**Background**

- Despite approval of immunotherapeutic agents in bladder patients, advanced bladder cancer has 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 exists a need for a more effective strategy.
- Adoptive cell therapy using tumor infiltrating lymphocytes (TIL) has demonstrated durable and complete responses with long-term survival of patients with metastatic melanoma.
- Previous attempts to generate TIL from bladder cancer showed various challenges: hence, the process required an extended manufacturing time (25–50 days).
- Iovance has developed a second-generation Good Manufacturing Practice (GMP) manufacturing process (Gen 2) with an extended time (2 days) to expand functional TIL from tumors, cervical, head, and neck tumors, as well as other tumor types.

**Study Objectives**

- The goal of this study was to determine the feasibility, success rate, and yield of generating TIL from patient-derived bladder cancer specimens using the 23-day iOvance Gen 3 manufacturing process.

**Materials and Methods**

- Manufacturing: The Gen 2 TIL manufacturing process for the resected bladder tumor samples includes a pre-Expansion Protocol (pre-RP) and Rapid Expansion Protocol (REP) over 22 days. Pre-RP (1010 cells) and REP (109 cells) were performed as follows: During the pre-RP: 1 mm tissue fragments were placed in media containing 2% SFM-IL 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 recombinant IFN-α, IL-2, and IL-15.
- Total Viable Cells (TVC) and Viability of the product were analyzed on Day 22.
- **Dose**: Final Harvested REP in-process were assayed for total nucleated cells, viable cells, and viability determined by Trypan blue/DAPI measurement using the NC-200 automated cell counter.
- **Identity**: Final harvested REP products were sampled and assayed for identity by immunophenotyping using Parent TIL Cells.
- **Functionality**: The ability of the harvested REP product to secrete IFN-γ and Granzyme B upon reactivation is measured to assess functional capacity of the clinical manufacturing scale.
- **Cytokine release**: Total Viable Cells (TVC) and Viability of the product were analyzed on Day 22.
- **Human ELISA Kit (Thermo Fisher)** was used to measure IFN-γ and Granzyme B levels respectively in the supernatant.

**Results**

**Figure 1. Iovance Cryopreserved Manufacturing Process**

**Figure 2. Total Viable Cells, Viability, and Identity of the TIL product**

**Figure 3. TIL Purity function measured by IFNγ and Granzyme B release**

**Figure 4. Multi-color flow cytometry was used to characterize TIL Purity and impurities on Day 22.**

**Figure 5. Iovance harvested TIL samples were thawed and incubated with antibody-coated beads as previously described. Data presented above illustrates the expression of the proteins of IFN-γ and Granzyme B.**

**Figure 6. Gen 2 process from bladder cancer for adoptive immunotherapy**

**Figure 7. TIL release for the 5 bladder tumors on Day 22.**

**Figure 8. Temporal analysis of IFN-γ and Granzyme B release.**

**Discussion and Conclusion**

- **Success rate of growth of TIL from bladder tumors was 100%**.
- **Yield of TIL from the 5 tumors was on average 292 million 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 any autologous cellular inkuday products to most bladder cancer patients.**

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**References**

- [Iovance Biotherapeutics](https://iovance.com)