Iovance Generation-2 Tumor-infiltrating Lymphocyte (TIL) Product is Reinvigorated During the Manufacturing Process

Michelle R. Simpson-Abelson, Angel Cedano-Hilton, Kenneth D'Arigo, Maria Fardis and Cécile Chartier

For more information, please contact
Michelle Simpson-Abelson
Michelle.Abelson@iovance.com

IOVANCE BIOTHERAPEUTICS
ADVANCING IMMUNO-ONCOLOGY

Introduction

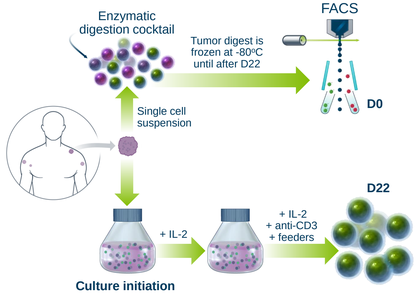
Background

- Adoptive cell therapy (ACT) is a therapeutic strategy that has emerged as a powerful and potentially curative therapy for several cancers. One premise of TIL therapy is that, by isolating the TIL from the immunosuppressive TME and activating them, the ex vivo expansion process produces reinvigorated tumor-specific T cells, which, when transferred back to the patient in high numbers, can drive a robust anti-tumor response.
- To determine whether Iovance's second generation (Gen2) TIL expansion protocol resulted in reinvigorated T cells, the TIL product (D22 TIL) was compared to T cells directly isolated from the tumor (D0 T cells) for phenotype, function, and tumor reactivity.

Methods

- Endogenous T cells (D0), isolated from digested biopsies from melanoma, cervical cancer, head and neck squamous cell carcinoma (HNSCC), and non-small cell lung cancer (NSCLC) were sorted using fluorescence-activated cell sorting (FACS).
- TIL from matching tumor biopsies were expanded according to the Generation- 2 process used to manufacture lifileucel.
- The phenotype of D0 and D22 cells was determined by flow cytometry, using markers of T cell lineage, memory, differentiation, activation/exhaustion, and regulatory properties.
- The secretion of immune molecules, including interferon gamma (IFNγ) and a 32-plex panel, in response to the activation of the T cell receptor (TCR) complex was assessed by enzyme-linked immunosorbent assay (ELISA) and IsoLight technology to determine the T cell effector state and polyfunctionality.
- The tumor reactivity of TIL was tested by co-culture with autologous tumor cells and monitored by IFNγ release.

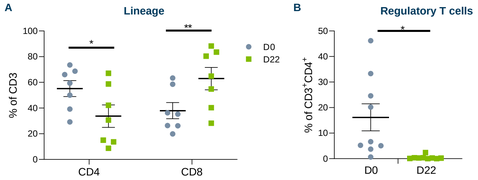
Figure 1: Comparing D0 TIL and Gen2-generated D22 TIL



Shown is the workflow involved in comparing Gen2 D22 TIL product with D0 T cells obtained from the same patient tumor sample.

Results

Figure 2. TIL expansion enriched the product for CD8$^+$ T cells, while excluding Treg

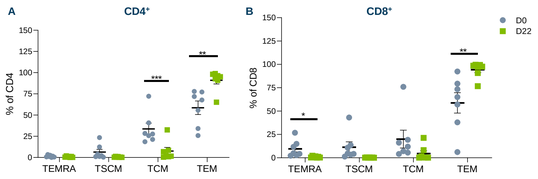


D0 T cells (black dots) and D22 TIL (gray squares) from 4 melanoma, 2 HNSCC, and 1 cervical tumor were assessed by flow cytometry for the cell surface expression of T cell markers representative of the CD4$^+$ and CD8$^+$ T cell lineages **(A)**. Paired D0 and D22 samples from 2 bladder, 1 cervical, 1 breast, 1 HNSCC, 1 NSCLC, and 3 ovarian cancer samples were assessed for the presence of regulatory T cells (Treg), defined as CD4$^+$, FoxP3$^+$, CD127^{low} and CD25^{high} **(B)**. Results expressed as percentage of CD3$^+$ and CD4$^+$ cells, respectively, are plotted for individual samples with mean percentages and standard errors.

Statistical significance was assessed by a paired student t-test; * designates a p value <0.05, ** designates a p value <0.01.

The TIL product is mostly comprised of T cells of a non-suppressive nature.

