Expansion of tumor-infiltrating lymphocytes (TIL) using Iovance's Gen 2 process from bladder cancer for adoptive immunotherapy

Qiang John Li[1], Khurshid Guru[1], Amit Lugade[1], Elizabeth Brese[1], Kunle Odunsi[1], Anand Veerapathran[2], Kenneth Onimus[2], Adrian Wells[2], Cecile Chartier[2], Arvind Natarajan[2], Maria Fardis[2], Gurkamal Chatta[1]

[1]Roswell Park Comprehensive Cancer Center, Buffalo, New York; [2]Iovance Biotherapeutics, 999 Skyway Road, STE 150, San Carlos, CA

ROSWELL PARK COMPREHENSIVE CANCER CENTER

IOVANCE BIOTHERAPEUTICS

ADVANCING IMMUNO-ONCOLOGY

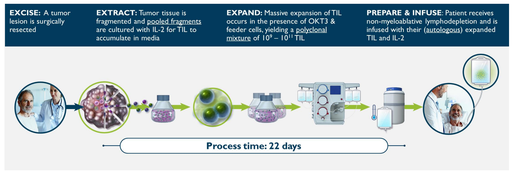
999 Skyway Road, STE 150, San Carlos, CA 94070

For more information, please contact Arvind Natarajan
Arvind.Natarajan@iovance.com

BACKGROUND

- Despite approval of immunotherapeutic agents in bladder, patients with advanced bladder cancer have limited therapeutic options, apart from cisplatin-based chemotherapy and use of immune checkpoint inhibitors (ICI).
- Median survival for patients with metastatic disease is 15 to 18 months. Hence, there remains a need for more effective therapies.
- Adoptive cell therapy using tumor infiltrating lymphocytes (TIL) has demonstrated durable and complete responses with long-term survival of > 10 years in patients with metastatic melanoma.
- Previous attempts to generate TIL from bladder cancer showed some success; however, the process required an extended manufacturing time (35–50+ days).
- Iovance has developed a second generation Good Manufacturing Practice (GMP) manufacturing process (Gen 2) with a significantly reduced time (22 days) to expand functional TIL from melanoma, cervical, head and neck tumors, as well as other tumor types.

Figure 1. Iovance Cryopreserved Manufacturing Process



STUDY OBJECTIVES

- The goal of this study was to determine the feasibility, success rate, and yield of generating TIL from patient-derived bladder cancer tumor specimens using the 22-day Iovance Gen 2 manufacturing process.
- To characterize the final harvested product for the following quality attributes:
  - Dose: Cell count and % viability
  - Identity: % T-cells
  - Functionality: Ability to secrete IFNγ and Granzyme B in response to stimulation with anti-CD3/CD28/CD137
  - Phenotype: Purity, Differentiation and Memory status

MATERIALS & METHODS

- **Manufacturing:** The Gen 2 TIL manufacturing process for the resected bladder tumor samples includes a pre-Rapid Expansion Protocol (pre-REP) and Rapid Expansion Protocol (REP) over 22 days. Pre-REP (1/10th scale) and REP (1/100th scale) were performed as follows: During the pre-REP, 1- 3 mm size tumor fragments were placed in media containing IL-2 for 11 days, and TIL were allowed to extravasate from the tumor. To further stimulate extravasated TIL growth, TIL were expanded using REP that included irradiated PBMC feeders, IL-2 and anti-CD3 for 11 days.
- **Dose:** Final Harvested REP and in-process samples were assayed for total nucleated cells, total viable cells, and viability determined by acridine orange / DAPI counterstain using the NC-200 automated cell counter.
- **Identity:** Final harvested REP products were sampled and assayed for identity by immunofluorescent staining. Percent T-cells was determined as the CD45+, CD3+ (double positive) population of viable cells.
- **Functionality**: The ability of the harvested REP product to secrete IFNγ and Granzyme B upon reactivation was measured following coculture with antibody coated beads (Thermo Fisher, anti-CD3, anti-CD28 & anti-CD137). After 24 hours of co-culture, culture supernatants were harvested, frozen, thawed, and assayed by ELISA. Quantikine IFNγ ELISA kit (R&D systems) and Granzyme- B Human ELISA Kit (Thermo Fisher) was used to measure IFNγ and Granzyme B levels respectively in the supernatant.
- **Phenotype:** Final harvested REP product were thawed and assayed for extended phenotypic markers using two Flow cytometry panels. Flow cytometry antibodies (CD3-BUV395, CD62LBV421, CD57-PB, CD11c-BV711, CD28-BB515, CD19-BUV563, CCR7-PE, CD123-BV605, CD27PE-CF594, CD14-BV-650, TCRγ/δ-APC, CD45-PerCP-Cy5.5, CD45RA-A700, CD56-BUV737, CD8-BV786, CD4-PE-Cy7AND CD16-APC-Vio770) differentiation and memory status. Stained samples products were acquired on the Bio-Rad ZE5 Cell Analyzer.

