Bispecific Anti-CD123 x Anti-CD3 ADAPTIR™ Molecules APVO436 and APVO437 Have Broad Activity Against Primary Human AML Cells *In Vitro*

Colin D. Godwin[1], Olivia M. Bates[2], George S. Laszlo[2], Rebecca Gottschalk[3], Michael R. Comeau[3], Gabriela H. Hoyos[3], and Roland B. Walter[2,4,5]

[1]Hematology/Oncology Fellowship Program, University of Washington, Seattle, WA, USA; [2]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [3]Aptevo Therapeutics, Inc., Seattle, WA, USA; [4]Department of Medicine, Division of Hematology, University of Washington, Seattle, WA, USA; [5]Department of Epidemiology, University of Washington, Seattle, WA, USA

Introduction

The IL-3 receptor α-chain CD123 is an attractive target for antibody-based therapies in acute myeloid leukemia (AML) because of its frequent expression on AML blasts and its reported overexpression on leukemic stem cells (LSCs) as compared to normal hematopoietic tissues. This has led to development of multiple antibody-based therapies for AML targeting CD123. Here, we present results using the novel bispecific anti-CD123 x anti-CD3 targeting ADAPTIR molecules APVO436 and APVO437 to target AML patient specimens *in vitro*.

Figure 1 - ADAPTIR Molecules Targeting CD123 and CD3

Figure 1 – 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.

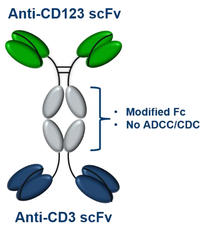

Table 1 – Characteristics of AML Patient Specimens

Patient #	Age	Disease stage	Gender (M/F)	Specimen source	Cytogenetics	NPM1 mutation status	FLT3-ITD status	CEBPA mutations status
1	65	Diagnosis	F	Apheresis	46,XX[10]	Pos	Pos	Neg
2	63	Diagnosis	F	Apheresis	46,XX,i(3;5)(q21;q22)[9]/46,XX[11]	Neg	Neg	Pos
3	76	Diagnosis	F	Apheresis	46,XX[5]	Pos	Neg	ND
4	58	Diagnosis	M	Apheresis	46,XY,t(6;11;17)(q25;q23;q21)[20]	Neg	Neg	Neg
5	52	Diagnosis	F	PB	46,XX[20]	Neg	Neg	Neg
6	60	Refractory	M	BM	46,XY[20]	Pos	Pos	ND
7	40	Relapse	F	PB	46,XX[20]	Pos	Pos	ND
8		Diagnosis	F	PB	46,XX[20]	Pos	Pos	ND
9	54	Diagnosis	M	PB	46,XY,inv(16)(p13.1q22)[20]	Neg	Neg	Neg
10	59	Relapse	M	PB	45,X,-Y,del(5)(q13q14),del(10)(q22q25.2)[10]/46,XY[10]	Neg	Neg	ND
11	74	Diagnosis	F	BM	91,XXXX,-5,del(6)(q21q25)[20].	Neg	Neg	ND
12	48	Refractory	M	PB	46,XY[20]	ND	Neg	ND
13	48	Refractory	M	PB	46,XY,t(11;12)(q13;p13)[9]	Neg	Neg	Neg
14	51	Diagnosis	M	PB	46,XY[20]	Pos	Pos	Neg
15	48	Relapse	M	BM	47,XY,+8[20]	Neg	Pos	ND
16	81	Diagnosis	M	Apheresis	46,XY[20]	Pos	Pos	ND
17	76	Relapse	M	BM	46,XY,t(2;11)(p21;q23)	Neg	Neg	ND
18	61	Diagnosis	M	PB	50,XY,t(8;12)(q21;p11.2),+del(9)(q21q31),+11,add(21)(q22),+mar1x[6]/50,x,add(2)(q37),add(4)(q22),+add(5)(q33)[8]/48,sl,+6,mar1x[2]/51,sl,+11[2]/46,XY[2]	Neg	Neg	ND
19	31	Diagnosis	M	PB	46,XY,inv(16) (p13.1q22)[20]	Neg	Neg	ND
20	69	Refractory	F	PB	46, XX[19]	Neg	Pos	Neg

Highlighted specimens are those indicated by the red box in Figure 2A.

