P357 SITC ANNUAL MEETING, NOVEMBER 8-12, 2017 | NATIONAL HARBOR, MD, USA

The T-cell Growth Factor Cocktail IL-2/IL-15/IL-21 Enhances Expansion and Effector Function of Tumor-Infiltrating T cells in a Novel Process Developed by Iovance

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BACKGROUND

Adoptive T cell therapy with autologous tumor infiltrating lymphocytes (TIL) has demonstrated clinical efficacy in patients with metastatic melanoma and cervical carcinoma.

In some studies, better clinical outcomes have positively correlated with the total number of cells infused and/or percentage of $CD8^+$ T cells.

Most current production regimens solely utilize IL-2 to promote TIL growth.

Enhanced lymphocyte expansion has been reported using IL-15 and IL-21-containing regimens.

This study describes the positive effects of adding IL-15 and IL-21 to IL-2 in a second generation TIL protocol recently developed by Iovance Biotherapeutics.

Generation of TIL using a novel process developed at Iovance

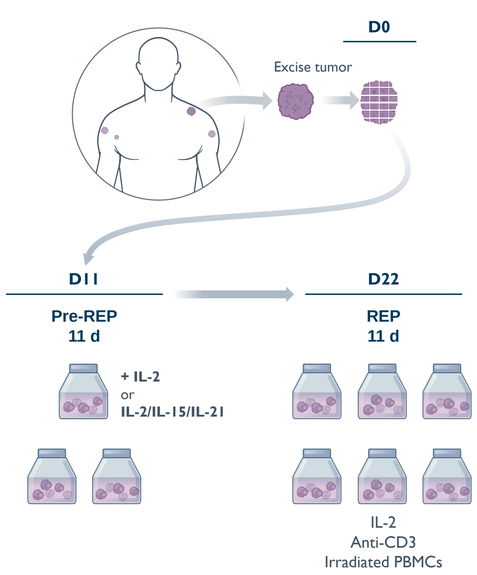

Figure 1. The tumor is excised from the patient and transported to the GMP Manufacturing facility or Iovance (for research purposes). Upon arrival the tumor is fragmented, placed into flasks with IL-2 for pre-Rapid Expansion Protocol (pre-REP) for 11 days. For the triple cocktail studies, IL-2/IL-15/IL-21 is added at the initiation of the pre-REP. For the Rapid Expansion Protocol (REP), TIL are cultured with feeders and anti-CD3 antibody for an additional 11 days.

ACKNOWLEDGMENT

The contributions of Michael Lotze to earlier studies on which this work is based are gratefully acknowledged.

DISCLOSURE & FUNDING STATEMENT

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

MATERIALS & METHODS

- The process of generating TIL includes a pre-Rapid Expansion Protocol (pre-REP), in which tumor fragments of 1-3 mm^3 size are placed in media containing IL-2.
- During the pre-REP, TIL emigrate out of the tumor fragments and expand in response to IL-2 stimulation.
- To further stimulate TIL growth, TIL are expanded through a secondary culture period termed the Rapid Expansion Protocol (REP) that includes irradiated PBMC feeders, IL-2 and anti-CD3 antibody.
- A shortened pre-REP and REP expansion protocol was developed at Iovance to expand TIL while maintaining the phenotypic and functional attributes of the final TIL product.
- This shortened TIL-generation protocol was used to assess the impact of IL-2 alone versus a combination of IL2/IL-15/IL-21 added to the pre-REP step.
- These two culture regimens were compared for the generation of TIL grown from colorectal, melanoma, cervical, triple negative breast, lung and renal tumors.
- At the completion of the pre-REP, cultured TIL were assessed for expansion, phenotype, function ($CD107a^+$ and IFNγ) and TCR Vβ repertoire.

RESULTS

Enhancement in expansion during the pre-REP with IL-2/IL-15/IL-21 in multiple tumor histologies

Tumor Histology	# of studies demonstrating >20% enhancement of growth using IL-2/IL-15/IL-21 (compared to IL-2)
Melanoma	**1/5 (20%)**
Lung	**3/8 (38%)**
Colorectal	7/11 (63%)
Cervical	1/1 (100%)
Pancreatic	2/2 (100%)
Glioblastoma	1/1 (100%)
Triple Negative Breast	1/2 (50%)

Table 1. Pre-REP cultures were initiated using the standard IL-2 protocol, or with IL-15 and IL-21 in addition to IL-2. Cells were assessed for expansion at the completion of the pre-REP. A culture was classified as having increased expansion over the IL-2 if the overall growth was enhanced by at least 20%. Melanoma and lung phenotypic and functional studies are presented herein (**bolded text**).

