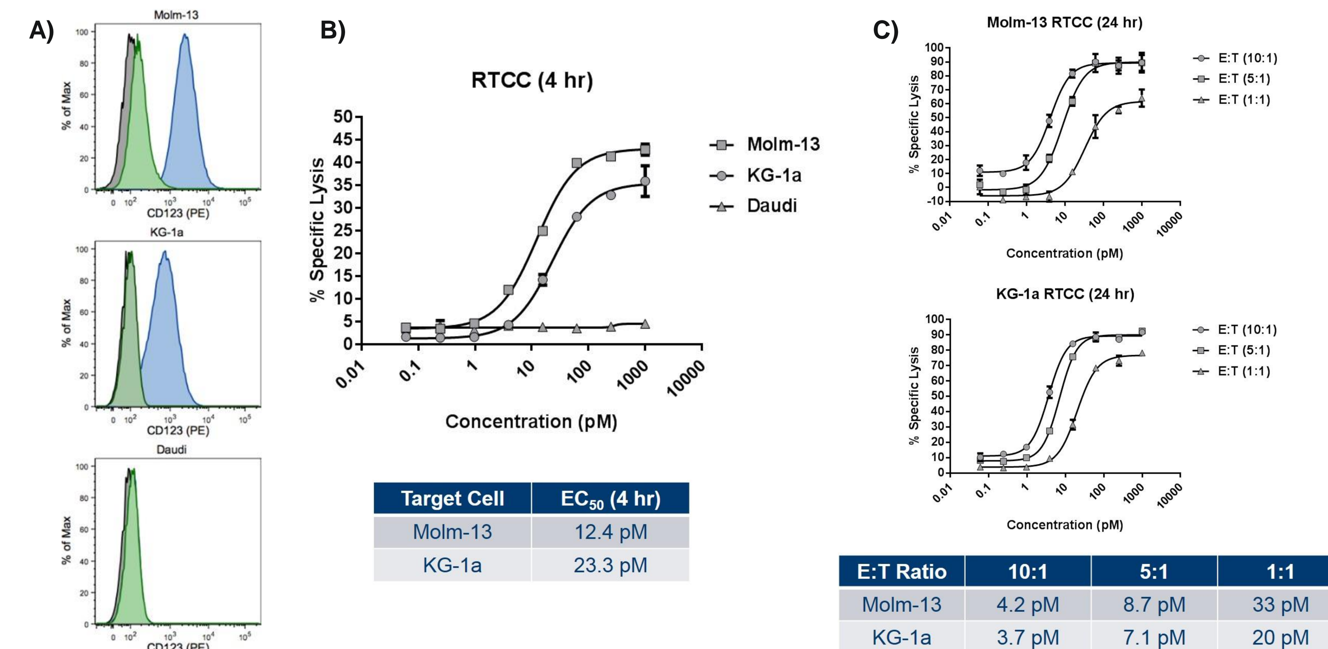


APVO436 Binds with High Affinity and Induces T-cell Activation and Proliferation



A) Binding of APVO436 to CD123 and CD3 was assessed by flow cytometry on human CD123 expressing Molm-13 cells and human CD3+ T-cells. B) T-cell activation and C) T-cell proliferation were assessed via multi-color flow cytometry after labeling with CD4, CD5, CD8, CD19, CD25, CD69 and 7AAD as appropriate at the end of the culture period. T-cell activation was quantified by measuring the percentages of CD4+ or CD8+ T-cells that had upregulated CD69 and CD25 after 20 hours of culture. To monitor proliferation PBMCs were labeled with CFSE at the start of the culture and after 96 hours 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 CFSE profile.

