Successful Manufacturing of Tumor-Infiltrating Lymphocyte (TIL) Cell Therapy From Cryopreserved Melanoma Tumors Shipped From Australia

Kenneth Onimus,[1] Adrian Wells,[1] Courtney Herman,[1] Ahmed Tawashi,[1] Georgina V. Long,[2,3] Richard A. Scolyer,[2,4,5,6] Rebecca Velickovic,[2] Robyn P. M. Saw,[2,4] Thomas E. Pennington,[2,4] Alexander M. Menzies,[2,3] Madan Jagasia,[1] Arvind Natarajan,[1] Anand Veerapathran[1]

[1]Iovance Biotherapeutics, Inc., San Carlos, CA, USA; [2]Melanoma Institute Australia (MIA), The University of Sydney, Sydney, Australia; [3]Royal North Shore Hospital, Sydney, Australia; [4]Royal Prince Alfred Hospital, Sydney, Australia; [5]NSW Health Pathology, Australia; [6]Faculty of Medicine and Health, The University of Sydney, Sydney, Australia

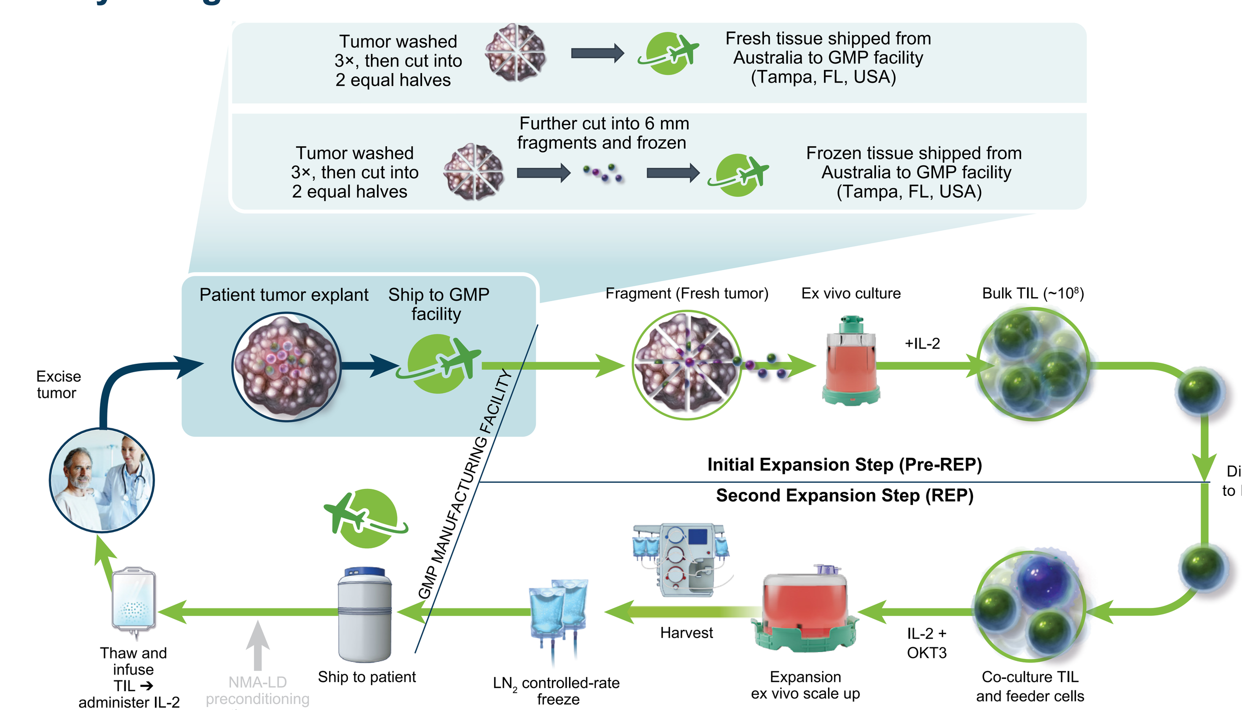
Copies of this poster obtained through Quick Response (QR) Code are for personal use only and may not be reproduced without permission from ASTCT™ & CIBMTR®, and the author of this poster