RESULTS

IL-2/IL-15/IL-21 enhances the percentage of $CD8^+$ cells in lung carcinoma, but not in melanoma

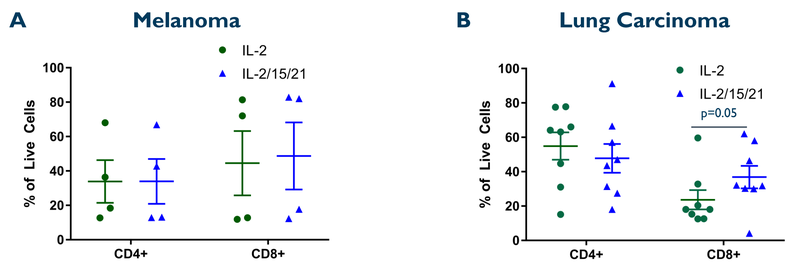

Figure 2. TIL derived from (**A**) melanoma (n=4), and (**B**) lung (n=7) were assessed phenotypically for $CD4^+$ and $CD8^+$ cells using flow cytometry post pre-REP. p value represents the difference between the IL-2 and IL-12/IL-15/IL-21 conditions using the student's unpaired t test.

Expression of CD27 is slightly enhanced in CD8+ cells in cultures treated with IL-2/IL-15/IL-21

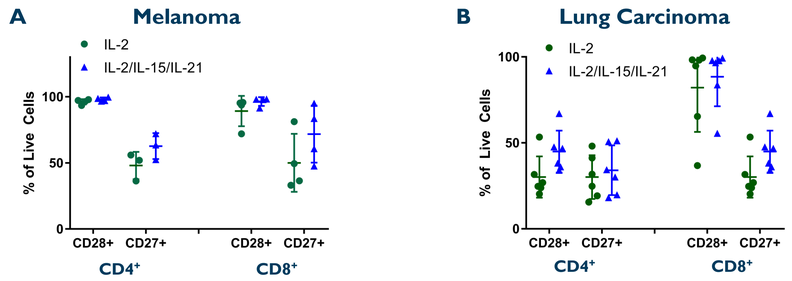

Figure 3. TIL derived from **(A)** melanoma (n=4), and **(B)** lung (n=7) were assessed phenotypically for $CD27^+$ and $CD28^+$ in the $CD4^+$ and $CD8^+$ cells using flow cytometry post pre-REP. Expression of CD27, a cellular marker associated with a younger phenotype that has correlated with outcomes to adoptive T cell therapy, is slightly enhanced in $CD8^+$ TIL derived from culture with IL-2/IL-15/IL-21 *vs.* IL-2 alone.

T cell subsets are unaltered with the addition of IL-15/IL-21

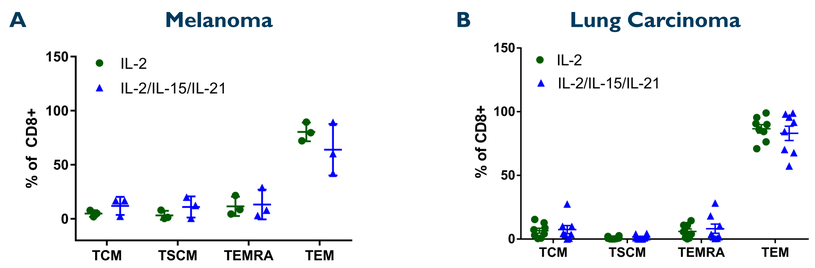

Figure 4. TIL were assessed phenotypically for effector/memory subsets (CD45RA and CCR7) in the $CD8^+$ and $CD4^+$ (data not shown) cells from (**A**) melanoma (n=4), and (**B**) lung (n=8) via flow cytometry post pre-REP. TEM = effector memory ($CD45RA^-$, $CCR7^-$), TCM = central memory ($CD45RA^-$, $CCR7^+$), TSCM = stem cell memory ($CD45RA^+$, $CCR7^+$), TEMRA = effector T cells ($CD45RA^+CCR7^-$).