APVO436 Induces Target-Dependent Cell Lysis at Low Effector to Target Ratios

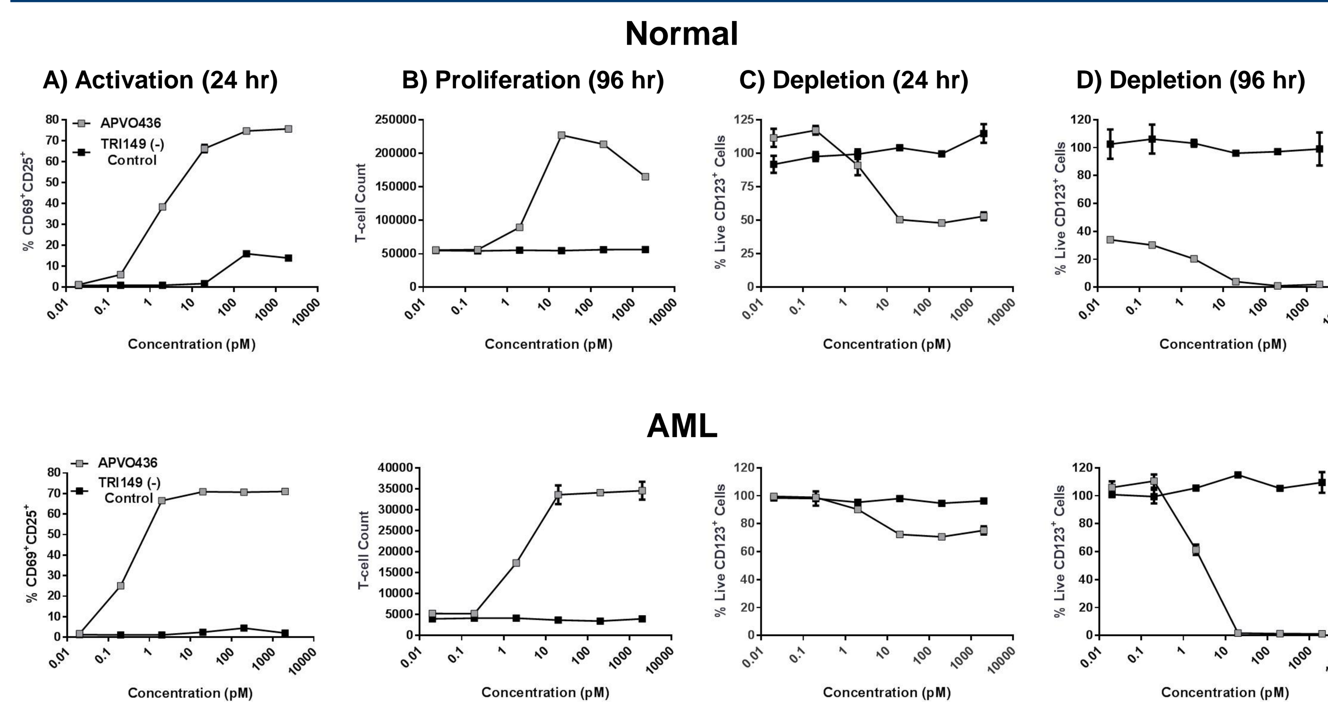


Target Cell	EC_{50} (4 hr)
Molm-13	12.4 pM
KG-1a	23.3 pM

E:T Ratio	10:1	5:1	1:1
Molm-13	4.2 pM	8.7 pM	33 pM
KG-1a	3.7 pM	7.1 pM	20 pM

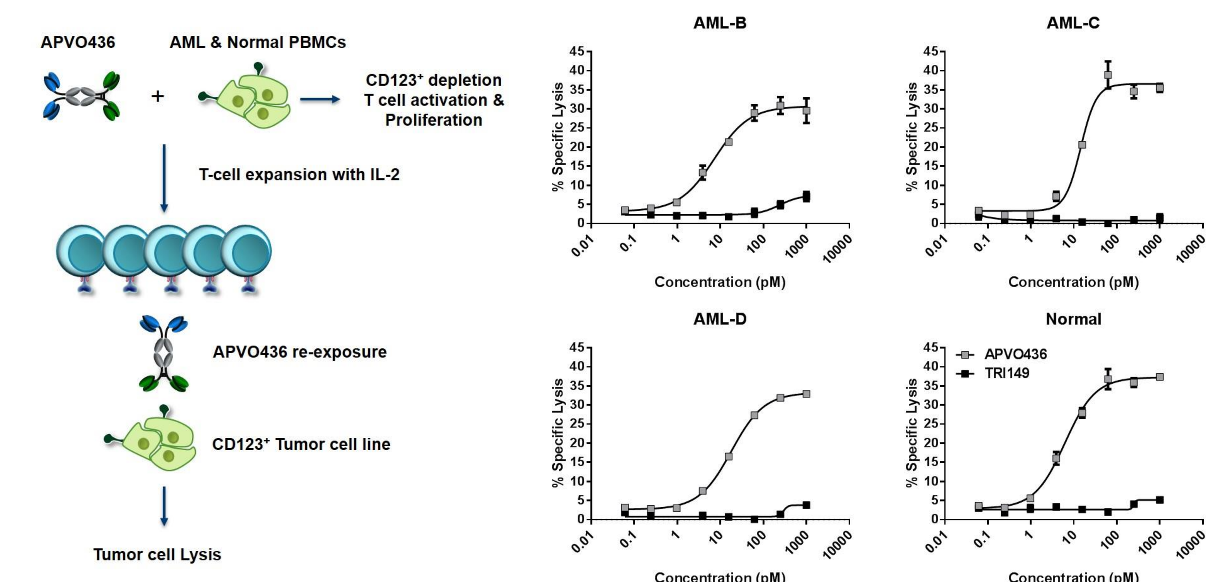
(A) CD123 expression levels on MOLM-13 and KG-1a AML tumor cells lines were compared to the CD123 negative Daudi Burkitt's lymphoma tumor cell line by flow cytometry. Positive CD123 expression is indicated in blue compared to isotype control in green or unstained cells in black. (B) Molm-13, KG-1a or Daudi cell lines were loaded with chromium-51 (^{51}Cr) and incubated with APVO436 and purified human T-cells pre-activated with Human T-Activator CD3/CD28 Dynabeads (Gibco) for 4 hours. (C) ^{51}Cr loaded Molm-13 and KG-1a cell lines were incubated with APVO436 and pre-activated purified human T-cells at several effector to target cell (E:T) ratios for 24 hours.

