Iovance Gen 2 TIL Manufacturing Process Produces Drug Products that Exhibit Favorable Quality Attributes for Adoptive Cell Transfer Across 5 Solid Tumor Indications

Seth Wardell[1], Maritza Lienlaf-Moreno[1], Michelle Blaskovich[1], Ian Frank[1], Virginia Van Grod[1], Maria Fardis[1], and Joseph Wypych[1]

[1]Iovance Biotherapeutics, Inc. Tampa, FL and San Carlos, CA, USA

IOVANCE BIOTHERAPEUTICS
ADVANCING IMMUNO-ONCOLOGY
999 Skyway Road, STE 150, San Carlos, CA 94070

For more information, please contact
seth.wardell@iovance.com

BACKGROUND

The Iovance Gen 2 manufacturing process improved over classical TIL expansion methods by dependably delivering potent cryopreserved drug products with a greatly reduced manufacturing cycle time. Drug products generated by this process displayed favorable quality attributes for adoptive transfer relative to the first-generation process, Gen 1. Here we demonstrate the Gen 2 process currently used to manufacture lifileucel for melanoma is reproducible across multiple solid tumor indications at clinical scale and present quality attributes associated with the resulting drug products.

STUDY OBJECTIVES

Drug products generated across multiple solid tumor indications with Iovance Gen 2 process were assayed to determine comparability in terms of the following quality attributes:

1. Manufacturing success rate
2. Dose
3. Viability
4. Drug product identity
5. Phenotypic expression of CD28 on T-cells
6. Ability to secrete IFNγ in response to mitogenic activation

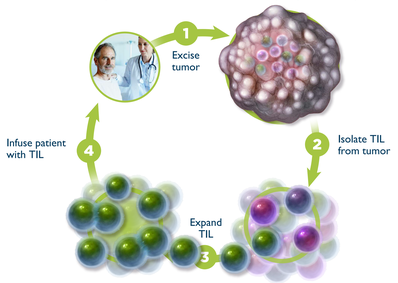
OVERVIEW OF TIL THERAPY PROCESS

EXTRACTION: Patient's TIL are removed from suppressive tumor microenvironment (via surgical resection of a lesion).

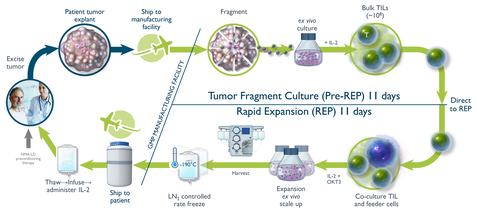
EXPANSION: TIL are expanded exponentially in culture with IL-2 to yield 10^9 – 10^{11} TIL before infusion into the patient.

PREPARATION: Patient receives NMA-LD (non-myeloablative lymphodepletion, cyclophosphamide: 60 mg/kg x 2 days and fludarabine: 25 mg/m^2 x 5 days) to eliminate potentially suppressive tumor microenvironment and maximize engraftment and potency of TIL therapy.

INFUSION: Patient is infused with their expanded TIL (lifieucel) and a short duration of high-dose of IL-2 (600,000 IU/kg for up to 6 doses) to promote activation, proliferation, and anti-tumor cytolytic activity of TIL.



IOVANCE GEN 2 TIL MANUFACTURING



METHODS

Starting Material:

- Advanced or metastatic lesions are excised via sterile resection and placed in transport media. Tumor tissue is maintained at 2-8°C during transport to the manufacturing facility. Upon receipt at the manufacturing facility, the tissue is further fragmented prior to entering culture to facilitate extravasation of the T-cells from the tissue into the growth medium.

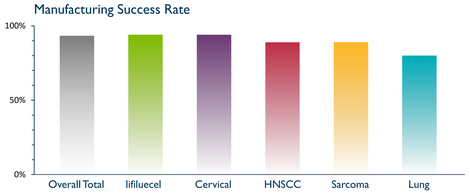
PRIMARY TUMOR INDICATION	CLINICAL SCALE MANUFACTURING REPLICATES
Malignant Melanoma (lifileucel)	64
Cervical Carcinoma	25
Head & Neck Squamous Cell Carcinoma (HNSCC)	10
Soft Tissue Sarcoma	3
Non-small Cell Lung – squamous, non-squamous, adenocarcinoma, large cell carcinoma (NSCLC)	3

Analytical Methods and Instrumentation:

- **Dose and Viability:** Final formulated products were sampled and assayed for total nucleated cells, total viable cells (TVC), and viability determined by acridine orange / DAPI counterstain using the NC-200 automated cell counter
- **Phenotypic markers:** Formulated drug 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. Frozen satellite or sentinel vials for each process were thawed and assayed for extended phenotypic markers including CD3, CD45, CD4, CD8, and CD28. Fresh drug products were acquired on the BD FACS Canto II, and extended phenotypic markers on thawed drug products were acquired on the Bio-Rad ZE5 Cell Analyzer.
- **Immune function:** The ability of the drug product to secrete IFNγ upon activation was measured following co-culture with anti-body coated beads. After 24-26 hours culture supernatants were harvested, frozen, thawed, and assayed by ELISA.

