AACR Annual Meeting April 2017 – Abstract Number 597

Bispecific anti-CD123 x anti-CD3 ADAPTIR™ Molecules for Redirected T-cell Cytotoxicity in Hematological Malignancies

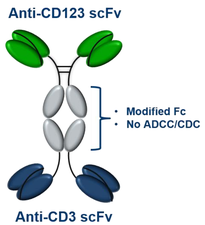
Michael R. Comeau, Danielle Mitchell, Rebecca Gottschalk, Lynda Misher, Mollie Daugherty, Lara Parr, Peter Pavlik, Brian Woodruff, Hang Fang, Megan Aguilar, Gary Li, Jeannette Bannink, Starrla Johnson, Robert E. Miller, Robert Bader, Nicole Zhang, Toddy Sewell, Maria Dasovich, Gabriela H. Hoyos, John W. Blankenship, Catherine McMahan, David Bienvenue and Jane A. Gross

Introduction

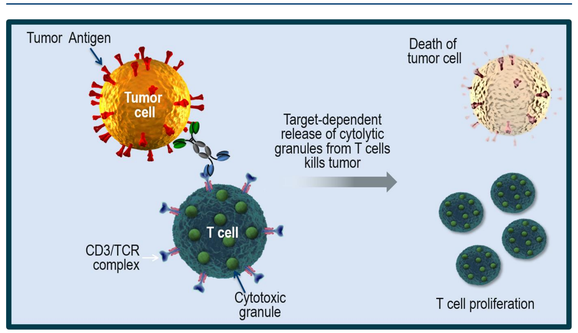
CD123 is a component of the IL-3 receptor expressed in several hematological malignancies including AML, ALL, HCL, and MDS. CD123 is a compelling target in AML due to its overexpression on AML blasts as well as leukemic stem cells, which are thought to be resistant to chemotherapy and may be responsible for relapse of disease following treatment[1, 2]. While CD123 is expressed by some normal leukocyte populations in circulation and hematopoietic progenitor cells in the bone marrow[3], the low frequency of expression on normal cell types provides a therapeutic window for targeting CD123 in tumor settings with the potential for durable response and reversible side effects. We have developed bispecific anti-CD123 x anti-CD3 ADAPTIR molecules APVO436 and APVO437 for redirecting T-cell cytotoxicity to CD123-expressing tumor cells. Results are presented that examine the *in vitro* and *in vivo* activity of these molecules in preclinical models of AML.

ADAPTIR Molecules Targeting CD123 and CD3

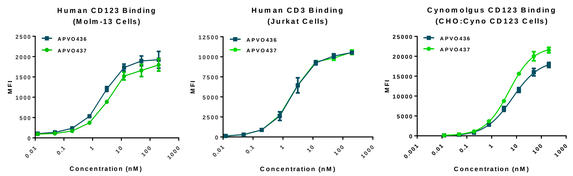
ADAPTIR molecules are bispecific antibody-like therapeutics containing two sets of binding domains linked to immunoglobulin Fc domains to extend the half-life of the molecule *in vivo*. The anti-CD123 x anti-CD3 ADAPTIR molecules bind both CD123 and CD3 to redirect T-cell cytotoxicity against CD123 expressing 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 adapted 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.



Redirected T Cell Cytotoxicity (RTCC) by anti-CD123 and anti-CD3



Binding Studies



A) Binding Affinity by Flow Cytometry

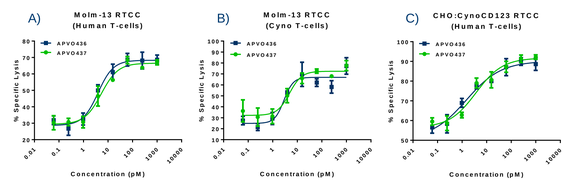
ADAPTIR	Molm-13 (Human CD123)	Jurkat (Human CD3)	CHO (Cyno CD123)
APVO436 (EC_{50})	2 nM	2 nM	7 nM
APVO437 (EC_{50})	3 nM	2 nM	5 nM

B) Binding Affinity by SPR

ADAPTIR	Ka (1/Ms)	Kd (1/s)	K_D (nM)
APVO436	1.6×10^5	3.7×10^{-4}	2
APVO437	1.7×10^5	1.8×10^{-3}	11

A) Binding of ADAPTIR molecules to CD123 and CD3 was assessed by flow cytometry on relevant target-expressing cell lines including human CD123 expressing Molm-13 cells, human CD3 expressing Jurkat cells and CHO cells stably expressing full length cynomolgus CD123 protein. B) ADAPTIR molecules binding to recombinant human CD123 ectodomain was assessed by surface plasmon resonance (SPR).