APVO436 Induces Endogenous T-cell Activation, Proliferation and Depletion of CD123+ Cells in Normal and AML Subject Samples



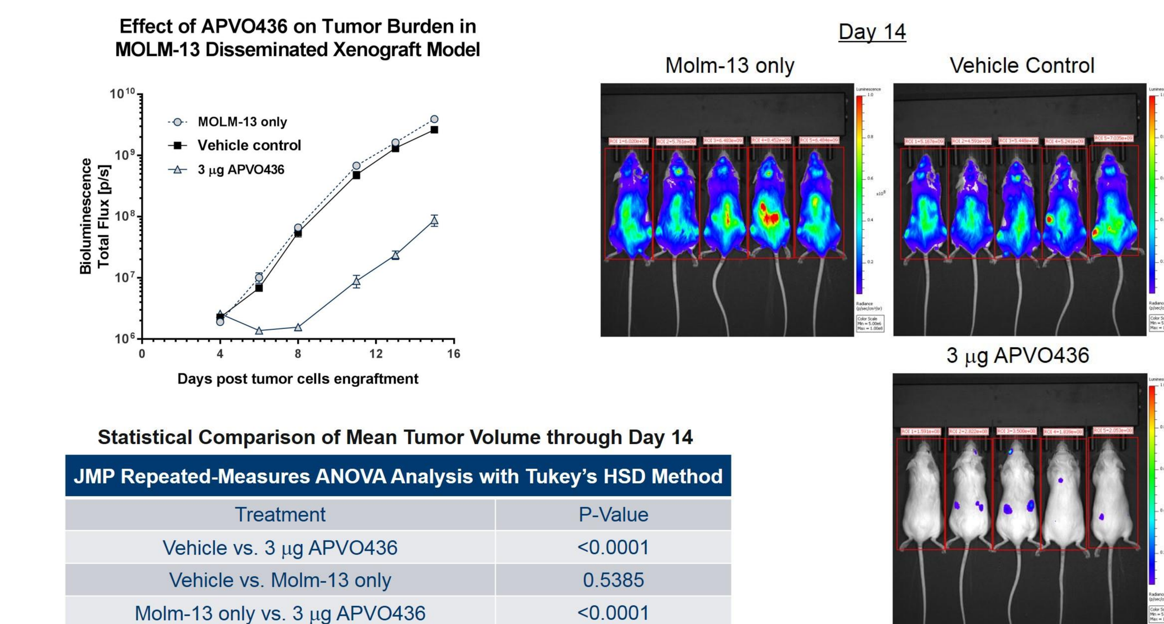
PBMC samples were cultured with APVO436 or the negative control ADAPTIR TRI149. T-cell activation, T-cell proliferation and depletion of CD123+ cells was assessed via multi-color flow cytometry after labeling with CD5-APC, CD19-PacBlue, CD25-PE-Cy7, CD33-AF700, CD69-FITC, CD123-PE and 7AAD. A) T-cell activation was quantified by measuring the percentage of CD69+CD25+ cells in the CD5+CD19- gate after 24 hours of culture. B) T-cell proliferation was measured by counting the total number of live CD5+CD19- T cells after 96 hours of culture. Depletion of CD123+ cells was assessed by gating on live CD123+ cells and normalizing to untreated sample at C) 24 hr and D) 96 hr.

