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

**Ann Forslund**, Khaja Syed, Amy Axell, Ronan McDaid, Yingzhe Li, Juan Ballesteros, Francois Gaudet, Ricardo M. Attar, Mark Salvati, Fei Huang, and Kate Sasser

Janssen Pharmaceuticals Research & Development, Spring House, PA; 
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 therapies 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.

**RESULTS**

Objectives:
- To determine whether AML blasts 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 cytokines present in BM. BM with CD123 receptor density on blast cells ranging from the lowest (1759 receptors/cell) to the highest (13115 receptors/cell) were evaluated following culturing with CD19598, aspirate and blood were extensively profiled by an automated multi-parameter flow cytometry system at Vivia.

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

<table>
<thead>
<tr>
<th>Sample</th>
<th>CD123 Density</th>
<th>E:T ratio</th>
<th>WBC</th>
<th>blasts</th>
<th>T cells</th>
<th>T cells</th>
<th>T cells</th>
<th>T cells</th>
<th>T cells</th>
<th>T cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>JN001</td>
<td>99.6</td>
<td>1:18.3</td>
<td>505</td>
<td>48.46</td>
<td>59.57</td>
<td>505</td>
<td>48.46</td>
<td>59.57</td>
<td>505</td>
<td>48.46</td>
</tr>
<tr>
<td>JN002</td>
<td>99.6</td>
<td>1:4.9</td>
<td>92.13</td>
<td>52.41</td>
<td>89.19</td>
<td>92.13</td>
<td>52.41</td>
<td>89.19</td>
<td>92.13</td>
<td>52.41</td>
</tr>
<tr>
<td>JN003</td>
<td>99.6</td>
<td>1:24.4</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN004</td>
<td>99.6</td>
<td>1:2.6</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN005</td>
<td>99.6</td>
<td>1:18</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN006</td>
<td>99.6</td>
<td>1:1.5</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN007</td>
<td>99.6</td>
<td>1:11.5</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN008</td>
<td>99.6</td>
<td>1:42.2</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
<tr>
<td>JN009</td>
<td>99.6</td>
<td>1:26</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
<td>70.53</td>
<td>52.41</td>
<td>89.19</td>
</tr>
</tbody>
</table>

**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

**Figure 3. Inter-patient Variability in CD123 Expression.**

*(A) % blasts CD123+; (B) CD123 receptor density.*

**CONTO 9958**

- Humanized IgG4-PAA bi-specific duobody
- 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

**Vivia Method**

Figure 2. Vivia ExviTech Platform.

**GOAL**

- Determine if CD123xCD3 duo Ab has efficacy in primary AML/MDS (myelodysplastic syndrome) samples
- 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 CONTO 9958 (CD123xCD3 Ab) in the majority of samples, but not with CONTO 9253 (CD123xCD1) 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 CONTO 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, BM, YL, RA

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

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

Employment and stock: FR

Employment and Johnson & Johnson equity: KS

Vivia Biotech

Employment and equity ownership: JB