Successful Generation of Tumor-Infiltrating Lymphocyte (TIL) Product From Renal Cell Carcinoma Tumors for Adoptive Cell Therapy

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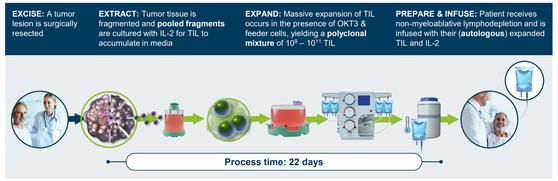
Introduction

Background

- Patients with renal cell carcinoma (RCC) may achieve remission with immune-checkpoint inhibitors (ICI); however, most patients will progress
- Adoptive cell therapy with autologous tumor-infiltrating lymphocytes (TIL) allows for expansion of T-cells from tumor tissue, leading to a polyclonal T-cell product with a diverse T-cell receptor (TCR) repertoire capable of recognizing an array of tumor antigens
- TIL therapy with centrally manufactured lifileucel demonstrated a 36% objective response rate in patients with ICI-refractory melanoma[1]
- We have developed a second-generation (Gen 2) Good Manufacturing Practice (GMP) manufacturing process with a substantially reduced time (22 days) to expand functional TIL from melanoma, cervical, head and neck, bladder, and lung tumors, as well as other tumor types
- Here we present our preclinical experience of TIL production in RCC using Gen 2 manufacturing

Figure 1. Cryopreserved Gen 2 GMP Manufacturing Process



Study Objectives

- To determine the feasibility of generating TIL from patient-derived RCC tumor specimens using a 22-day Gen 2 GMP manufacturing process
- To characterize the final harvested product for the following quality attributes:
  1. Dose: Cell count and % viability
  2. Identity: % T-cells
  3. Functionality: Ability to secrete IFNγ and Granzyme B in response to stimulation with anti-CD3, -CD28, -CD137 and anti-CD3, -CD28, respectively
  4. Phenotype: Purity, differentiation, and memory status

Methods

Manufacturing

- The Gen 2 TIL manufacturing process for the resected RCC tumor samples includes pre-rapid expansion protocol (pre-REP) and rapid expansion protocol (REP) over 22 days
  - During pre-REP (1/10th scale), 1- to 3-mm tumor fragments were placed in media containing IL-2 for 11 days, and TIL were allowed to leave the tumor tissue
  - To further stimulate TIL growth, TIL were expanded using REP (1/100th scale) that included irradiated peripheral blood mononuclear cell feeders, IL-2, and anti-CD3 for 11 days

Methods

Dose

- Final harvested TIL and in-process samples were assayed for total nucleated cells, total viable cells (TVC), and viability determined by acridine orange / DAPI counterstain using the NucleoCounter® NC-200™ (ChemoMetec, Lillerød, Denmark) automated cell counter

Identity

- Final harvested TIL products were sampled and assayed for identity by immunofluorescent staining
- Percent T-cells was determined as the percentage of CD45^{+}CD3^{+} (double positive) population of viable cells

Functionality

- The ability of the harvested TIL product to secrete IFNγ and Granzyme B upon reactivation was measured following coculture with antibody-coated beads (IFNγ: anti-CD3, anti-CD28, and anti-CD137; Granzyme B: anti-CD3 and anti-CD28; Thermo Fisher, Waltham, MA)
- After 24 hours of co-culture, culture supernatants were harvested, frozen, thawed, and assayed by ELISA

Phenotype

- Final harvested TIL products were thawed and assayed for extended phenotypic markers using two flow cytometry panels
- Multicolor flow cytometry was performed to characterize TIL purity, identity, memory subset, activation, and exhaustion status
- Data were acquired from stained sample products on the ZE5 (Bio-Rad, Hercules, CA) cell analyzer

Results

Table 1. Baseline Demographics and Tumor Characteristics

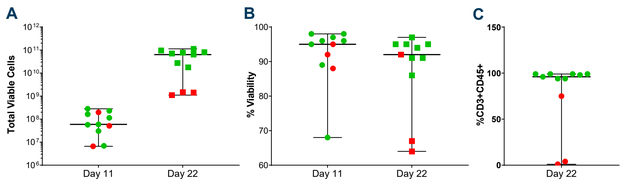
Characteristic	N = 11
Age, years, median (IQR)	59 (52–68)
Sex, n (%)	
Male	10 (91)
Race, n (%)	
White	9 (82)
Histology, n (%)	
Clear cell	8 (73)
Papillary	2 (18)
Chromophobe	1 (9)
Tumor site, n (%)	
Kidney	9 (82)
Adrenal	1 (9)
Lung	1 (9)

Table 2. Summary of Product Attributes

Tumor ID	Histology	Tumor	Tissue Type	Treatment History	Acceptance Criteria	TVC (×10^{9})	Viability (%)	CD45^{+} CD3^{+} (%)	CD4^{+} (%)	CD8^{+} (%)	CD4^{+}/ CD8^{+}
K7024	Clear cell	Lung	Lung	Axitinib, IL-2, X4P-001	Not met	1	64	0		-*	
K7025	Clear cell	Primary	Kidney	None	Met	77	97	99	48	34	1
K7026	Clear cell	Primary	Kidney	None	Met	96	95	96	21	72	0
K7028	Clear cell	Primary	Kidney	None‡	Not met	1	68	0		-*	
K7029	Clear cell	Primary	Kidney	None	Met	71	91	99	77	18	4
K7030	Papillary	Primary	Kidney	None	Met	63	95	98	58	29	2
K7031	Papillary	Primary	Kidney	None	Met	81	94	99	97	2	49
K7032	Clear cell	Primary	Kidney	Local cryoablation	Not met	1	92	75		-†	
K7033	Chromophobe	Primary	Kidney	None	Met	27	91	94	72	25	3
K7034	Clear cell	Primary	Kidney	None	Met	113	95	99	65	29	2
K7035	Clear cell	Adrenal	Adrenal gland	IL-2, sunitinib, nivolumab	Met	18	86	94	80	18	4

*No CD3^{+} T-cell subset. †Product not available to test. ‡Patient received ocrelizumab for multiple sclerosis.

