Expanding Iovance's tumor infiltrating lymphocytes (TIL) from core biopsies for adoptive T cell therapy using a 22-day manufacturing process

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INTRODUCTION

- Iovance's TIL products lifileucel and LN-145 have demonstrated remarkable clinical activity in melanoma and cervical cancer utilizing Iovance's proprietary 22-day manufacturing process.[1,2]
- Current protocol for generating TIL requires surgical resection of tumor lesions ~1.5-cm diameter.
- Using small tumor biopsies obtained by core needle biopsy procedures would allow a greater number of patients to benefit from TIL therapy.
- We asked whether a streamlined manufacturing process could be implemented to produce therapeutically relevant TIL from multiple histologies starting with a core biopsy.

[1] Jazaeri AA, Zsiros E, Amaria RN, Artz AS, Edwards RP, Robert Michael Wenham RM, et al. Safety and efficacy of adoptive cell transfer using autologous tumor infiltrating lymphocytes (LN-145) for treatment of recurrent, metastatic, or persistent cervical carcinoma. *Clin Oncol.* 2019;37:15:2538 (suppl).

[2] Sarnaik A, Khushalani NI, Chesney JA, Kluger HM, Curti BD, et al. Safety and efficacy of cryopreserved autologous tumor infiltrating lymphocyte therapy (LN-144, lifileucel) advanced metastatic melanoma patients who progressed on multiple prior therapies including anti-PD-1. *J Clin Oncol.* 2019;37:15:2518 (suppl).

MATERIALS & METHODS

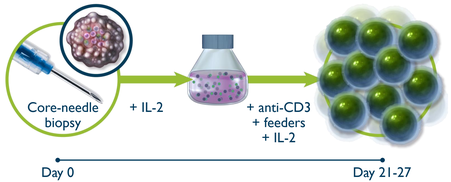
- Core biopsies obtained from 2 pancreatic, 4 melanoma, 2 breast, 2 ovarian, and 1 lung tumors were processed *in vitro*, using a 2-step expansion method by which TIL are recovered in the presence of IL-2 prior to being rapidly expanded in the presence of anti-CD3 antibody and allogeneic PBMCs.
- Core biopsy-derived TIL were assessed for total viable cell count, expansion, phenotype based on the expression of markers associated with T cell lineage, memory, youth/differentiation, activation, and exhaustion markers, and function based on IFNγ secretion and CD107a mobilization in response to non-specific stimuli.
- Results were compared with the average characteristics of melanoma TIL obtained from regular biopsies, using Iovance's current Gen 2 process.

Table 1. TIL derived from one core were expanded to therapeutic numbers in 22 days

TUMOR	CORES/ CONDITION	DURATION OF CULTURE	FINAL CELL COUNT
Pancreas 1	4	25	1.45e10
Pancreas 2	2	27	1.45e10
Melanoma 1	2	21	1.23e10
Melanoma 2	2	23	7.47e10
Melanoma 3	2	21	2.05e10
Melanoma 4	1	22	3.21e10
Breast 1	2	25	2.90e10
Breast 2	2	22	17.8e10
Ovarian 1	2	21	9.08e10
Ovarian 2	0.25	22	5.23e8
Lung 1	1	22	1.38e10

Cores from pancreatic (n=2), melanoma (n=4), breast (n=2), ovarian (n=2), and lung (n=1) were used to derive TIL using Iovance's Core expansion protocol. The number of cores used for expansion and cell counts are indicated.

Figure 1. Development of a novel process for the *ex vivo* expansion of TIL from core biopsies



One to two fresh core biopsies were placed in culture media + IL-2. TIL were then expanded with OKT3 (30ng/ml) and irradiated PBMCs (feeders). Durations of 21-27 days were tested during the optimization phase of the project.

RESULTS

Figure 2. Core-derived TIL were mostly comprised of non-differentiated, activated effector memory T cells

Core-derived TIL from pancreatic (n=2), melanoma (n=4), breast (n=2), ovarian (n=2), and lung (n=1) tumors were assessed by flow cytometry for the cell surface expression of T cell markers representative of lineage **(A)**, memory **(B)**, differentiation **(C)**, activation **(D)**, and exhaustion **(E)**. Average percent positive T cells are shown as green ($CD4^+$) and blue ($CD8^+$) bars topped with standard error lines. Memory subsets were identified based on the levels of CD45RA and CCR7 on the $CD4^+$ and $CD8^+$ T cells.

Abbreviations: TEM, effector memory (CD45RA-, CCR7-); TCM, central memory (CD45RA-, CCR7+); Tnaive/TSCM, naive/stem cell memory ($CD45RA^+$, $CCR7^+$); TEMRA, CD45RA+ effector memory ($CD45RA^+$, $CCR7^-$).

