P2.18-02 2023 World Conference on Lung Cancer | 9–12 September 2023 | Singapore

Successful generation of tumor-infiltrating lymphocytes (TIL) for adoptive cell therapy from mesothelioma

Dean A Fennell,[1] Charlotte Poile,[1] Aleksandra Bzura,[1] Joanna Dzialo,[1] Apostolos Nakas,[2] Kudzayi Kutywayo,[2] Arvind Natarajan,[3] Courtney Herman,[3] Ryan Kovatch,[3] Brittany Bunch,[3] and Anand Veerapathran[3]

[1]University of Leicester, Leicester, UK; [2]University Hospitals of Leicester NHS Trust, Leicester, UK; [3]Iovance Biotherapeutics, Inc., San Carlos, CA, USA

Background

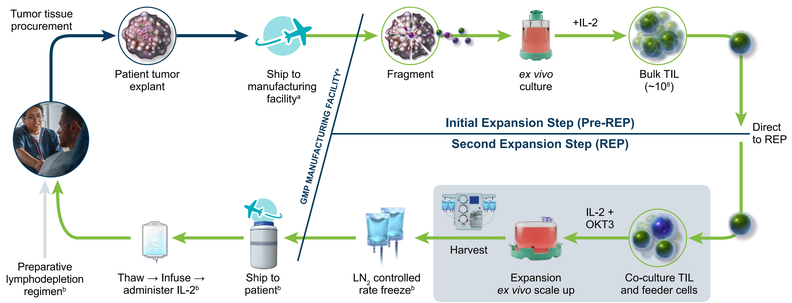
- **Patients diagnosed with mesothelioma have a poor prognosis with a median overall survival of ~18 months**
- **First-line standard of care for patients diagnosed with mesothelioma is immune checkpoint inhibitor (ICI) therapy using ipilimumab and nivolumab[1] followed by a second-line of care using systemic platinum-based chemotherapy**
  - **Often, patients progress within 7 months of receiving these treatments**
- **Adoptive cell therapy (ACT) 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 patient-specific tumor neoantigens**
- **TIL cell therapy has shown efficacy (including complete responses) in other solid tumor indications[2-4] and may be a viable treatment option in this setting**
- **Here, we describe the successful generation of TIL product from mesotheliomas and subsequent phenotypic and functional characterization of the TIL**

Methods

Manufacturing

- A small-scale 22-day manufacturing process was used, including pre-rapid expansion protocol (pre-REP) and rapid expansion protocol (REP) for the generation of TIL from mesotheliomas (**Figure 1**)
  - During pre-REP (1/10th scale), tumor fragments were placed in media containing IL-2 for 11 days and TIL migrated from the tumor tissue
  - To further stimulate TIL growth, cells were expanded using REP (1/50th scale) that included irradiated peripheral blood mononuclear cells (iPBMC), IL-2, and anti-CD3 for 11 days
  - Final TIL product was characterized to determine whether acceptance criteria were met for the following attributes
    - Total viable cells (TVC), purity (% viability), identity (%CD45$^+$CD3$^+$), activity (IFN-γ), and extended phenotyping using multicolor flow cytometry

Figure 1. TIL Product Manufacturing for Autologous TIL Cell Therapy



[a]In this analysis, TIL manufacturing was performed in a development laboratory.
[b]Step was not performed in this analysis because patients did not undergo TIL infusion.

Product Release

- Final TIL product was characterized for
  - Total viable cells (TVC) and purity (% viability), determined by acridine orange/4',6-diamidino-2-phenylindole (DAPI) counterstain using the NucleoCounter® NC-200™ (ChemoMetec, Lillerød, Denmark) automated cell counter
  - Identity (%CD45$^+$CD3$^+$ phenotype), assayed by immunofluorescence staining and flow cytometry
  - Activity (interferon-γ [IFNγ] release), assayed by ELISA using the Quantikine® IFNγ ELISA kit (R&D Systems, Minneapolis, MN, USA)

Phenotype

- Final TIL products were assayed for extended phenotypic markers using a multicolor flow cytometry panel to characterize TIL purity, identity, memory subset, activation, and exhaustion status