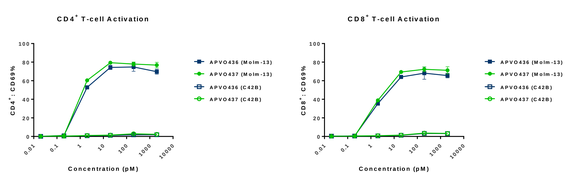
In Vitro: Redirected T-cell Cytotoxicity (RTCC)



ADAPTIR	Molm-13 (Hu T-cells)	Molm-13 (Cyno T-cells)	CHO:Cyno CD123 (Hu T-cells)
APVO436 (EC_{50})	4 pM	2 pM	3 pM
APVO437 (EC_{50})	5 pM	4 pM	5 pM

Molm-13 (human CD123$^+$) target cells (A,B) or CHO cells stably expressing cynomolgus CD123 (C) were loaded with chromium-51 (^{51}Cr) and incubated with anti-CD123 x anti-CD3 ADAPTIR proteins with either human or cynomolgus T-cells. The percentage of target cell lysis was measured by specific ^{51}Cr release into the supernatant as compared to total target cell lysis with NP-40. The Y-axes in each graph start at the observed level of % lysis with effector + target cells alone.

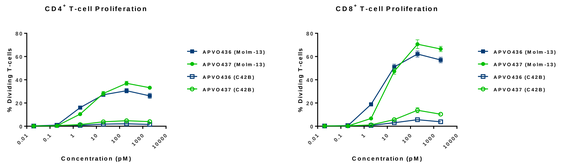
In Vitro: Target Dependent T-cell Activation



ADAPTIR	CD4$^+$ T-cells	CD8$^+$ T-cells
APVO436 (EC_{50})	1 pM	2 pM
APVO437 (EC_{50})	1 pM	2 pM

T-cell activation was assessed via flow cytometry by culturing the CD123(+) Molm-13 cell line, and the CD123(-) C42B cell line with purified human T-cells. After 20 hours, cells were labeled with CD69-FITC, CD5-PE, CD8-Pacific Blue, CD4-APC, CD25-PE-Cy7 antibodies and 7AAD for flow cytometric analysis prior to acquisition using a BD LSRII flow cytometer. The sample files were analyzed using FlowJo software to calculate the percentages of CD4$^+$ (CD8$^-$)or CD8$^+$ T-cells that had upregulated CD69, by gating sequentially on forward vs side scatter, 7AAD$^-$, CD5$^+$, CD4$^+$ or CD8$^+$ T-cells (7AAD$^-$, CD5$^+$ CD4$^+$ or 7AAD$^-$ CD5$^+$ CD8$^+$, respectively).

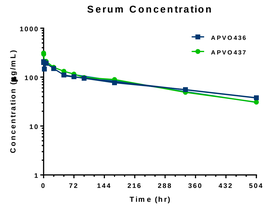
In Vitro: Target Dependent T-cell Proliferation



ADAPTIR	CD4$^+$ T-cells	CD8$^+$ T-cells
APVO436 (EC_{50})	2 pM	4 pM
APVO437 (EC_{50})	5 pM	11 pM

T-cell proliferation was assessed via flow cytometry by culturing the CD123(+) Molm-13 cell line, and the CD123(-) C42B cell line with purified human T-cells. Molm-13 and C42B cells were irradiated to prevent cell division. Proliferation was assessed by labeling isolated T-cell populations with CFSE. After 4 days, cells were labeled with CD4-APC, CD5-PE, CD8-Pacific Blue, CD25-PE-Cy7 antibodies and 7AAD for flow cytometric analysis prior to acquisition using a BD LSRII flow cytometer. The sample files were analyzed using FlowJo software to calculate the percentages of CD4$^+$ (CD8$^-$) or CD8$^+$ T-cells that had undergone at least one cell division, according to their CFSE profile, by gating sequentially on forward vs side scatter, 7AAD$^-$, CD5$^+$, CD4$^+$ or CD8$^+$ T-cells (7AAD$^-$, CD5$^+$ CD8$^-$ or 7AAD$^-$ CD5$^+$ CD8$^+$, respectively).

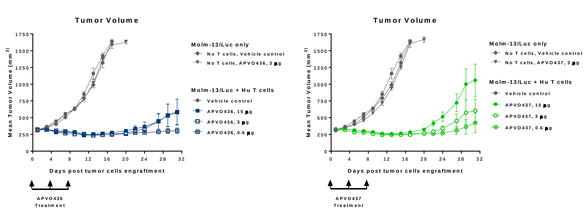
In Vivo: APVO436 and APVO437 Have Antibody-Like Half-Lives in Balb/c Mice



Parameter	APVO436	APVO437
T ½	301 hours (12.5 days)	229 hours (9.5 days)
Clearance	0.186 ml/hr/kg	0.204 ml/hr/kg
Volume	80.84 ml/kg	67.54 ml/kg
AUC	37309 hr*µg/ml	38750 hr*µg/ml

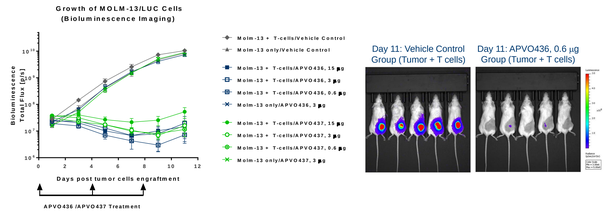
To determine the pharmacokinetic activity of bispecific molecules, female Balb/c mice were injected intravenously (IV) with 200 µg (~10 mg/kg) of either APVO436 or APVO437 (n=30 mice for each molecule). At each time point, blood was collected by cardiac puncture from 3 mice. Time points were: 15 minutes, 2, 6, 24, 48, 72, 96, 168, 336 and 504 hours. Blood was processed to serum, aliquoted and frozen. APVO436 and APVO437 concentrations in serum samples were determined by enzyme linked immunosorbent assay (ELISA). Serum concentrations over time were used to determine PK parameter estimates by non-compartmental analysis (NCA) and compartmental analysis. Serum concentration over time profiles were analyzed with Phoenix 64 software.