Corresponding Author: Anandaraman Veerapathran; Anand.Veerapathran@iovance.com

Background

- Lifileucel, an investigational adoptive cell therapy utilizing tumor-infiltrating lymphocytes (TIL), has demonstrated safety and activity in treating patients with advanced melanoma (objective response rate of 36% and duration of response not reached at a median follow-up of 33.1 months) using fresh (non-cryopreserved) tumors shipped to a central Good Manufacturing Practice (GMP) facility[1,2]
- Australia has the highest incidence rate of melanoma in the world[3]; however, shipping duration from Australia to the US can be lengthy and may impact the ability to manufacture TIL from a fresh tumor sample
- The current study was designed to (1) determine the feasibility of receiving fresh tumors from Australia versus freezing tumors at the clinical site prior to shipment, and (2) determine whether TIL can be manufactured from tumors in each of these conditions using a proprietary tumor cryopreservation process

Study Design



Methods

Manufacturing

- Tumors were resected from 4 patients with metastatic melanoma treated at Melanoma Institute Australia (MIA). Tumor samples were shipped fresh (2ºC–8ºC) ("Fresh") or cryopreserved prior to shipment ("Frozen")
  - Fresh tumor fragments were processed using a Gen 2 (22-day) manufacturing process, including an 11-day pre-Rapid Expansion Protocol (pre-REP) and 11-day REP duration
  - Frozen tumor manufacturing process was executed with a pre-REP duration of 7 days and REP duration of 14 days

Dose (Total Viable Cells) and Purity

- Final harvested REP and in-process samples were assayed for total nucleated cells, total viable cells, 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

- Final harvested REP and in-process samples were assayed for identity by immunofluorescent staining. Percentage of T cells was determined as the CD45⁺CD3⁺ (double-positive) population of viable cells

Potency

- The ability of the harvested REP product to secrete interferon-γ (IFNγ) and Granzyme B upon reactivation was measured following co-culture with antibody-coated beads (IFNγ: anti-CD3, anti-CD28, and anti-CD137; Granzyme B: anti-CD3 and anti-CD28; ThermoFisher, Walther, MA, USA). After 24 hours of co-culture, culture supernatants were harvested, frozen, thawed, and assayed by ELISA. Quantikine® IFNγ ELISA kit (R&D Systems, Minneapolis, MN, USA) was used to measure IFNγ in the supernatant

Phenotype

- Final harvested REP and in-process samples were assayed for extended phenotypic markers using 2 flow cytometry panels
- The following flow cytometry antibodies were used to characterize T-cell content, TIL memory subset, activation, and exhaustion status:
  - CD3, CD62L, CD57, CD11c, CD28, CD19, CCR7, CD123, CD27, CD14, TCRγ/δ, CD45, CD45RA, CD56, CD8, CD4, and CD16
  - CD3, PD-1, 2B4/CD244, CD8, CD25, BTLA, KLRG1, TIM-3, CD194/CCR4, CD4, TIGIT, CD183, CD69, CD95, CD127, CD103, and LAG-3
- Stained sample products were acquired on the FACS Canto II™ (BD Biosciences, Franklin Lakes, NJ, USA) cell analyzer