RESULTS

Figure 2. Total Viable cells, Viability, & Identity of the TIL product

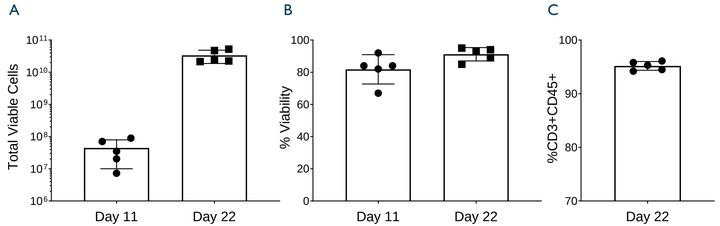


Figure 2. On Day 11 (Pre-REP) and 22 (REP), Total Viable cells (A) and Viability (B) of the product were analyzed on the NC-200 automated cell counter as previously described. Total Viable Cells (TVC) presented in the figure were extrapolated to full-scale for comparison with the clinical manufacturing scale. All 5 samples had > 5e6 Pre-REP cells to initiate REP. The average TVC count and % Viability of Pre-REP harvested on Day 11 were 4.5e7 cells (range 7e6 - 90e6 cells) and 82% (range: 67 - 92%). All the 5 harvested REP had >5e9 cells and >85% viability. The 5 bladder tumors on Day 22 harvest had an average of 24e9 cells (range: 5e9 - 47e9 cells). The average % viability was 91% (range: 85 - 95%). TIL identity was determined on Day 22 using CD3, CD45 markers (C). All the 5 harvested TIL showed greater than 90% %CD3+CD45+ TIL (range: 94-96%).

Figure 3. TIL Purity by Multicolor flow cytometer

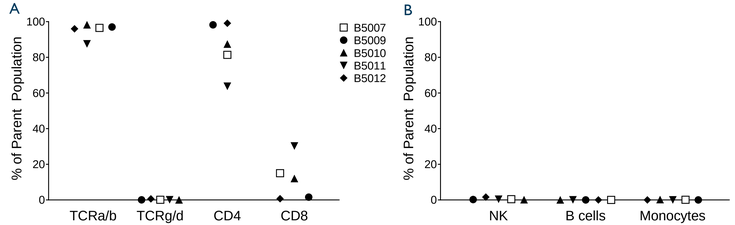


Figure 3. Multicolor flow cytometer was used to characterize TIL Purity and impurities (non- T cells). Most human CD3+ T-cells express the receptors formed by α and β chains that recognize antigens in an MHC restricted manner. A) All the TIL had greater expression of TCR αβ and less than 1% TCR γ/δ cells. Most of the Bladder TIL were CD4+ dominant. B) Non-T cell population including B cells (CD19), monocytes (CD14) and NK (CD56+/CD16+) cells were < 4%.

Figure 4. TIL Differentiation & Memory status

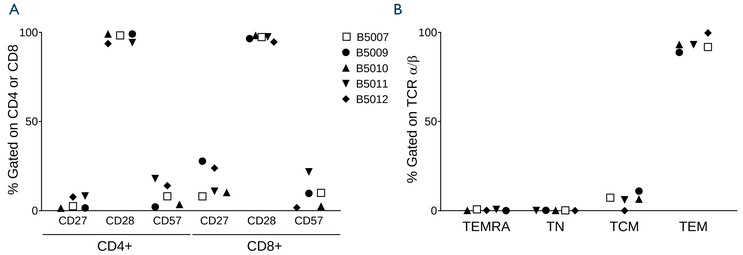


Figure 4. Multicolor flow cytometer was used to characterize TIL Differentiation (A) and Memory status (B). Costimulatory molecules such as CD27 and CD28 are required to supply secondary and tertiary signaling necessary for effector cell proliferation upon T-cell receptor engagement. Most of the Bladder TIL expressed CD28 and displayed Effector memory phenotype. TEMRA- TIL Effector Memory CD45 RA+ (CD45RA+CCR7-), TN- TIL Naïve (CD45RA+CCR7+), TCM- TIL Central Memory (CD45RA-CCR7+), TEM- TIL Effector Memory (CD45RA-CCR7-)

Figure 5. TIL function measured by IFNγ & Granzyme B release

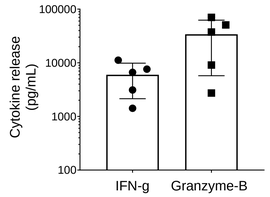


Figure 5. Cryopreserved Final REP products were thawed and incubated with antibody-coated beads as previously described. Data presented above are expressed as the amount of IFNγ or Granzyme-B produced by 5e5 viable cells in 24hrs. TIL expanded from bladder cancer samples released IFNγ in the range of other TIL product generated by Gen 2 at Iovance. The average IFNγ release for the 5 bladder tumors was 5993 pg/mL (1418 - 11197 pg/mL). The average Granzyme B release for the 5 bladder tumors was 34053 pg/mL (range 9078 - 70274 pg/mL).

CONCLUSIONS

- Success rate of growth of viable TIL from bladder tumors were 100%
- Yield of TIL from the 5 tumors was an average of 24 billion viable cells.
- Bladder TIL were generally comparable in function and phenotype to TIL generated from other indications.
- The excellent success in growth of TIL from bladder cancer allows for the application of Iovance's Gen 2 manufacturing process for an autologous cellular infusion product to treat bladder cancer patients.
- Based on this data, a Phase 2 trial has been initiated investigating LN-145 TIL in combination with pembrolizumab for the treatment of patients with unresectable or metastatic urothelial carcinoma who have failed cisplatin-based chemotherapy (NCT03935347).

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