In Vivo: APVO436 and APVO437 Inhibit Molm-13 Tumor Growth



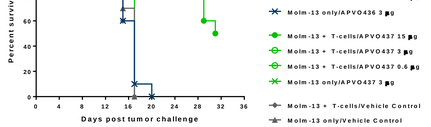
Female NOD SCID mice were implanted subcutaneously with 2 x 10^6 MOLM-13/LUC cells co-mixed with 1 x 10^6 human T-cells in Matrigel on Study Day 0. Animals were treated in groups of 10 mice per group with vehicle, APVO436 or APVO437 on study Day 0, 4 and 8 at doses of 15, 3 and 0.6 µg of protein (total volume of 200 µl). Animals were monitored daily for health condition and body weight measurements were taken 2-3 time per week. Tumor growth measures were taken with calipers 3 times per week. Tumor volumes were calculated using the formula: TV=1/2[length x (width)^2]. Endpoint = Tumors volume reached more than 1500 mm^3. Control groups reached endpoint by day 13; treated groups terminated on day 31.

In Vivo: APVO436 and APVO437 Inhibit Molm-13 Tumor Growth



Female NOD SCID mice were implanted subcutaneously with 2 x 10^6 MOLM-13/LUC cells co-mixed with 1 x 10^6 human T-cells in matrigel on Study Day 0. Animals were treated in groups of 10 mice per group with vehicle, APVO436 or APVO437 on study Day 0, 4 and 8 at doses of 15, 3 and 0.6 µg of protein (total volume of 200 µl). Tumor growth measures were taken by IVIS Bioluminescent imaging 3 times per week. Representative images of APVO436, 0.6 µg treated group are shown.

In Vivo: APVO436 and APVO437 Prolong Survival in Molm-13 Tumor Model



Comparison of Survival Curves: Log-rank (Mantel-Cox) test

Treatment Group	Median Survival Time (Days)	Survival Relative to Molm-13 + T-cells/Vehicle Control (P value)
Molm-13 + T-cells/Vehicle	17	(-)
Molm-13 + T-cells/APVO436 15 µg	Undefined	<0.0001
Molm-13 + T-cells/APVO436 3 µg	Undefined	<0.0001
Molm-13 + T-cells/APVO436 0.6 µg	Undefined	<0.0001
Molm-13 + T-cells/APVO437 15 µg	Undefined	0.0004
Molm-13 + T-cells/APVO437 3 µg	Undefined	<0.0001
Molm-13 + T-cells/APVO437 0.6 µg	Undefined	<0.0001
Molm-13 only/Vehicle	17	0.5138
Molm-13 only/APVO436	17	0.0772
Molm-13 only/APVO437	17	0.5310

Summary and Conclusions

- APVO436 and APVO437 bound human and cynomolgus CD123-expressing cells with EC_{50} values in the low nM range and SPR studies demonstrated that both ADAPTIR proteins bound human CD123 protein with high affinity.
- Both APVO436 and APVO437 induced concentration-dependent lysis of CD123$^+$ AML cell lines with primary human effector T-cells, accompanied by T-cell activation and proliferation. Comparable redirected T-cell cytotoxicity function was observed using primary cynomolgus macaque T-cells. These activities were dependent on the expression of CD123 by the tumor target cells.
- In vivo, pharmacokinetic analysis demonstrated serum half-lives of 12.5 days for APVO436 and 9.5 days for APVO437 in Balb/c mice and growth of AML tumor cells was inhibited by treatment with low doses of APVO436 and APVO437, significantly improving host survival.

Conclusions: These studies demonstrate the anti-CD123 x anti-CD3 ADAPTIR molecules APVO436 and APVO437 are capable of potently inducing redirected T-cell killing of AML tumor lines both *in vitro* and *in vivo* and suggest further investigation of these proteins as potential therapeutics in hematological malignancies is warranted.

References
1. Jordan *et al* The interleukin-3 receptor alpha chain is a unique marker for human acute myelogenous leukemia stem cells. Leukemia. 2000 Oct;14(10):1777-84.
2. Busfield *et al* Targeting of acute myeloid leukemia in vitro and in vivo with an anti-CD123 mAb engineered for optimal ADCC. Leukemia. 2014 Nov;28(11):2213-21.
3. Testa *et al* CD 123 is a membrane biomarker and a therapeutic target in hematologic malignancies. Biomarker Research (2014) 2:4.