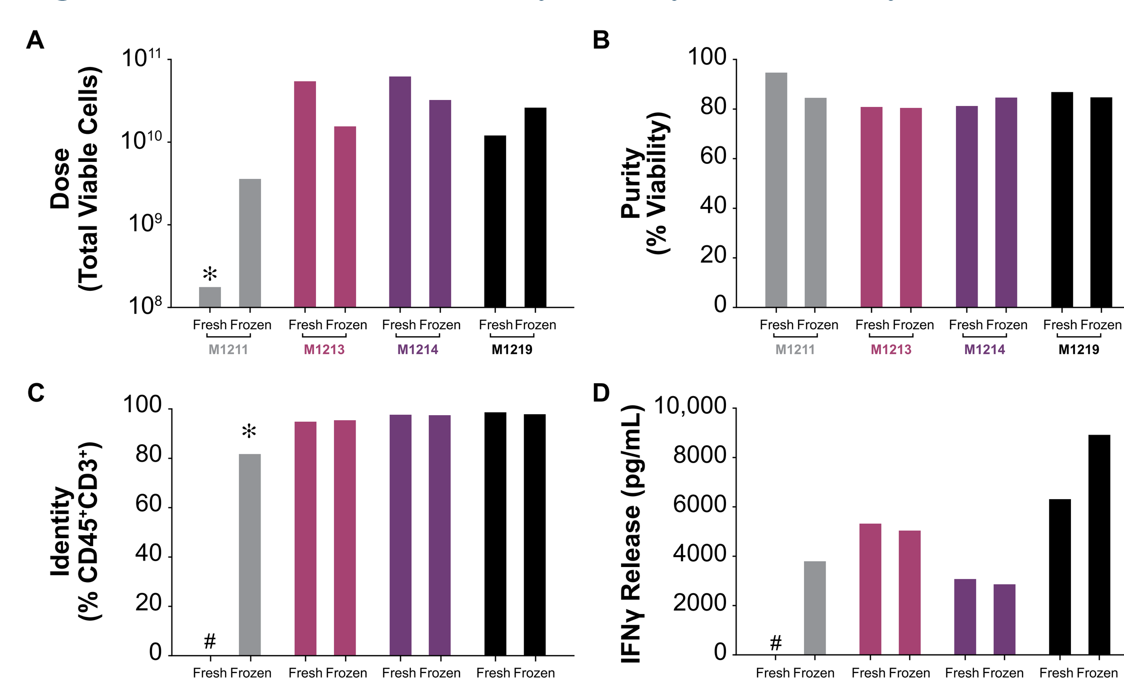
Statistical Analysis

- Differences in phenotype, late-stage apoptosis, CD107a granulation, and Granzyme B were analyzed by the unpaired Student t-test, and $P < 