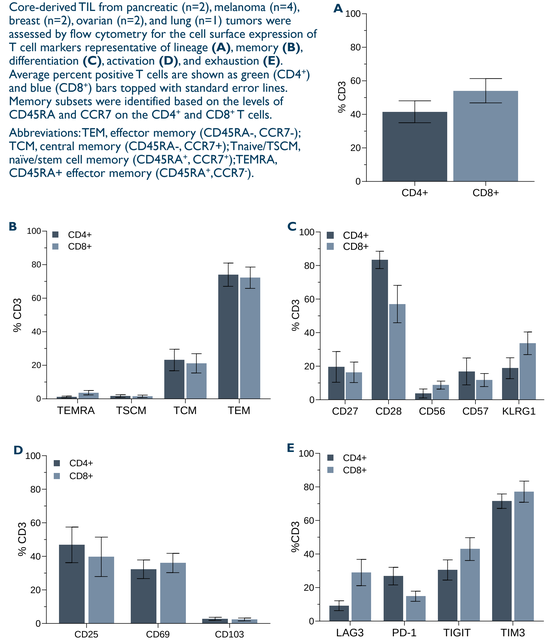


Figure 3. Core-derived TIL were functional as determined by IFNγ secretion and CD107a mobilization in response to non-specific stimulation

Core-derived TIL from pancreatic (n=2), melanoma (n=4), breast (n=2), ovarian (n=2), and lung (n=1) tumors were assessed for IFNγ secretion **(A)** and CD107a mobilization **(B)** in response to stimulation with αCD3/αCD28/αCD137-coated beads and PMA/Ionomycin, respectively. Percentages of $CD4^+$ and $CD8^+$ cells expressing CD107a were assessed using flow cytometry and plotted for each TIL sample. Horizontal lines represent the mean percentages of each group and vertical lines represent the standard errors.

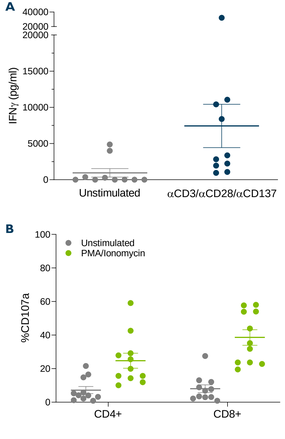


Figure 4. TIL derived from melanoma core biopsies were comparable to Gen 2-derived TIL from melanoma resections

TIL expanded from 4 melanoma core biopsies using Iovance's Core process and 5-7 unmatched melanoma resections were assessed by flow cytometry for cell surface expression of T cell markers representative of lineage **(A)**, memory **(B)**, differentiation **(C)**, activation **(D)**, and exhaustion **(E)**. Percentages of $CD4^+$ and $CD8^+$ T cells are shown for each individual TIL product. Bars represent the mean percentages of the TIL preparations and vertical lines represent the standard errors. Statistical significance was assessed by an unpaired student t-test **p<0.01.

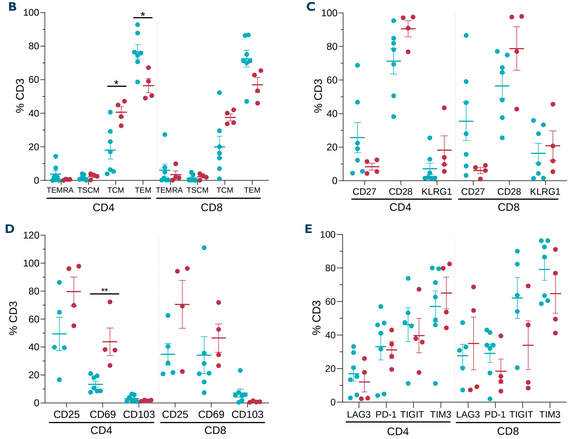
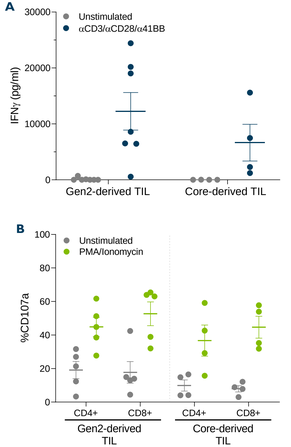


Figure 5. TIL derived from core biopsies and Gen 2-derived TIL from tumor resections were functionally comparable in melanoma

TIL derived from 4 melanoma core biopsies using Iovance's Core process and 5-7 unmatched melanoma resections were assessed for IFNγ secretion **(A)** and CD107a mobilization **(B)** in response to stimulation with αCD3/αCD28/αCD137-coated beads and PMA/Ionomycin, respectively. Percentages of $CD4^+$ and $CD8^+$ cells expressing CD107a were assessed using flow cytometry and plotted for each individual TIL sample. Horizontal lines represent the mean percentages of each group and vertical lines represent the standard errors.



SUMMARY

- A protocol has been developed for the *in vitro* expansion of TIL to clinically relevant numbers from core-biopsies derived from pancreatic, melanoma, breast, ovarian, and lung cancer.
- Core-derived TIL products phenotypically represented a non-differentiated, activated, effector memory T cell population that was comparable with Iovance's current TIL product derived from melanoma.
- Core-derived TIL products were highly functional, as shown by CD107a mobilization and IFNγ secretion.

CONCLUSIONS

- This work demonstrates that the 22-day manufacturing method developed at Iovance can reliably expand TIL from a single core biopsy to therapeutically relevant numbers from multiple tumor histologies.
- TIL derived using core biopsies were shown to be comparable to products generated using Iovance's Gen 2 process, in melanoma.
- Iovance anticipates implementing this process in the clinic in the near future.

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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