T Cells Demonstrate Potent RTCC Activity upon Re-exposure to APVO436 and CD123+ Tumor Cells



PBMC samples from AML subjects or normal donors were cultured with APVO436 or the negative control ADAPTIR TRI149 for 96 hours at which point the cultures were harvested for various assessments. Expanded T cells from the cultures were incubated with human IL-2 for an additional 5 days then co-cultured with ^{51}Cr loaded Molm-13 cells and APVO436 or the negative control ADAPTIR TRI149 at an E:T ratio of 5:1 for 4 hours.

APVO436 Significantly Reduces Established Disseminated Skeletal Tumor Burden in NSG Mice

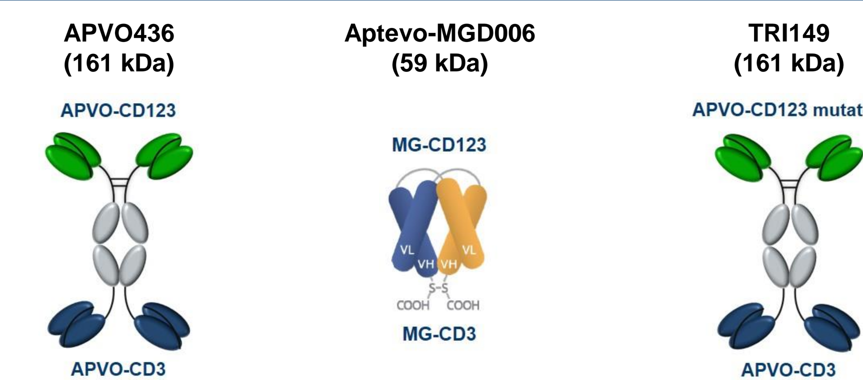


Statistical Comparison of Mean Tumor Volume through Day 14

JMP Repeated-Measures ANOVA Analysis with Tukey's HSD Method	
Treatment	P-Value
Vehicle vs. 3 µg APVO436	<0.0001
Vehicle vs. Molm-13 only	0.5385
Molm-13 only vs. 3 µg APVO436	<0.0001

