Ex Vivo Activity Profile of the CD123xCD3 Duobody® Antibody Against Primary Acute Myeloid Leukemia Bone Marrow Samples

Ann Forslund[1], Khaja Syed[1], Amy Axel[1], Ronan McDaid[1], Yingzhe Li[1], Juan Ballesteros[2], Francois Gaudet[1], Ricardo M. Attar[1], Mark Salvati[1], Fei Huang[1], and Kate Sasser[1]

[1]Janssen Pharmaceuticals Research & Development, Spring House, PA; [2]Vivia Biotech, Madrid, Spain

BACKGROUND

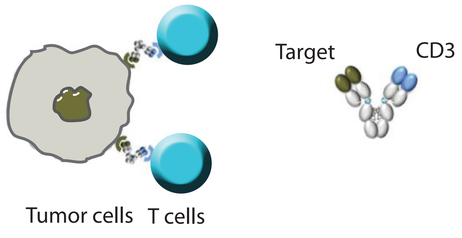
Acute myeloid leukemia (AML) is a hematologic malignancy that affects the normal production of neutrophils, red blood cells, and platelets. CD123 (IL-3 receptor alpha) is over-expressed on AML leukemic stem cells (LSCs) and blasts compared with normal hematopoietic progenitor cells, and represents a promising target of antibody therapies for AML (Jordon et al. Leukemia 2000;14:1777-84).

The outcome for patients with high-risk AML remains poor, and effective therapeutics are desperately needed in this patient population. Janssen is exploring CD123 as a potential target for the generation of a promising new bi-specific antibody (Ab) that recruits T cells to tumor cells through a tumor-specific antigen binding arm and a CD3-specific arm.

CNTO 9958

- Humanized IgG4-PAA bi-specific duobody **(Fig. 1)**
- GenMab Technology
- Recognizes and inhibits CD123 signaling on myeloid cells
- Recognizes and binds CD3 on T cells
- Binding and proximity activates cytotoxic T cells resulting in lysis of AML blasts and LSCs

Figure 1. CNTO 9958, humanized IgG4-PAA bi-specific duobody.



- CNTO 9253 (NullxCD3) was used as a negative control

METHODS

Goal:

- Determine if CD123xCD3 duo Ab has efficacy in primary AML/MDS (myelodysplastic syndrome) samples

Objectives:

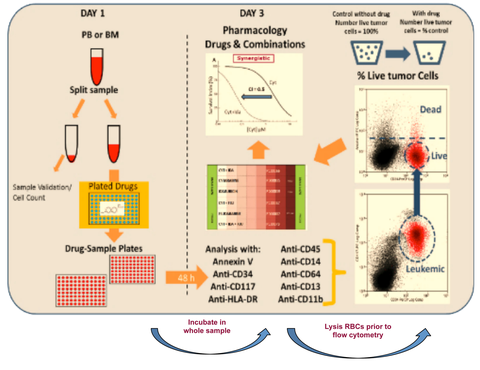
- To determine whether AML blast cells are depleted by CD123xCD3 Ab
- To determine whether T cells are activated by CD123xCD3 Ab
- To investigate the effect of different effector to target cell (E:T) ratios on efficacy of CD123xCD3 Ab
- To explore the selective effect of the CD123xCD3 Ab compared with the NullxCD3 Ab to deplete CD123+ AML cells

Vivia Method

To evaluate ex vivo effects of these bi-specific Abs on primary samples from AML patients, bone marrow (BM) samples were collected, received within 24–48 h at Vivia Biotech, and analyzed by Vivia's proprietary ExviTech flow cytometry system **(Fig. 2)**.

This platform preserves the whole BM native environment, retaining all cell types, matrix, and proteins important for long-term growth of primary human BM with CD123 receptor density on blast cells ranging from the lowest (1759 receptors/cell) to the highest (13315 receptors/cell). Following culturing with CNTO 9958, aspirate and blood were extensively profiled by an automated multi-parameter flow cytometry system at Vivia.

Figure 2. Vivia ExviTech Platform.



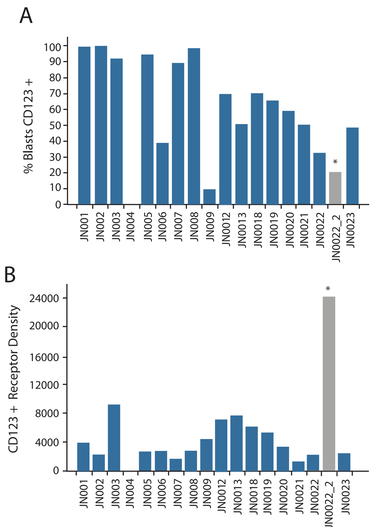
Parameters measured by Vivia Experiments

- CD123 expression (MFI, receptor density)
- E:T ratio
- T-cell panel: CD4, CD8, CD25
- Proliferation/depletion of AML blasts and T cells
- Time points for analysis were 72 h, 96 h, and 120 h

RESULTS

- There was marked inter-patient variability in CD123 expression and receptor density at baseline **(Fig. 3, Table 1).**

Figure 3. Inter-patient Variability in CD123 Expression. (A) % blasts CD123+; (B) CD123 receptor density.



*The gray bars for sample JN0022 (JN0022_2) represent the same sample, but 2 different blast populations.

Table 1. CD123 Expression and E:T ratios for 17 Fresh AML Bone Marrow Samples.