0.05$ was considered statistically significant

© 2022, Iovance Biotherapeutics

Results

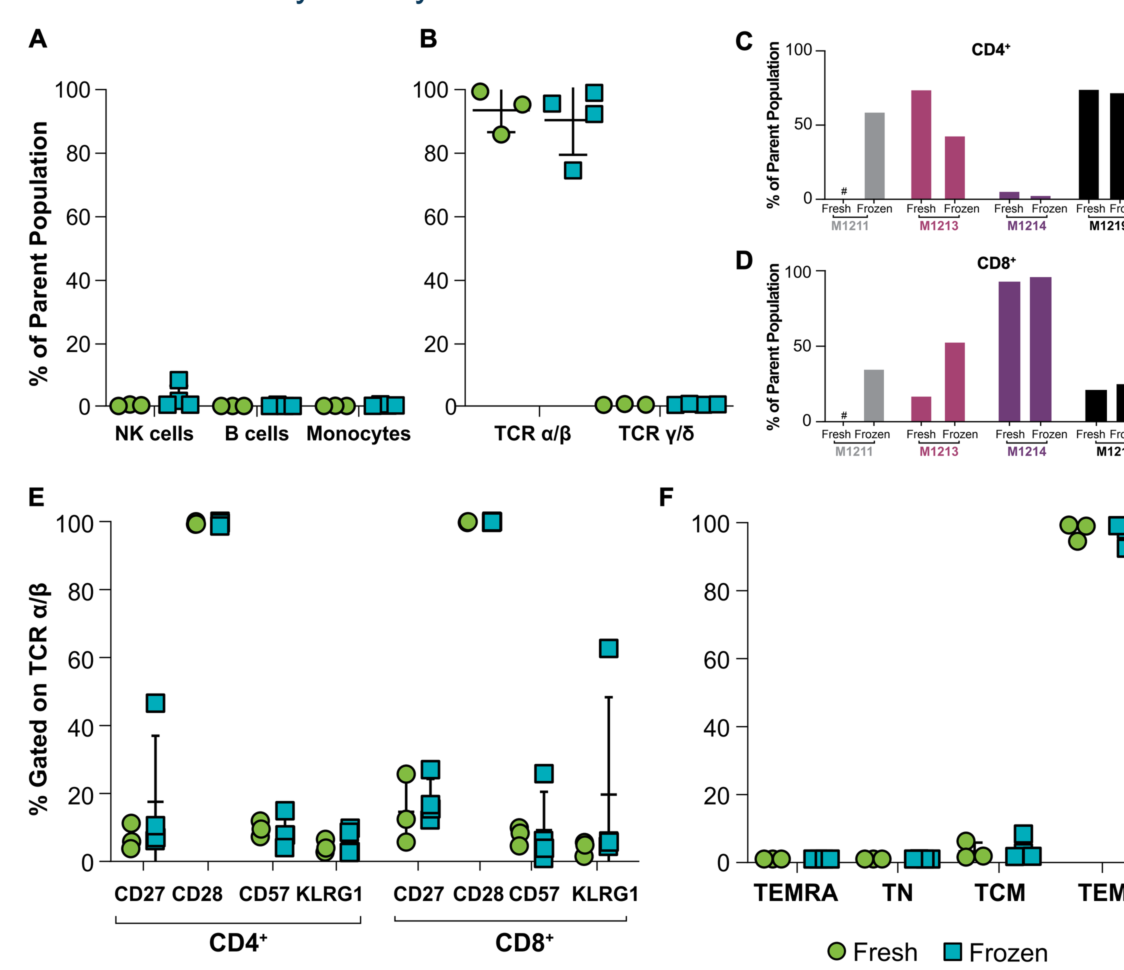
Figure 1. Viable Cell Dose, Purity, Identity, and Potency of TIL Product