Statistical Analysis

- Unpaired Student t-test was used to analyze differences in phenotype; $p < 0.05$ was considered statistically significant

Results

Table 1. Patient Baseline Demographics and Tumor Characteristics

Characteristic	N = 6
Median age, years (range)	69 (55–83)
Sex, (n) %	
Male	5 (83)
Histology, n (%)	
Mesothelioma	6 (100)
Tumor site, n (%)	
Left pleura	3 (50)
Right pleura	3 (50)

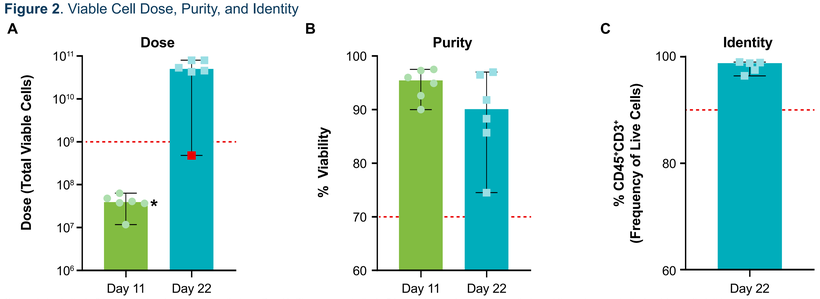
Table 2. Summary of Product Attributes

Tumor ID	Histology	Tumor	Tissue Type	Treatment History	Acceptance Criteria	TVC (x10^9)	Viability (%)	CD45$^+$ CD3$^+$ (%)	CD4$^+$ (%)	CD8$^+$ (%)
ME18002	Epithelial Mesothelioma	L Pleura	Lung	None	Met	79.7	96.5	98.9	74.5	23.3
ME18003	Epithelial Mesothelioma	R Pleura	Lung	None	Met	45.9	74.5	96.4	22.6	71.8
ME18004	Mesothelioma	R Pleura	Lung	None	Met	79.6	97.0	99.0	75.7	19.9
ME18006	Mesothelioma	R Pleura	Lung	None	Not Met	0.5	85.7	*	*	*
ME18007	Epithelioid Malignant Mesothelioma	L Pleura	Lung	None	Met	53.4	91.8	97.5	93.7	4.0
ME18008	Epithelial Mesothelioma	L Pleura	Lung	None	Met	42.8	88.3	98.8	7.5	90.7

*Product failed to meet criteria for TVC. Not available for testing.

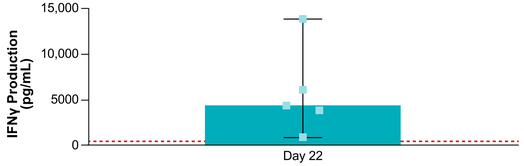
- Final TIL products met acceptance criteria for 5 of the 6 tumor samples (83%)
  - Median (range) TVC was 53.4×10^9 cells/mL (42.8×10^9 – 79.7×10^9 cells/mL) for passing lots (**Figure 2A**)
  - Median (range) purity was 90.1% (74.5–97.0%) for passing lots (**Figure 2B**)
  - Median (range) identity phenotype was 98.8% (96.4–99.0%) for passing lots (**Figure 2C**)

Figure 2. Viable Cell Dose, Purity, and Identity



Green circles indicate 6 tumors that met acceptance criteria on Day 11. Blue squares indicate 5 tumors that met acceptance criteria and the red square indicates 1 tumor that did not meet acceptance criteria on Day 22. Median (range) are displayed.
Red dotted line indicates Day 22 acceptance criteria.
*Corresponds to sample that did not meet TVC on Day 22.

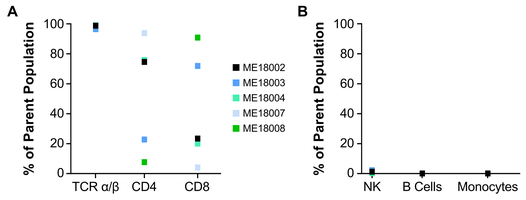
Figure 3. TIL Activity



Blue squares indicate the 5 individual tumor samples that met acceptance criteria.
Red dotted line indicates acceptance criteria.