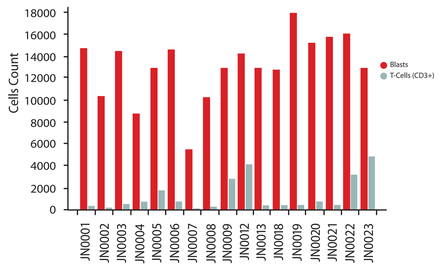
Sample	CD123+ve Blasts: % Blasts CD123+ve	CD123+ve Blasts: RD	Blasts: n	Blasts: % WBC	T Cells (CD3+): n	T Cells (CD3+): % WBC	Ratio E:T
JN001	99.3	3981	14712	91.47	318	1.98	1:46.3
JN002	99.54	2233	10370	51.28	191	0.94	1:54.3
JN003	92.13	9265	14449	70.53	591	2.88	1:24.4
JN004	0		8714	58.82	756	5.1	1:11.5
JN005	94.5	2708	12911	60	1839	8.55	1:7
JN006	38.8	2783	14598	82.86	796	4.52	1:18.3
JN007	89.19	1759	5477	86.4	163	2.57	1:33.6
JN008	98.12	2757	10259	82.09	306	2.45	1:33.5
JN009*	9.32	4398	12909	41.97	2846	9.25	1:4.5
JN0012	69.62	7035	14203	59.57	4169	17.49	1:3.4
JN0013	50.3	7721	12922	69.61	427	2.3	1:30.3
JN0018	69.81	6120.5	12810	40.96	493.5	1.58	1:26
JN0019	65.43	5360	17917.5	72.33	425	1.72	1:42.2
JN0020	58.93	3415.75	15215	60.06	846.5	3.34	1:18
JN0021	49.8	13315.5	15747	79.31	505	2.54	1:31.2
JN0022	32.5	2280.25	16022	50.86	3237.5	10.13	1:4.9
JN0023	48.46	2449.75	12891	52.41	4941.5	20.39	1:2.6

*JN0009 was only assessed at 4 h and 24 h.

E:T ratio = effector to target cell ratio; RD = receptor density; WBC = white blood cells.

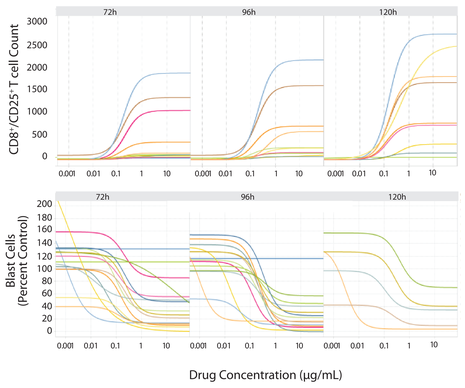
- Marked inter-patient variability in absolute number of T cells and blasts, as well as E:T ratio was found at baseline **(Fig. 4, Table 1)**
  - T cells: 163–4941
  - Blasts: 5477–17917
  - E:T ratios: 1:2.6–1:54.3

Figure 4. Inter-patient Variability in T cells and Blasts at Baseline.



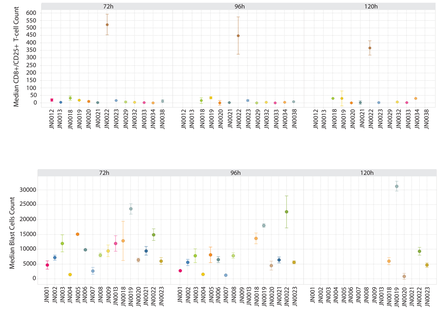
- CNTO 9958 resulted in time- and dose-dependent tumor cell depletion **(Fig. 5, Fig. 7)**

Figure 5. Tumor Cell Depletion by CNTO 9958 by Time and Dose (N=17).



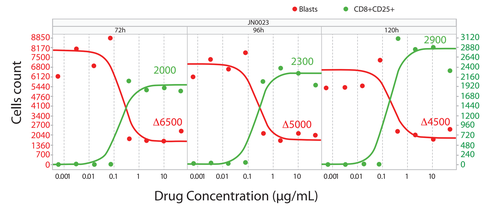
- There was no activation of T cells after incubation with the control compound CNTO 9253 (NullxCD3) (Figure 6)

Figure 6. Effect of a Single Dose of CNTO 9253 (50 µg/mL)



- Correlation of T-cell activation and blast cell depletion in a representative sample (JN0023)

Figure 7. Correlation of T cell activation and blast cell depletion in a representative sample (JN0023).



SUMMARY AND CONCLUSIONS

- Demonstrated T-cell recruitment, activation, and proliferation with CNTO 9958 (CD123xCD3 Ab) in the majority of samples, but not with CNTO 9253
- CNTO 9958 (CD123xCD3) demonstrated AML blast killing in BM samples in a time- and dose-dependent manner in 16/17 samples
- Correlation between activation/proliferation of T cells and blast depletion
- Marked inter-patient variability in E:T ratios and CD123 expression were seen, but no clear correlation between E:T ratio and CNTO 9958 efficacy
- Modulation of data and inclusion of more markers may clarify relationships between marker and efficacy

Disclosure Statements

Janssen Pharmaceuticals R & D

Employment: AF, KS, AA, RM, YL, RA

Employment, stock options, and patents and royalties (pending, not yet issued): FG

Employment, stock options, and patents and royalties (patent): MS

Employment and stock: FH

Employment and Johnson & Johnson equity: KS

Vivia Biotech

Employment and equity ownership: JB

Presented at the 58th Annual Meeting of the American Society of Hematology, December 3-6, 2016, San Diego, CA.