Iovance Peripheral Blood Lymphocytes (PBL): A Potential Cell Therapy Strategy For The Treatment of Chronic Lymphocytic Leukemia

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BACKGROUND

• Complete remissions in chronic lymphocytic leukemia (CLL) are rare and patients relapsing after treatment with brutinib are in need of novel improved therapeutic strategies.

• Adoptive cell therapies (ACT), including idiopathic antigen receptor (CAR) T cells are under development for the treatment of CLL; however, these therapies are typically genetically modified products and are monoclonal. ACT using a polyclonal, non-genetically altered product, may provide a more effective cell therapy.

• Generating T cell product for ACT is a complex manufacturing process as a high percentage of T cells in common adult hematologic malignancies including CLL or small lymphocytic lymphoma (SLL) are in an exhausted dysfunctional state.

• Brutinib, a Bruton’s tyrosine kinase (BTK) inhibitor, is known to improve proliferative and effector functions of T cells by inhibiting IL-2 inducible T cell kinase (ITK). This study presents preliminary data on successful generation of peripheral blood lymphocytes (PBLs) as bulk T cell product (product name: IOV-2001) from brutinib-treated patients with CLL.

• Clinically relevant doses of IOV-2001 can be produced with 50 mL blood, with no need for leukapheresis.

• First-in-patient testing of IOV-2001 is planned.

STUDY OBJECTIVES

• To develop a short and efficient method for the generation of PBL from peripheral blood mononuclear cell (PBMC) of brutinib-treated patients with CL.

• To demonstrate autologous tumor-killing capability in the expanded PBLs.

MATERIALS AND METHODS

• Patients samples: Cryopreserved peripheral blood mononuclear cells (PBMC) were obtained from three different groups of chronic lymphocytic leukemia (CLL) patients (i.e. treatment-naive, brutinib-naive (or pre-brutinib), post-brutinib). Clinical samples were provided by The Ohio State University.

• Flow cytometry: PBL were analyzed for memory subsets using flow cytometry.

• ELispot: IFNγ production by PBL in response to non-specific TCR engagement was measured following stimulation with mAb-coated Dynabeads (CD3/CD28/CD137). IFNγ secretion was assessed by ELispot (ImmunoSpot CTL) and IFN+ cells were enumerated using ImmunoSpot 56 analyzer.

• Autologous tumor killing assay: Cytotoxicity of PBL was measured by flow cytometry-based method. Briefly, effector (E) cells (PBLs) were labeled with carboxyfluorescein succinyl ester (CFSE) and Target (T) cells (autologous CD19+ cells/Leukemia cells) were labeled with CellTrace violet (CTV). E and T cells were mixed at different ratios and incubated for 24 hours. Cells were harvested following co-culture and stained with annexin-V and propidium iodide (PI). Target cell killing was assessed by calculating percent CTV+ Annexin-V+ P+ cells from background wells.

• Gene expression analysis using nanoString nCounter® system: nCounter CAR T characterization panel (nanoString, Seattle) was used. Data were normalized by scaling with geometric mean of the built-in control gene probes for each sample.

RESULTS

Figure 1. IOV-2001 Manufacturing Process

9-14 days expansion process

CD3+/CD19- cells

CD3+/CD19+ cells

Cryopreserved PBL

Day 1

Day 3

Day 7

Day 10

Day 14

Day 16

Cryopreserved PBL obtained from peripheral blood of CLL patients were enriched for T cells. Enriched fractions were expanded for a duration of 9-14 days in the presence of αCD3/αCD28 beads and interleukin-2 (IL-2) to obtain IOV-2001 product.

Table 1. Phenotype of PBL was performed to identify percent of viable T cells and their subsets. Using flow cytometry samples were evaluated for the presence of CD4+ and CD8+ T cell subsets and memory T cell subsets.

<table>
<thead>
<tr>
<th>Subset</th>
<th>Treatment naïve PBL</th>
<th>Post-ibrutinib PBL (IOV-2001)</th>
<th>Treatment naïve PBL</th>
<th>Post-ibrutinib PBL (IOV-2001)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4+ T cells (% of viable cells)</td>
<td>81</td>
<td>80</td>
<td>81</td>
<td>69</td>
</tr>
<tr>
<td>Memory T cell subsets (% CD4+ T cells)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Central (CLM)</td>
<td>0.7</td>
<td>0.8</td>
<td>0.7</td>
<td>0.8</td>
</tr>
<tr>
<td>EMRA</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>TEFF</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Memory T cell subsets (% CD8+ T cells)</td>
<td>28</td>
<td>14</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>Central (CLM)</td>
<td>4</td>
<td>6</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>EMRA</td>
<td>7</td>
<td>10</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

IOV-2001 is comprised of >97% T cells and the majority of T cells are effector memory phenotype.

Figure 2. Cryopreserved PBL were obtained from three different groups of CLL patients: treatment naïve, pre-brutinib (brutinib-naive) and post-brutinib (minimum 2 cycles of brutinib treatment) PBL were expanded as described in Figure 1. Fold expansion is representative of total number of T cells in final PBL product over number of T cells in enriched fraction. Mean fold expansion of each group is shown in box plots. Pure post-ibrutinib patients samples are color matched. Statistical significance was assessed by a Mann-Whitney U-test. **p<0.01

Figure 3. IFNγ secretion by different groups of PBL in response to non-specific TCR engagement was assessed by Enzyme-Linked Immunosorbent Spot (ELISpot) assay. Data is shown as IFNγ+ T cells per million PBL. Mean number of IFNγ+ T cells per million PBL in each group is shown in parentheses. Paired pre- and post-ibrutinib patients samples are color matched. Statistical significance was assessed by a Mann-Whitney U-test: ***p<0.001. **p<0.01, *p<0.05

IOV-2001: Beneficial effect of prior brutinib exposure on IFNγ secretion

Figure 4. Cytotoxicity of PBL against autologous CD19+ cells (Leukemia cells) was measured using flow cytometry-based cell-killing assay. Specific cytotoxicity of post-ibrutinib PBL (IOV-2001) against autologous leukemia cells is shown. Pvalues A & B, C & D, E & F & G & H represent paired sample tests.

Figure 5. Target specificity (autologous CD19+PBL cells) of IOV-2001 was determined by HLA blockade experiments.

CONCLUSIONS

• IOV-2001 is a non-genetically modified, polyclonal T cell product called PBL.

• IOV-2001 can be reproduced from 50 mL of blood over a 9-day manufacturing duration to yield billions of PBL.

• Compared to pre-brutinib and treatment-naïve PBL, IOV-2001 has high fold expansion from initial limited clinical starting material (simple blood draw, no pheresis required) and secretes high levels of IFNγ in response to non-specific TCR stimulation.

• IOV-2001 demonstrated superior cytotoxicity against autologous tumor (leukemia) cells.

• First-in-patient testing of IOV-2001 is planned for the treatment of CLL/SLL patients.

• Future testing of this approach in broader array of hematologic malignancies is being explored.

DISCLOSURE

• The study and poster are sponsored by iovance Biopharmaceuticals, Inc.

ACKNOWLEDGMENTS

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