Figure 3. Both CD4$^+$ and CD8$^+$ T cell lineages became mostly of the effector memory phenotype during the ex vivo expansion

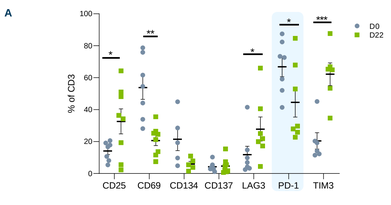


D0 T cells (black dots) and D22 TIL (gray squares) from 4 melanoma, 2 HNSCC, and 1 cervical cancer samples were assessed by flow cytometry for the cell surface expression of memory T cell markers on both CD4$^+$ **(A)** and CD8$^+$ T cells. Memory subsets were identified based on the levels of CD45RA and CCR7. TEM=effector memory (CD45RA$^-$, CCR7$^-$), TCM=central memory (CD45RA$^-$, CCR7$^+$), Tnaive/TSCM= naive/stem cell memory (CD45RA$^+$, CCR7$^+$), TEMRA=CD45RA$^+$ effector memory (CD45RA$^+$,CCR7$^-$). Results expressed as percentage of CD3$^+$ cells are plotted for individual samples with mean percentages and standard errors.

Statistical significance was assessed by a paired student t-test; * designates a p value <0.05, ** designates a p value <0.01, and *** designates a p value <0.001.

The TIL product contains both helper and cytotoxic T cells with rapid effector capabilities.

Figure 4. Ex vivo expansion induced the expression of multiple activation/exhaustion markers, while downregulating PD-1 levels

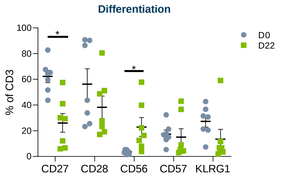


D0 T cells (black dots) and D22 TIL (gray squares) from 4 melanoma, 2 HNSCC, and 1 cervical cancer samples were assessed by flow cytometry for the cell surface expression of T cell markers of activation and exhaustion **(A)**. Results expressed as percentage of CD3$^+$ cells are plotted for individual samples with mean percentages and standard errors. PD-1-positive D0 T cells from 4 melanoma, 7 NSCLC, and 2 HNSCC were sorted by FACS prior to ex vivo expansion **(B)**. PD-1 expression was measured by flow cytometry on D0 T cells (black dots) and D22 TIL (gray square). Unselected TIL were used as controls. Results expressed as mean percentages of CD3$^+$ cells are plotted with standard errors.

Statistical significance was assessed by a paired student t-test; * designates a p value <0.05, ** designates a p value <0.01, *** designates a p value <0.001 and **** designates a p value <0.0001.

The TIL product is comprised of highly activated T cells. This is due to down-regulation of PD-1.
The differential expression of TIM3 and LAG3 in Gen2-expanded TIL is indicative of highly activated cells, rather than markers of exhaustion.

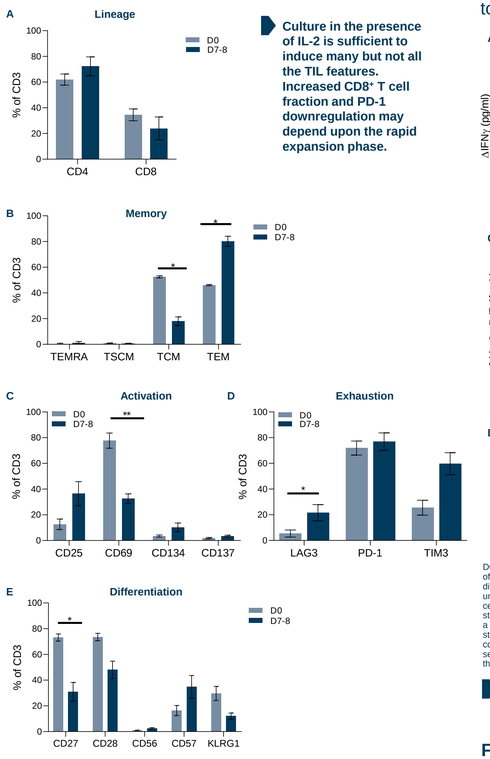
Figure 5. Ex vivo expansion did not push the T cells toward a terminal state of differentiation and preserved T cell youth



D0 T cells (black dots) and D22 TIL (gray squares) from 4 melanoma, 2 HNSCC, and 1 cervical tumor were assessed by flow cytometry for the cell surface expression of the cell youth markers CD27 and CD28, differentiation markers CD56 and CD57, and senescence marker KLRG1. Results expressed as percentage of CD3$^+$ cells are plotted for individual samples with mean percentages standard errors. Statistical significance was assessed by a paired student t-test; * designates a p value <0.05.

The short duration of the Gen2 process maintains the T cells in a low state of differentiation, compatible with long-term in vivo persistence.