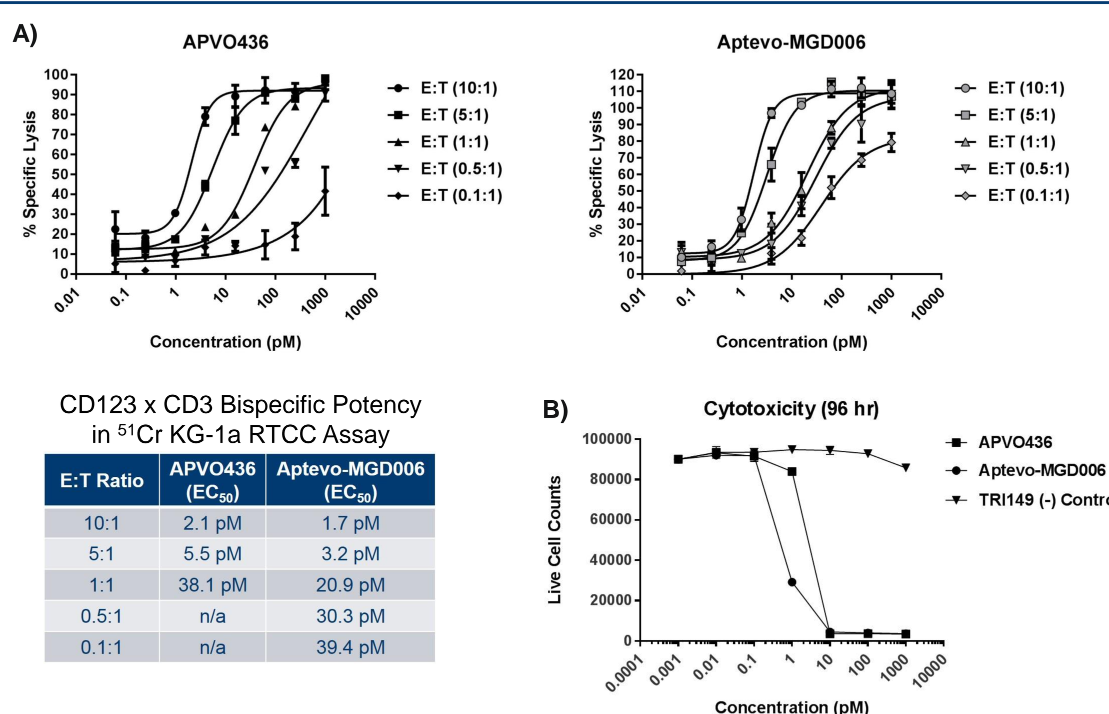
MOLM-13 Luc cells (1 x 10^5/mouse) were injected intravenously into the lateral tail vein of 24 NSG male mice. MOLM-13 Luc cells were allowed to establish in the mice for 4 days prior to initiation of treatments. Mice were assigned into 3 groups of 8 mice each. Treatments consisted of PBS vehicle control or APVO436 at 3 µg on days 4, 8 and 12. One group received no additional treatment as a control. Treatments were administered intravenously via the tail vein in 0.2 mL of Dulbecco's Phosphate Buffered Saline (PBS) containing no molecule (vehicle control) or 3 µg of APVO436. For the day 4 treatment only, 7 million purified human T cells were co-administered with treatment. Tumor growth measures were taken using IVIS Bioluminescent imaging.

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 flotuzumab (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)