PB, peripheral blood; BM, bone marrow; ND, not done.

Conflict of Interest Disclosure

Disclosures: Gottschalk: *Aptevo Therapeutics:* Employment, Equity Ownership. **Comeau:** *Aptevo Therapeutics:* Employment, Equity Ownership. **Hoyos:** *Aptevo Therapeutics:* Employment, Equity Ownership. Research Funding: *Aptevo Therapeutics*. All other authors declare no conflicts of interest.

Figure 2 – Characteristics of AML Patient Specimens

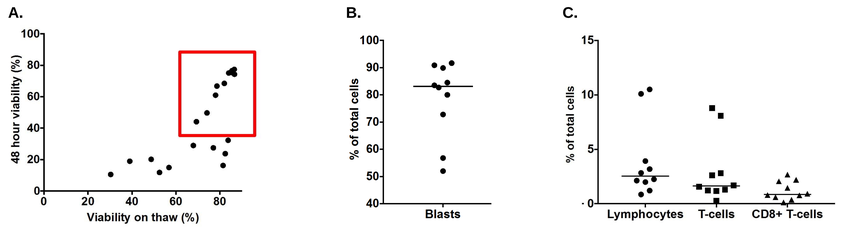

Figure 2 – A. Specimen viability on thaw and at 48 hours. Red box shows samples with thaw viability >60% and 48 hour viability >40% which are included in subsequent analysis. **B.** Percentage of blasts in patient samples, median is shown. **C.** Percentage of lymphocytes and subsets in patient samples, median is shown.

Figure 3 – APVO436 and APVO437 Broadly Induce Cytotoxicity in AML Patient Specimens in a Concentration and E:T-dependent Manner

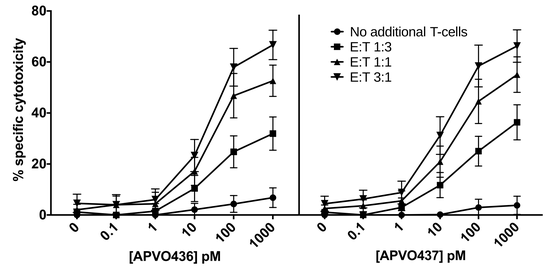

Figure 3 – AML specimens were incubated for 48 hours with APVO436 (left panel) and APVO437 (right panel) with AML patient specimens and healthy donor T-cells at the effector:target (E:T) ratios and drug concentrations shown. Mean and S.E.M. for the 10 specimens included in the red box in figure 2A are plotted.

Figure 4 – Number of Endogenous T Cells Does Not Correlate With Cytotoxicity in the Absence of Exogenous T Cells

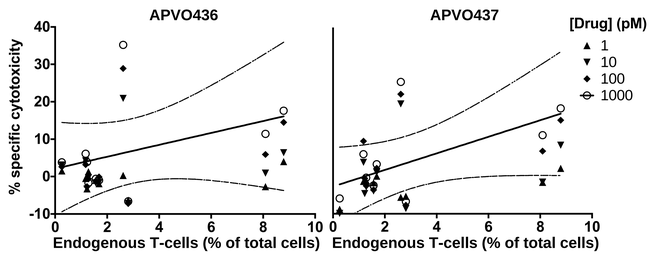

Figure 4 – AML specimens were incubated for 48 hours with APVO436 (left panel) and APVO437 (right panel) at the concentrations shown, with cytotoxicity plotted on the y-axis and the percentage of endogenous T-cells plotted on the x-axis. Linear regressions (solid lines) and 95% confidence intervals (dotted lines) correlating cytotoxicity to T-cell number for the 1000 pM drug dose for the 10 specimens included in the red box in figure 2A are shown. For APVO436, R^2=0.15 (p=0.26) and for APVO437, R^2=0.34 (p=0.80)

Materials and Methods

Frozen peripheral blood or bone marrow specimens were obtained from adults with AML. AML samples were incubated with various concentrations of ADAPTIR constructs and healthy donor T-cells at different effector:target (E:T) cell ratios. Cytotoxicity was measured using DAPI to detect non-viable cells. T-cell activation, proliferation, depletion of CD123$^+$ cell populations, CD123 expression, and lymphocyte populations in AML specimens were assessed using multi-color flow cytometry.