*Sample did not meet the acceptance criteria.
Not tested.

- All Frozen products met the release criteria for total dose (**Figure 1A**), viability (**Figure 1B**), and potency (**Figure 1D**). All Frozen tumors except M1211 met the prespecified release criteria for identity (**Figure 1C**)
  - M1211 (Frozen) had a higher proportion of natural killer (NK) cells in the final product (8%)
- Time to ship Fresh tumors from Australia was ≥ 3 days, which is longer than the usual Gen 2 process tumor shipping time. Three of four Fresh products met the prespecified release criteria. Failure of M1211 Fresh product could be due to longer exposure of the tumor to Hypothermosol medium or exposure to conditions outside of our established shipment stability standards
- No statistically significant differences were observed between Fresh and Frozen samples in terms of dose, purity (% viability), identity, and IFNγ release

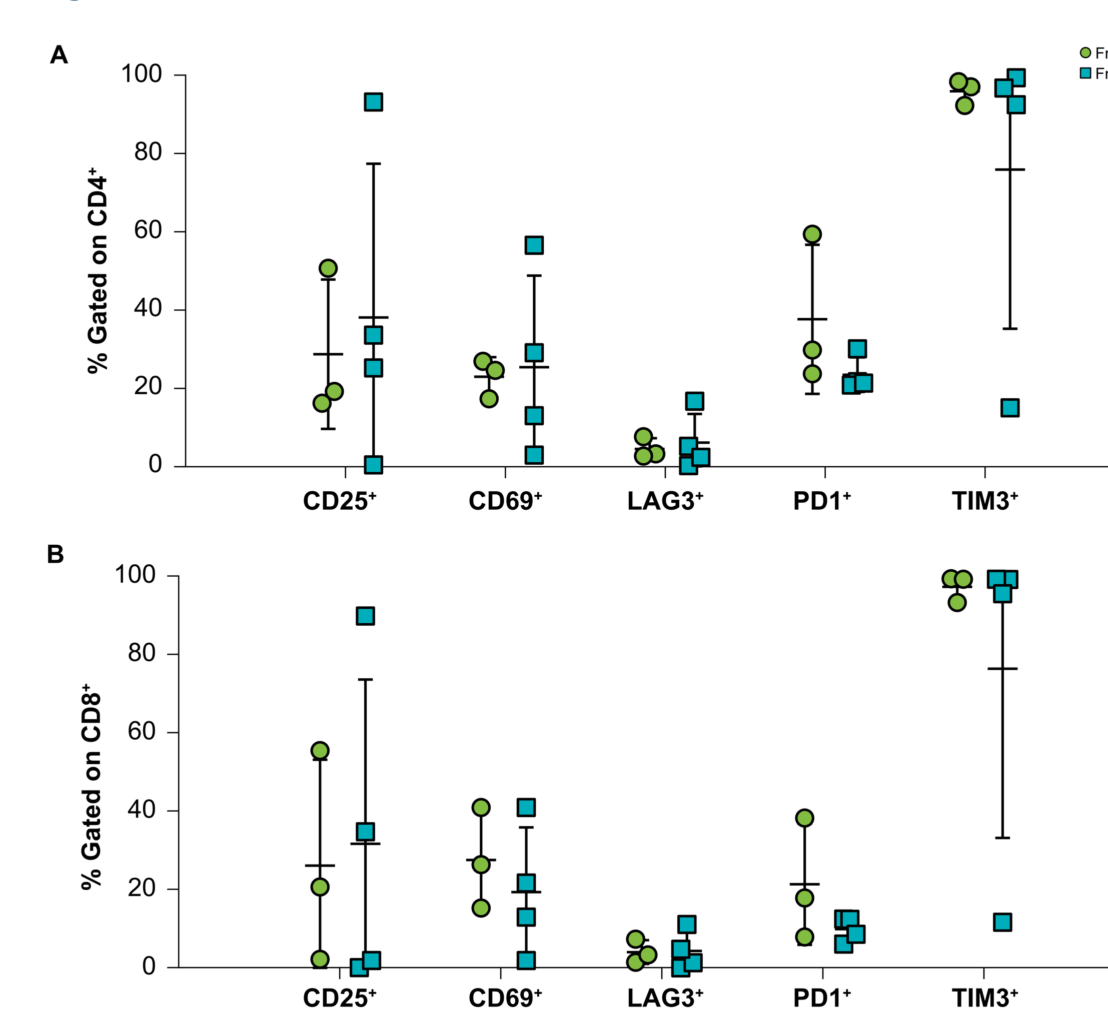
Figure 2. T-Cell Content, TIL Memory, and Differentiation Markers by Multicolor Flow Cytometry



Not tested.
Values are displayed as mean ± SD.

- Frozen tumor TIL exhibited comparable purity, identity, memory, and differentiation phenotype relative to Fresh control
  - Non–T-cell contaminants such as NK cells, B cells, and monocytes were < 8% (**Figure 2A**)
  - Both Fresh and Frozen samples were predominantly T-cell receptor α/β⁺ (TCRα/β⁺) (**Figure 2B**) with variable CD4⁺ and CD8⁺ populations. No statistically significant difference was observed between the 2 conditions for the CD4⁺/CD8⁺ ratio (**Figures 2C** and **2D**)
  - TIL from both conditions highly expressed CD28 co-receptors and moderately expressed other differentiation markers such as CD27, CD57, and KLRG1; no statistically significant difference was observed between Fresh and Frozen samples regarding expression of differentiation markers (**Figure 2E**)
  - Naïve (TN), central memory (TCM), effector memory (TEM), and effector memory RA⁺(TEMRA) T-cell subsets were defined using CD45RA and CCR7. A majority of the TIL lots displayed predominantly effector memory phenotype (**Figure 2F**)