APVO436 and Aptevo-MGD006 Induce Comparable T-cell Cytotoxicity

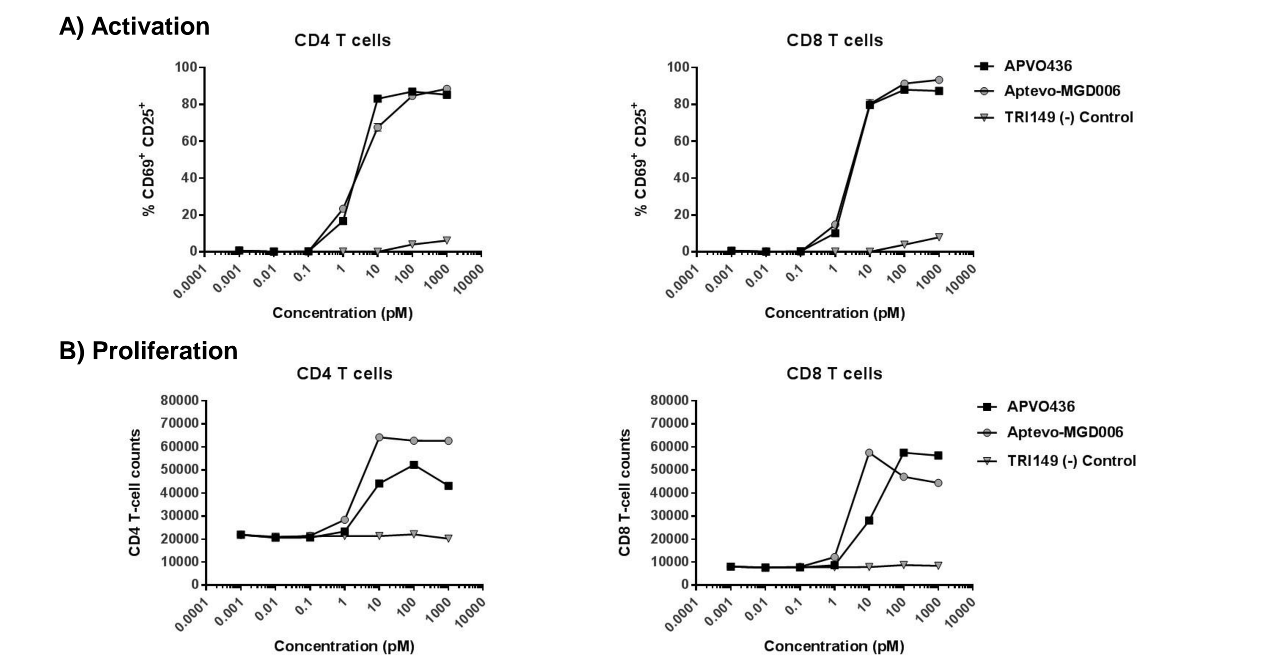


CD123 x CD3 Bispecific Potency in ^{51}Cr KG-1a RTCC Assay

E:T Ratio	APVO436 (EC_{50})	Aptevo-MGD006 (EC_{50})
10:1	2.1 pM	1.7 pM
5:1	5.5 pM	3.2 pM
1:1	38.1 pM	20.9 pM
0.5:1	n/a	30.3 pM
0.1:1	n/a	39.4 pM

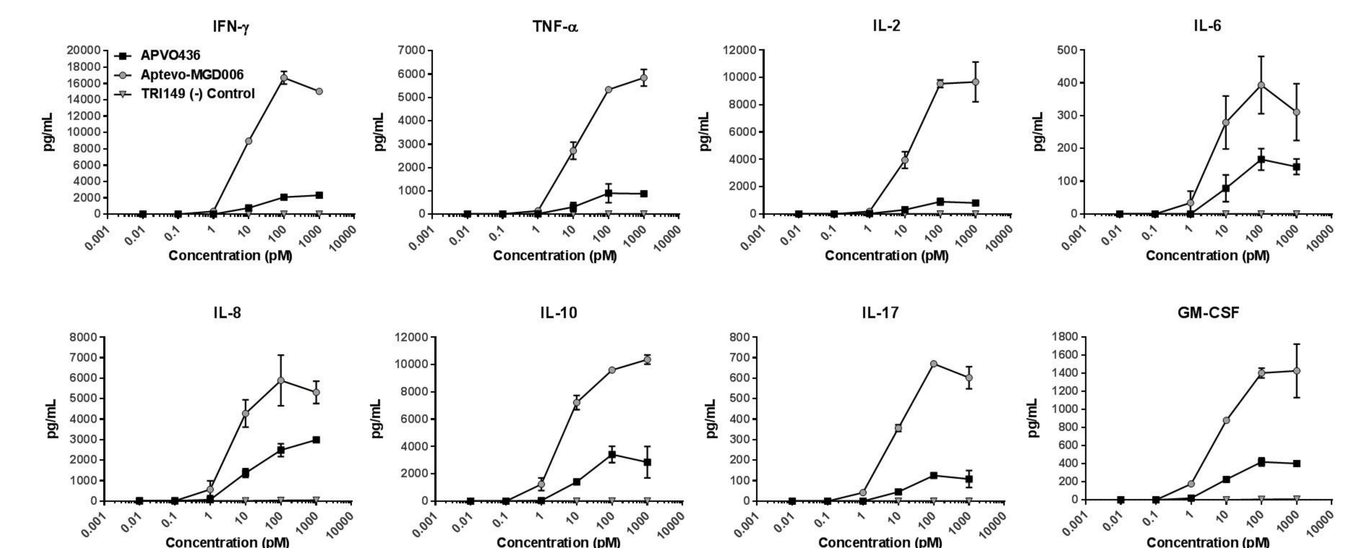
(A) KG-1a cells were loaded with chromium-51 (^{51}Cr) and cultured with APVO436 or Aptevo-MGD006 and purified T cells isolated from normal donor peripheral blood at various effector to target cell ratios for 48 hours. (B) Purified T cells isolated from normal donor peripheral blood were cultured with Molm-13 tumor cells and APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149. Cytotoxicity of Molm-13 target cells in the cultures was assessed using multi-color flow cytometry. After 4 days, live Molm-13 target cell numbers were counted by gating on 7AAD negative CD5 negative cells.