RESULTS

Figure 1: Gen 2 Manufacturing Success Rates Across Five Solid Tumor Indications



Manufacturing success rate is calculated for each primary indication analyzed. Manufacturing success rate is calculated as manufacturing starts that yield a drug product that meets release specifications over manufacturing starts.

Figure 2: Dose and Viability of Drug Products generated across tumor indications with Gen 2

Upon formulation the drug product is dispensed into multiple final product containers. Each container is sampled and the resulting pooled specimen is submitted for testing. **(A)** Samples are analyzed on the NC-200 automated cell counter as previously described. Total viable cell density is determined by the mean of quadruplicate counts. Minimum release specification set as ≥ 1x10^9 viable cells. Mean ± 95% CI shown. **(B)** Cell viability was assessed as previously described. Release specification is set to ≥70% of nucleated cells. Mean ± 95% CI shown.

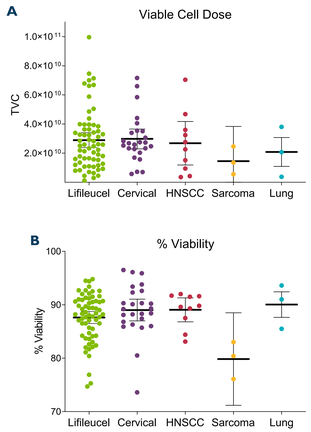


Figure 3: Identity of Viable Cells within Drug Products

Formulated drug products were assayed for identity by flow cytometry for release. Gen 2 processes across all indications produce high purity T-cell cultures as defined by CD45+,CD3+ (double positive) phenotype.

Figure 4: CD8+ T cells as a Percentage of Viable Cells across Tumor Indications

Cryopreserved satellite vials of drug product were thawed and assayed for extended phenotype by flow cytometry as previously described. Mean percentage of total viable cells expressing CD8 ± 95% CI are displayed.

Figure 5: Expression of Costimulatory Molecule CD28 on Viable CD8+ and CD4+ T cells

Cryopreserved satellite vials of formulated drug product were thawed and assayed for extended phenotype by flow cytometry as previously described. **(A)** Mean percentage of viable CD8+ cells expressing CD28 ± 95% CI displayed. **(B)** Mean percentage of viable CD4+ cells expressing CD28 ± 95% CI displayed.

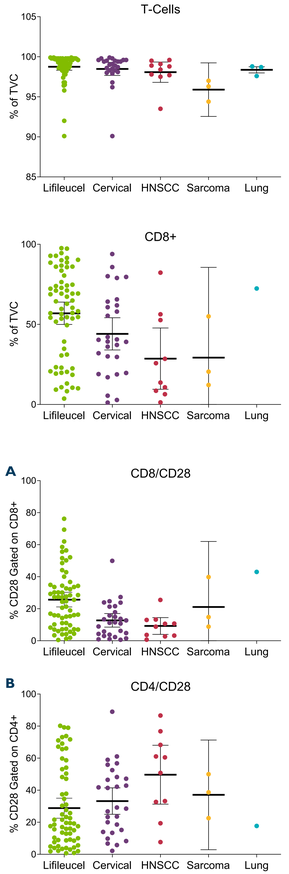
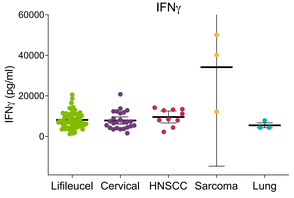


Figure 6: Gen 2 drug products secrete cytokine in response to CD3, CD28, and CD137 engagement

Cryopreserved drug products were thawed and incubated with anti-body coated beads as previously described. Data is expressed as the amount of IFNγ produced by 5x10^5 viable cells in 24-26 hrs. Gen 2 drug products across all primary indications exhibit a robust functional capacity to produce IFNγ upon reactivation. The ability of the drug product to be reactivated and secrete cytokine is a surrogate measure of *in-vivo* functional capacity upon TCR binding to cognate antigen in the context of HLA.



SUMMARY

- Manufacturing success defined as the number of drug products that meet release specifications over the number of manufacturing starts indicate the Iovance Gen 2 process is applicable to a wide range of solid tumor indications
- The mean viable cell dose and viability of drug products across the additional 5 tumor indications were highly comparable to lifileucel
- Iovance Gen 2 process produces high purity T-cell cultures across all indications as defined by CD45+,CD3+ double positive phenotype
- Absolute numbers of CD8+ cells in the final drug product remained comparable to lifileucel with broadly overlapping confidence intervals. Indications with predominant CD4 populations display increased expression of CD28 on the CD4 T-cell subset
- Gen 2 drug products across all primary indications exhibit a robust capacity to produce IFNγ upon reactivation indicating a highly functional T-cell population

DISCLOSURE

- This study and poster are sponsored by Iovance Biotherapeutics, Inc.
- All authors are employees of Iovance Biotherapeutics, Inc. and may have stock options.

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