Figure 5 – APVO436 and APVO437 Induce Robust Cytotoxicity in Samples with Low Blast CD123 Expression

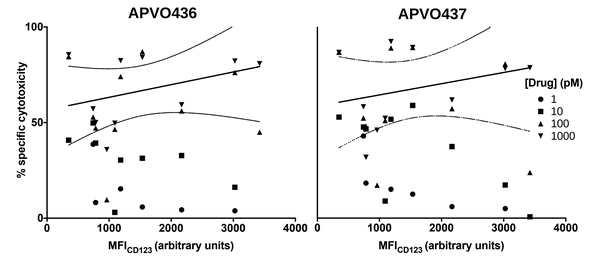

Figure 5 – AML specimens were incubated for 48 hours with APVO436 (left panel) and APVO437 (right panel) at the concentrations shown with healthy donor T-cells at an E:T ratio of 3:1, with cytotoxicity plotted on the y-axis and the median fluorescence intensity of CD123 (MFI_{CD123}) on blasts plotted on the x-axis. Linear regressions (solid lines) and 95% confidence intervals (dotted lines) correlating cytotoxicity to $MFI_{CD123}$for the 1000 pM drug dose are shown. For APVO436, R^2=0.14 (p=0.29) and for APVO437, R^2=0.08 (p=0.411).

Figure 6 – APVO436 Induces Endogenous T-cell Activation, Proliferation and Depletion of CD123$^+$ Cells in Normal and AML Samples

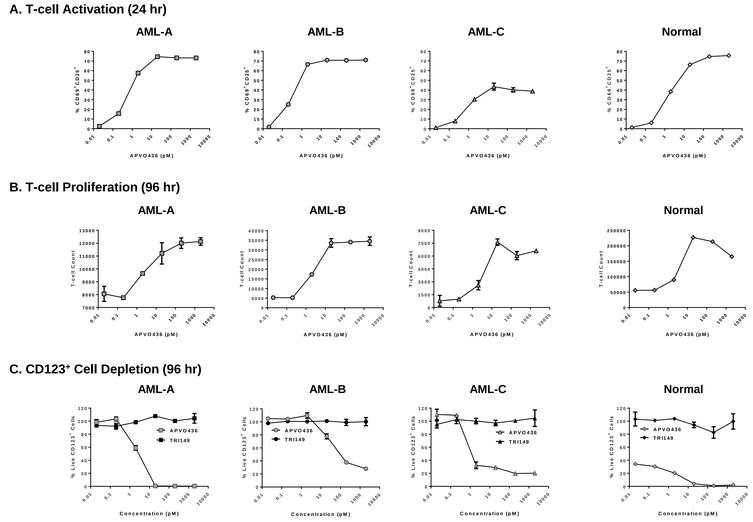

Figure 6 - PBMC samples were cultured with APVO436 or the negative control ADAPTIR TRI149. T-cell activation, T-cell proliferation and depletion of CD123$^+$ cells was assessed via multi-color flow cytometry after labeling with CD5-APC, CD19-PacBlue, CD25-PE-Cy7, CD33-AF700, CD69-FITC, CD123-PE and 7AAD. **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. **C.** Depletion of CD123$^+$ cells was assessed by gating on live CD123$^+$ cells and normalizing to untreated sample.

Summary and Conclusions

APVO436 and APVO437 induce target cell cytolysis in a dose-dependent, target-antigen-dependent, and E:T-dependent manner. Broad activity was seen in primary human AML samples and both constructs were effective at inducing cytotoxicity across a range of blast CD123 expression levels, including specimens with very limited CD123 expression. Endogenous T-cell number was not clearly correlated with cytotoxicity in the absence of exogenous T-cells possibly due to the small sample size. Furthermore, endogenous T-cell activation and proliferation accompanied by CD123$^+$ cell depletion demonstrate similar activity of anti-CD123 x anti-CD3 ADAPTIRs in both normal and AML subject samples. These data are supportive of further investigation of anti-CD123 x anti-CD3 ADAPTIR molecules as a potential treatment option for AML.