APVO436 and Aptevo-MGD006 Induce Efficient Activation and Proliferation of Purified T cells



Purified T cells isolated from normal donor peripheral blood were cultured with Molm-13 tumor cells and APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149. (A) 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) Proliferation of T cells in the cultures was quantified after 4 days using multi-color flow cytometry by gating on CD4+ or CD8+ T cells and enumerating total T cell counts for each population.

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



Purified T cells isolated from normal donor peripheral blood were cultured with Molm-13 tumor cells and APVO436, Aptevo-MGD006 or the negative control ADAPTIR TRI149 for 24 hours. Levels of several cytokines commonly produced by activated T cells were measured in the 24-hour culture supernatants using multiplexed analyte assays. Not shown IL-4, IL-12, IL-13 and IL-1β.

Summary and Conclusions

- APVO436 bound human CD123 and CD3-expressing cells with EC_{50} values in the low nM range and demonstrated potent target specific activity against CD123 expressing tumor cell lines at low effector to target ratios.
- APVO436 induced endogenous T-cell activation and proliferation accompanied by CD123+ cell depletion in experiments with primary AML subject and normal donor samples. The resulting expanded T cells from both normal and AML subject samples demonstrated potent cytotoxic activity in the presence of CD123+ tumor cells upon re-exposure to APVO436.
- In male NSG mice with established disseminated Molm-13 tumors and IV-implanted human T cells, APVO436 treatment resulted in a rapid and significant reduction in skeletal tumor burden indicating migration and engagement of T cells at the tumor site.
- APVO436 was compared to an Aptevo-generated version of Macrogenics' CD123 x CD3 dual-affinity re-targeting (DART) molecule MGD006. Across multiple T-cell donors, APVO436 and MGD006 induced comparable tumor lysis and T-cell activation. However, APVO436 induced lower levels of multiple cytokines when T cells were stimulated in the presence of CD123+ tumor cells.

Conclusions: These studies demonstrate the anti-CD123 x anti-CD3 ADAPTIR molecule APVO436 is a potent inducer of redirected T-cell killing of AML tumor cells both *in vitro* and *in vivo*. APVO436 potently induces T-cell activation, proliferation and CD123+ cell depletion with limited levels of T-cell cytokine release compared to another CD123 x CD3 targeting bispecific format suggesting a potential safety advantage. These data are supportive of further investigation of APVO436 as a potential treatment option for AML and other hematological malignancies. GLP toxicology studies have been completed in non-human primates and APVO436 is advancing to clinical testing.