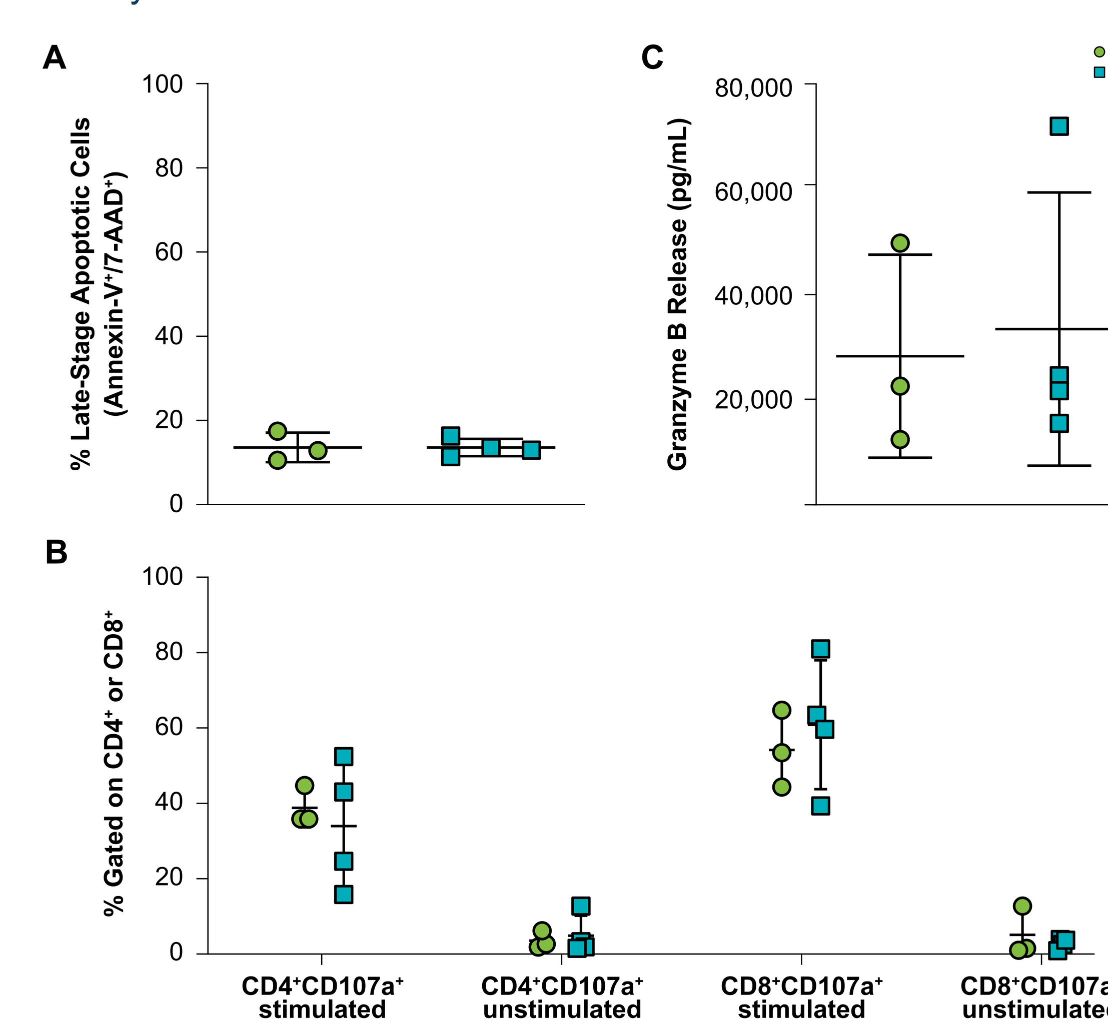
Figure 3. TIL Activation and Exhaustion Status



Values are displayed as mean ± SD.