- TIL released IFNγ in response to polyclonal bead stimulation; results are similar to historical results in melanoma
- Median (range) IFNγ levels was 4454 pg/mL (913–13850 pg/mL) (**Figure 3**)

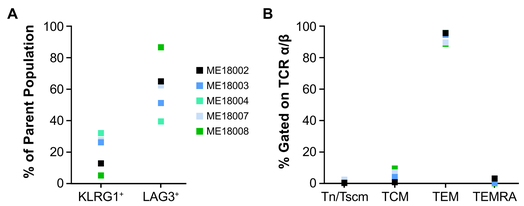
Figure 4. TIL Purity by Multicolor Flow Cytometry



Squares indicate the 5 individual tumor samples that met acceptance criteria.

- TIL predominantly expressed TCR α/β and mixed populations of CD4$^+$ and CD8$^+$ T-cells
  - The median (range) proportion of CD4$^+$ cells was 74.5% (7.5–93.7%) and CD8$^+$ cells was 23.3% (4.0–90.7%) (**Figure 4A**)
- B cells and monocyte populations were not observed in the final product
  - The median (range) proportion of NK cells was 0.6% (0.2–2.1%) (**Figure 4B**)

Figure 5. TIL Activation/Exhaustion, Differentiation, and Memory Markers



Squares indicate the 5 individual tumor samples that met acceptance criteria.

- TIL generally expressed higher levels of activation/exhaustion markers than differentiation markers (LAG3$^+$ and KLRG1$^+$, respectively)
  - Post REP, following expansion with OKT-3 and iPBMCs, LAG3 has been interpreted as an activation signal[5]
  - The median proportion of LAG3$^+$ cells was 62.4% and KLRG1$^+$ cells was 26.2% (**Figure 5A**)
- TIL populations expressed predominantly effector memory phenotype (CD45RA$^-$, CCR7$^-$) (**Figure 5B**)

Conclusions

- **5 of 6 final TIL products (83%) manufactured from mesotheliomas showed acceptable TIL product attributes**
- **Median yield of TIL from the 5 tumors on Day 11 was 41 × 10^6 and on Day 22 was 53 × 10^9 viable cells**
- **TIL generated from mesotheliomas using a 22-day (Gen 2) manufacturing process showed acceptable TIL product attributes and were generally comparable in function and phenotype to TIL generated from other tumor types**
- **These feasibility data suggest that viable TIL can be successfully expanded from mesothelioma tumor tissue**
- **This manufacturing process can be used to support potential clinical investigation of TIL cell therapy in patients with mesothelioma**

Abbreviations

DAPI, 4',6-diamidino-2-phenylindole; ICI, immune-checkpoint inhibitors; IFN, interferon; IL-2, interleukin-2; Gen, generation; GMP, good manufacturing practice; NK, natural killer; PBMC, peripheral blood mononuclear cell; REP, rapid expansion protocol; TCM, central memory T-cells; TCR, T-cell receptor; TEM, effector memory T-cells; TEMRA, effector memory RA$^+$ T cells; TIL, tumor-infiltrating lymphocytes; TN, naïve T-cells; TVC, total viable cells.

References

1. Baas P, et al. *Lancet*. 2021;397:375–386.
2. Sarnaik A, et al. Presented at: 2022 SITC Annual Meeting. November 8–12, 2022: Boston, MA. Abstract 789.
3. Chesney J, et al. *J Immunother Cancer*. 2022;10: e005755.
4. Schoenfeld AJ, et al. Presented at: 2021 SITC Annual Meeting. November 10–14, 2021: Washington, DC. Abstract 458.
5. Simpson-Abelson MR, et al. Presented at: 2020 ESMO Annual Meeting. September 19–21, 2020: Virtual. Abstract 1053P.

Acknowledgments

- The authors would like to thank the participating patients and their families for donation of material used in this study
- This study was sponsored by Iovance Biotherapeutics, Inc. (San Carlos, CA, USA)
- Editorial support was provided by Peloton Advantage, LLC, an OPEN Health company, and funded by Iovance

Disclosures

- All authors meet the criteria for authorship set forth by the International Committee of Medical Journal Editors
- AN, CH, RK, BB, and AV are current or former employees of Iovance and may have stock and/or stock options