The functional capacity of TIL is differentially enhanced with IL-2/IL-15/IL-21

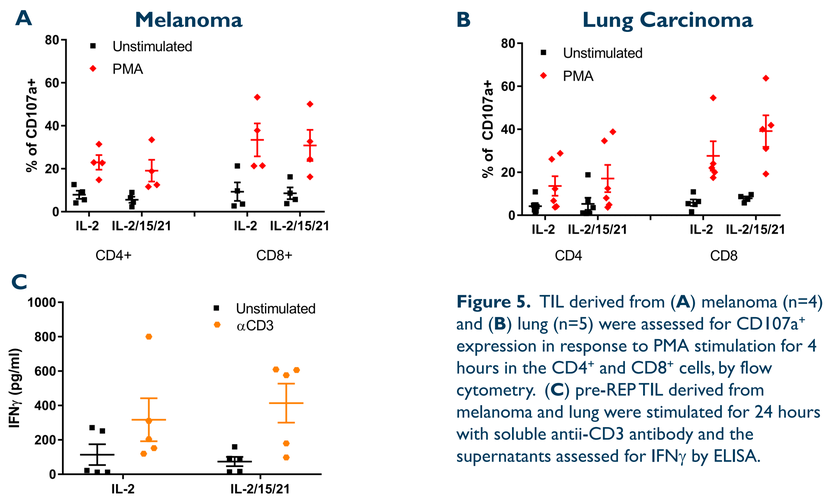

Figure 5. TIL derived from (**A**) melanoma (n=4) and (**B**) lung (n=5) were assessed for $CD107a^+$ expression in response to PMA stimulation for 4 hours in the $CD4^+$ and $CD8^+$ cells, by flow cytometry. (**C**) pre-REP TIL derived from melanoma and lung were stimulated for 24 hours with soluble antii-CD3 antibody and the supernatants assessed for IFNγ by ELISA.

The relative frequency of the TCRvβ repertoire is altered in response to IL-2/IL-15/IL-21 in lung, but not in melanoma

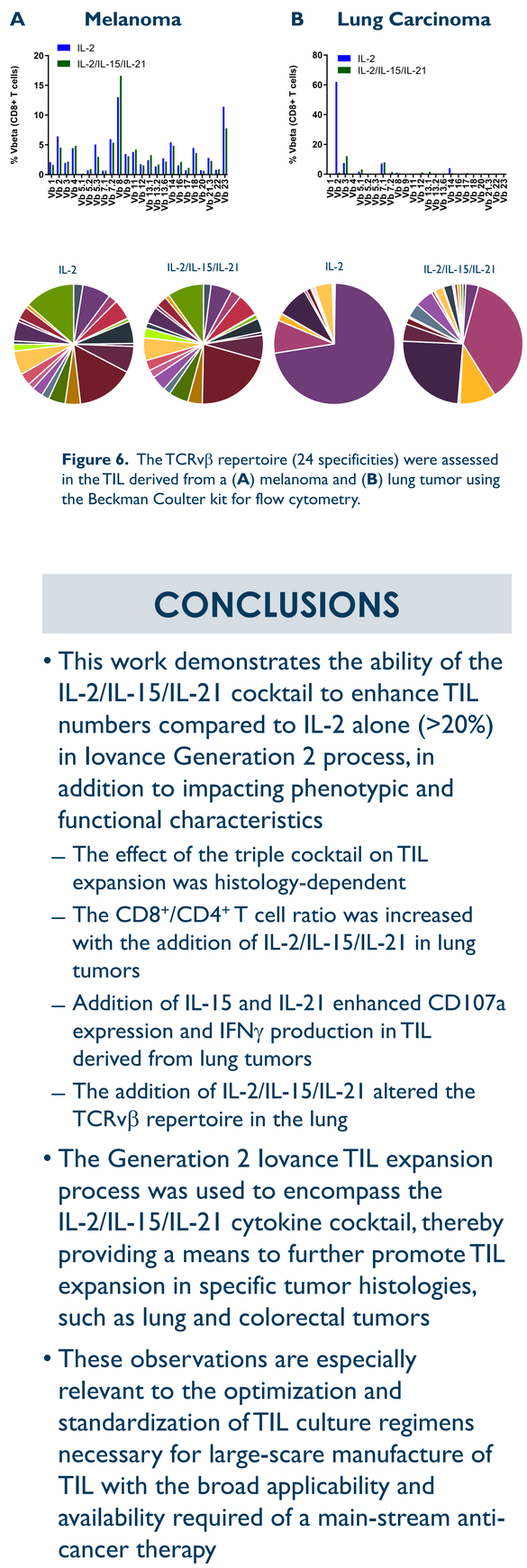

Figure 6. The TCRvβ repertoire (24 specificities) were assessed in the TIL derived from a (**A**) melanoma and (**B**) lung tumor using the Beckman Coulter kit for flow cytometry.

CONCLUSIONS

- This work demonstrates the ability of the IL-2/IL-15/IL-21 cocktail to enhance TIL numbers compared to IL-2 alone (>20%) in Iovance Generation 2 process, in addition to impacting phenotypic and functional characteristics
  - The effect of the triple cocktail on TIL expansion was histology-dependent
  - The $CD8^+/CD4^+$ T cell ratio was increased with the addition of IL-2/IL-15/IL-21 in lung tumors
  - Addition of IL-15 and IL-21 enhanced CD107a expression and IFNγ production in TIL derived from lung tumors
  - The addition of IL-2/IL-15/IL-21 altered the TCRvβ repertoire in the lung
- The Generation 2 Iovance TIL expansion process was used to encompass the IL-2/IL-15/IL-21 cytokine cocktail, thereby providing a means to further promote TIL expansion in specific tumor histologies, such as lung and colorectal tumors
- These observations are especially relevant to the optimization and standardization of TIL culture regimens necessary for large-scare manufacture of TIL with the broad applicability and availability required of a main-stream anti-cancer therapy