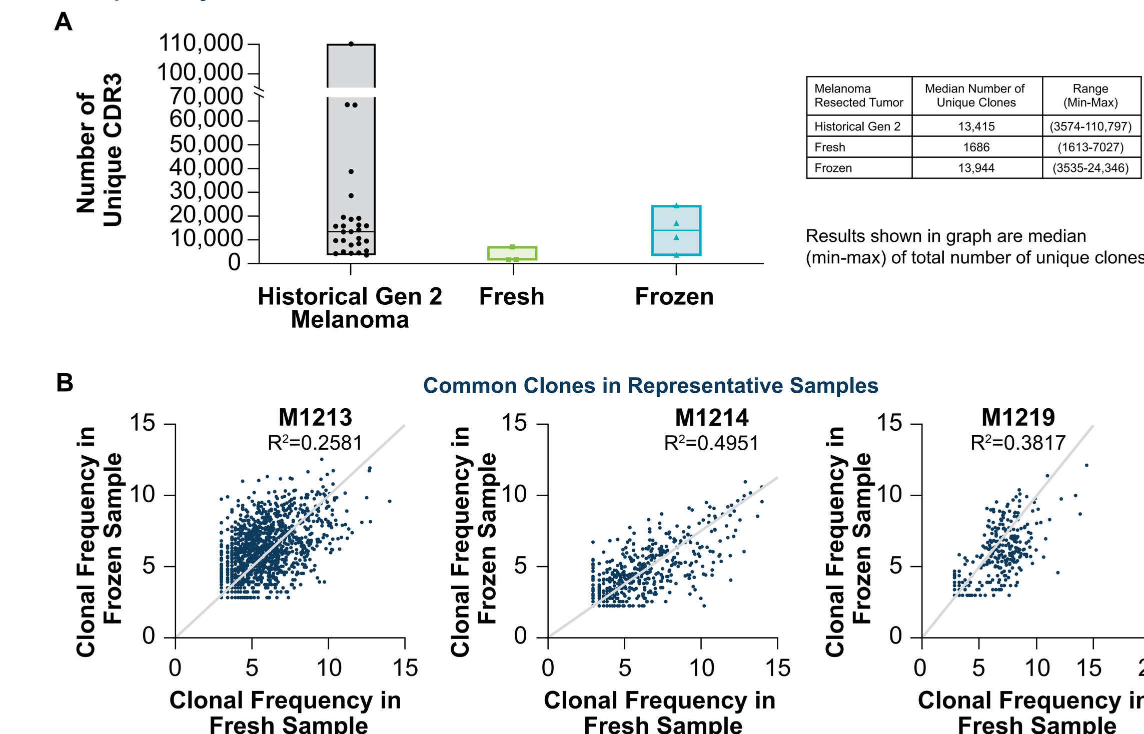
- Multicolor flow cytometry was used to characterize TIL activation and exhaustion status of CD4⁺ (**Figure 3A**) and CD8⁺ TIL (**Figure 3B**). Activation and exhaustion were comparable between the Fresh and Frozen samples, and comparable to prior results in multiple tumor types (data on file)

Figure 4. Late-Stage Apoptotic Cells, CD107a Degranulation, and Granzyme B Release of TIL Product



- Both Fresh and Frozen TIL product showed very low late-stage apoptotic cells and comparable CD107a degranulation and Granzyme B release
  - Final TIL product produced from both conditions had <17% late-stage apoptotic cells (**Figure 4A**)
  - CD4⁺CD107a⁺ and CD8⁺CD107a⁺ levels for Fresh and Frozen tumor TIL were comparable (**Figure 4B**)
  - Granzyme B release for Fresh and Frozen tumor TIL product was comparable except for M1219 (**Figure 4C**)
  - No statistically significant differences were observed between Fresh and Frozen tumor TIL product in terms of late-stage apoptosis, CD4⁺/CD8⁺ CD107a secretion, and Granzyme B release

Figure 5. Summary of Final Product TCR V-Beta Clonotype and Frequency Distribution



Melanoma Resected Tumor	Median Number of Unique Clones	Range (Min-Max)
Historical Gen 2	13,415	(3574-110,797)
Fresh	1686	(1613-7027)
Frozen	13,944	(3535-24,346)

Results shown in graph are median (min-max) of total number of unique clones.