Figure 6. Most phenotypic changes occurred during the initial phase of the culture

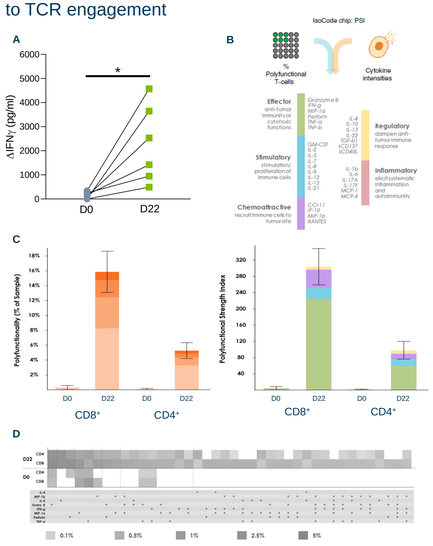


Culture in the presence of IL-2 is sufficient to induce many but not all the TIL features.
Increased CD8$^+$ T cell fraction and PD-1 downregulation may depend upon the rapid expansion phase.

D0 T cells and D3-4 and D7-8 TIL from 3 HNSCC, 2 NSCLC and 1 cervical were assessed by flow cytometry for the cell surface expression of T cell markers representative of lineage **(A)**, memory **(B)**, activation **(C)**, exhaustion **(D)**, and differentiation **(E)**. Results are plotted with the black bars representing the D0 T cells, the gray bars representing the D7-8 TIL.

Standard errors are shown as vertical lines. Statistical significance was assessed by a paired student t-test; * designates a p value <0.05, ** designates a p value <0.01.

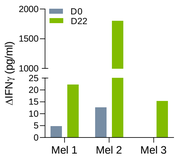
Figure 7. Ex vivo expansion restored the TIL ability to secrete multiple cytokines in response to TCR engagement



D0 T cells and D22 TIL from 3 melanoma, 2 HNSCC, and 1 cervical tumor were assessed for the secretion of IFNγ in response to stimulation with αCD3/αCD28/αCD137-coated beads. Results are expressed as the difference in IFNγ concentration measured upon stimulation minus the concentration measured for the unstimulated control (ΔIFNγ) and plotted for individual samples, with the black dots representing the D0 T cells and the gray squares representing the D22 TIL. Statistical significance was assessed by a paired student t-test; * designates a p value <0.05 **(A)**. Schematic of polyfunctionality assessment of T cells, using a 32-plex single cell platform (IsoPlexis, CT) **(B)**. Single cell polyfunctionality (top) and polyfunctionality strength index (PSI)(bottom) **(C)**. Heat map of single-cell cytokine combinations by each group. Each column corresponds to combination of cytokines, and the orange squares represent the frequency of the secreted cytokines **(D)**. PSI = percentage of polyfunctionality cells in sample multiplied by the intensities of the secreted cytokines.

T cell culture and expansion resulted in highly functional, reinvigorated T cells.

Figure 8. Tumor reactivity was increased upon ex vivo culturing of the TIL



D0 T cells and D22 TIL were co-cultured with autologous digest for 24-72 hours and assessed for IFNγ secretion, by ELISA. Difference in IFNγ concentration measured with and without an HLA class I blocking antibody (ΔIFNγ) is shown for 3 melanoma samples (Mel) at D0 (black bars) and D22 (gray bars). Positive values reflect HLA-specific anti-tumor responses, while null or negative values reflect non-specific responses.

The high functionality of the T cells as well as their enhanced tumor reactivity represents a measure of TIL in vivo activation potential.

Summary

- Iovance's 22-day process imparted to the TIL product the main features known to be associated with *in vivo* antitumor activity with:
  - T cells from both the CD4$^+$ and CD8$^+$ T cell lineages, skewed toward a high fraction of CD8$^+$ cells and depleted of Tregs;
  - An effector memory phenotype;
  - Concurrent expression of activation markers with low levels of differentiation markers and downregulation of the canonical PD-1 exhaustion marker; and
  - High functionality in response to pan and tumor-specific stimulation.

Conclusion

- Ex vivo expansion of TIL, using Iovance's Gen2 process, reverses the dysfunctional state of the T cells from the tumor microenvironment by improving their phenotypic, functional, and tumor-reactive profile.
- Reinvigoration of the T cells that comprise the TIL product may be critical to the induction of a potent and effective anti-tumor response *in vivo*. The remaining key for the efficacy of TIL lies within its tissue of origin, the tumor itself, naturally enriched for the polyclonal and patient-specific repertoire of tumor neoantigen-recognizing T cells.

Disclosure and Funding Statement
This study and poster are sponsored by Iovance Biotherapeutics, Inc.
All authors are employees of Iovance Biotherapeutics, Inc.