Successful Generation of Tumor-Infiltrating Lymphocyte (TIL) Product From Renal Cell Tumors for Adoptive Cell Therapy

Brian Halbert,1 David Einstein,1 David McDermott,1 Emanuelle Andrianopoulos,1 Mamba Gupta,1 Virginia Seery,1 Kenneth Onimus,2 Courtney Herman,2 Adrian Wells,2 Arvind Natarajan,2 Anand Veerapathran,2 Rupal S. Bhatt1

1Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02215; 2Iovance Biotherapeutics, Inc., 999 Skyway Road, Suite 150, San Carlos, CA 94070.

© 2021, Iovance Biotherapeutics

Introduction

Background
- Patients with renal cell carcinoma (RCC) may achieve remission with immune-checkpoint inhibition (ICI); however, most patients will progress
- Adoptive cell therapy with autologous tumor-infiltrating lymphocytes (TIL) allows for expansion of T-cells from tumor tissue, leading to a polyclonal T-cell product with a diverse T-cell receptor (TCR) repertoire capable of recognizing an array of tumor antigens
- TIL therapy with centrally manufactured products demonstrated a 36% objective response rate in patients with RCC histology, and metastases1
- We have developed a second-generation Gen 2 Good Manufacturing Practice (GMP) manufacturing process with a substantially reduced time (22 days) to expand functional TIL from melanoma, cervical, head and neck, bladder, and lung tumors, as well as other tumor types
- Here we present the preliminary experience of TIL production in RCC using Gen 2 manufacturing

Methods

Dose
- Final harvested TIL and in-process samples were assessed for total nucleated cells, total viable cells (TVC), and viability determined by sorache orange dye/DAPI counterstain using the NucleoCounter NC-200™ (ChemoMetec, Linder, Denmark) automated cell counter

Identification
- Of the harvested TIL products and evaluated for identity by immunofluorescent staining

Functionality
- The ability of the harvested TIL product to secrete IFN-γ and Granzyme B upon restimulation was measured following co-culture with antibody-coated beads (IFN-γ, Granzyme B, and CD137, Granzyme B, and anti-CD3 and anti-CD28). Thermo Fisher, Waltham, MA
- After 24 hours of co-culture, culture supernatants were harvested, frozen, thawed, and assayed by ELISA

Phenotype
- Final harvested TIL products were thawed and assessed for phenotype using two flow cytometry panels
- Multiflow cytometry was performed to characterize TIL purity, identity, memory subset, activation, and exhaustion status
- Data were acquired from stained sample products on the ZE5 (Bio-Rad, Hercules, CA) cell analyzers

Table 1. Baseline Demographics and Tumor Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 11</th>
<th>Sex, n (%): M/F</th>
<th>Age, years: Median (IQR)</th>
<th>Race, n (%): White/Black/Other</th>
<th>Histology, n (%): Clear Cell/Papillary</th>
<th>Treatment, n (%): Pre-treatment/Metastatic</th>
<th>Tissue, n (%): Kidney/Urinary Bladder/Lung/Adrenal/Pancreas/Gallbladder/Gastroesophageal Junction/Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years: Median (IQR)</td>
<td>59 (52–68)</td>
<td>9/2</td>
<td>53 (30–72)</td>
<td>9/2</td>
<td>11/0</td>
<td>10/1</td>
<td>7/4</td>
</tr>
</tbody>
</table>

Table 2. Summary of Product Attributes

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N = 11</th>
<th>TVC, Mean ± SD (pg/mL)</th>
<th>% Viability</th>
<th>% CD45+CD3+</th>
<th>% CD45RA+CD3+</th>
<th>% CD45RA–CD3+</th>
<th>% CD45RA–CD28+CD3+</th>
<th>% CD45RA+CD28–CD3+</th>
<th>% CD45RA–CD28–CD3+</th>
<th>% CD45RA+CD45RA+CD3+</th>
<th>% CD45RA–CD45RA–CD3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>TVC, Mean ± SD (pg/mL)</td>
<td>58 ± 29</td>
<td>60 ± 77</td>
<td>68 ± 0</td>
<td>29 ± 2</td>
<td>29 ± 2</td>
<td>39 ± 8</td>
<td>30 ± 4</td>
<td>18 ± 5</td>
<td>48 ± 12</td>
<td>34 ± 14</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>% Viability</td>
<td>68 ± 0</td>
<td>64 ± 0</td>
<td>64 ± 0</td>
<td>77 ± 18</td>
<td>97 ± 4</td>
<td>99 ± 4</td>
<td>48 ± 6</td>
<td>34 ± 7</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>% CD45+CD3+</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td></td>
</tr>
<tr>
<td>% CD45RA+CD3+</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td></td>
</tr>
<tr>
<td>% CD45RA–CD3+</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td>11 ± 4</td>
<td></td>
</tr>
<tr>
<td>% CD45RA–CD28+CD3+</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td>18 ± 5</td>
<td></td>
</tr>
<tr>
<td>% CD45RA+CD28–CD3+</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td>4 ± 2</td>
<td></td>
</tr>
<tr>
<td>% CD45RA–CD45RA+CD3+</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td>6 ± 1</td>
<td></td>
</tr>
<tr>
<td>% CD45RA–CD45RA–CD3+</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td>1 ± 1</td>
<td></td>
</tr>
</tbody>
</table>

Results

Figure 3. TIL Purity by Multicolor Flow Cytometry

Figure 4. TIL Differentiation and Memory Status

Figure 5. TIL Activation and Exhaustion Markers

Figure 6. TIL Function Measured by IFN-γ and Granzyme B Release

Conclusions
- 8 of 11 TIL products (73%) showed acceptable TIL product attributes
- Yield of TIL from the 8 tumors was an average of 74 × 10⁸ viable cells
- TIL generated from RCC samples using the Gen 2 process met all acceptance criteria and were generally comparable in function and phenotype to TIL generated from other tumor types
- These feasibility data suggest that TIL can be successfully expanded ex vivo from RCC samples (including pre-treated and metastatic tumors) and may support clinical investigation of TIL in patients with RCC

Acknowledgements
- KO, CH, AW, AN, and AV are employees of Iovance and may have stock options
- Graphics support was provided by Cognition Studio, Inc. (Seattle, WA) and funded by Iovance Biotherapeutics

References