- The median number of unique complementarity-determining region 3 (uCDR3) clones from Frozen samples was similar to those from historical Gen 2 melanoma cryopreserved samples[4] (**Figure 5A**)
- Fewer uCDR3 clones were observed in Fresh samples than in Frozen samples, which could possibly be due to the longer exposure of Hypothermosol medium (**Figure 5A**)
- 48%–93% of the uCDR3 clones in the TIL produced from Fresh samples were also present in the TIL produced from Frozen samples (**Figure 5B**)

Conclusions

- Long shipment times may limit the feasibility of manufacturing TIL cell therapy using fresh tumors, but tumors frozen locally prior to shipping can overcome shipping constraints related to geography
  - Tumors frozen locally in Australia using a proprietary cryopreservation process prior to shipment consistently produced sufficient doses for TIL treatment (4/4 samples)
  - TIL manufacturing using fresh tumors shipped from Australia was less reliable for TIL treatment (3/4 samples) compared with tumors frozen locally (4/4 samples)
  - Final TIL product generated from the frozen samples did not differ significantly in dose, viability, or phenotype compared with TIL produced from the fresh samples
  - 48%–93% of the unique clones in the TIL produced from fresh samples were composed of clones also present in the TIL produced from frozen samples
- Product characteristics of frozen tumor products were similar to those of fresh tumors per prior US and European experiences
- These data support further evaluation of TIL expansion from frozen tumor fragments to allow for greater manufacturing flexibility and suggest the potential for clinical manufacturing of TIL product for Australia at the US facility

References

1. Sarnaik AA, Hamid O, Khushalani AI, et al. *J Clin Oncol*. 2021;39(24):2656-2666.
2. Larkin JMG, Sarnaik A, Chesney JA, et al. *J Clin Oncol*. 2021;39(suppl 15):Abstract 9505.
3. World Cancer Research Fund & American Institute for Cancer Research. https://www.wcrf.org/dietandcancer/skin-cancer-statistics/
4. Gontcharova V, Suzuki S, Simpson-Abelson MR, et al. *Cancer Res*. 2019;79:Abstract LB-069.

Abbreviations

DAPI, 4',6-diamidino-2-phenylindole; GMP, Good Manufacturing Practice; IFNγ, interferon-γ; IL-2, interleukin-2; LAG3, lymphocyte activation gene-3; LN_2, liquid nitrogen; MIA, Melanoma Institute Australia; NK, natural killer cells; NMA-LD, nonmyeloablative lymphodepletion; PD-1, programmed cell death protein-1; REP, rapid expansion protocol; TCM, central memory cells; TCR, T-cell receptor; TEM, effector memory T cells; TEMRA, effector memory RA⁺ T cells; TIL, tumor-infiltrating lymphocytes; TIM3, T-cell immunoglobulin domain and mucin domain-3; TN, naïve T cells; uCDR3, unique complementarity-determining region 3.

Acknowledgments

- The authors would like to thank the patients and their families, as well as the investigators and study site team members who are participating in the study
- This study is sponsored by Iovance Biotherapeutics, Inc. (San Carlos, CA, USA)
- Editorial support was provided by Second City Science (Chicago, IL, USA) and funded by Iovance Biotherapeutics

Disclosures

- GVL is a consultant advisor for Amgen Inc, Agenus, Array Biopharma Inc, Boehringer Ingelheim, Bristol Myers Squibb, Evaxion, Hexel, Highlight Therapeutics, Merck Sharpe & Dohme, Novartis, OncoSec, Pierre Fabre, QBiotics, Regeneron, and Specialised Therapeutics Australia
- RAS has received fees for professional services from Evaxion, Provectus Biopharmaceuticals Australia, Qbiotics, Novartis, Merck Sharp & Dohme, NeraCare, AMGEN Inc, Bristol Myers Squibb, Myriad Genetics, and GlaxoSmithKline
- RV has no financial disclosures other than being an employee of MIA
- RPMS has received honoraria for advisory board participation from MSD, Novartis, and Qbiotics and speaking honoraria from BMS and Novartis
- TEP has no conflicts of interest to declare
- AMM is on the advisory boards for BMS, MSD, Novartis, Roche, Pierre-Fabre, and Qbiotics
- KO, AW, CH, AT, MJ, AN, and AV are employees of Iovance Biotherapeutics, Inc and have stock options