Figure 2. TIL Dose, Viability, and Identity

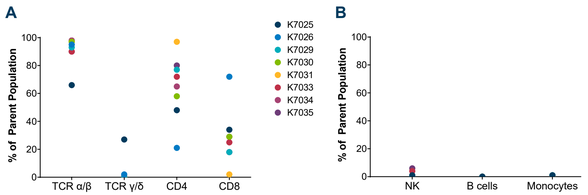


Green circles indicate 8 tumors that met acceptance criteria and red circles indicate 3 tumors that did not meet the acceptance criteria. Median (range) are displayed.

- 8 of 11 tumors met acceptance criteria, including TVC, % viability, and %CD3^{+}CD45^{+}

Results

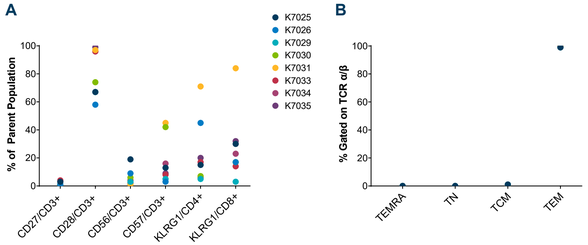
Figure 3. TIL Purity by Multicolor Flow Cytometry



Circles indicate the 8 individual tumor samples that met acceptance criteria. In panel B, samples were highly overlapped.

- TIL predominantly expressed TCR α/β and CD4, with lesser populations of TCR γ/δ and moderate populations of CD8^{+} TIL
- Few contaminating non–T-cell immune populations were observed

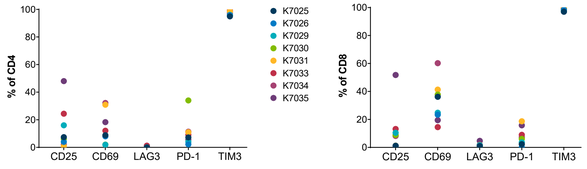
Figure 4. TIL Differentiation and Memory Status



Memory subsets were identified based on the levels of CD45RA and CCR7. TEM=effector memory (CD45RA^{-}, CCR7^{-}), TCM=central memory (CD45RA^{-}, CCR7^{+}), TN=naïve (CD45RA^{+}, CCR7^{+}), TEMRA=CD45RA+ effector memory (CD45RA^{+},CCR7^{-}). Circles indicate the 8 individual tumor samples that met acceptance criteria. In panel B, samples were highly overlapped.

- TIL mostly expressed CD28, required for activation of effector cells upon TCR engagement
- TIL were predominantly effector memory phenotype (CD45RA^{-}, CCR7^{-})

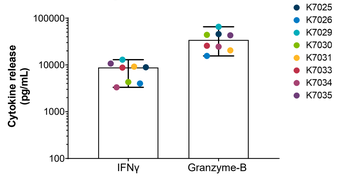
Figure 5. TIL Activation and Exhaustion Markers



Circles indicate the 8 individual tumor samples that met acceptance criteria.

- TIL generally expressed activation but not exhaustion markers, comparable to prior results in other tumor types (data on file)

Figure 6. TIL Function Measured by IFNγ and Granzyme B Release



Median (range) are displayed in the figure. Circles indicate the 8 individual tumor samples that met acceptance criteria.

- TIL released IFNγ and Granzyme B in response to anti-CD3, -CD28, and -CD137 beads, similar to prior results in melanoma[2]

Conclusions

- 8 of 11 TIL products (73%) showed acceptable TIL product attributes
- Yield of TIL from the 8 tumors was an average of 74 × 10^{9} viable cells
- TIL generated from RCC samples using the Gen 2 process met all acceptance criteria and were generally comparable in function and phenotype to TIL generated from other tumor types
- These feasibility data suggest that TIL can be successfully expanded ex vivo from RCC samples (including pre-treated and metastatic tumors) and may support clinical investigation of TIL in patients with RCC

References

1. Sarnaik AA, et al. Lifileucel, a Tumor-Infiltrating Lymphocyte Therapy, in Metastatic Melanoma. *J Clin Oncol*. 2021;JCO.21.00612.
2. Wardell S, et al. Iovance Gen 2 TIL Manufacturing Process Produces Drug Products that Exhibit Favorable Quality Attributes for Adoptive Cell Transfer Across 5 Solid Tumor Indications. SITC Annual Meeting 2019 (abstract P226).

Abbreviations

DAPI, 4′,6-diamidino-2-phenylindole; ICI, immune-checkpoint inhibitors; IFN, interferon; IL-2, interleukin-2; Gen, generation; GMP, good manufacturing practice; MHC, major histocompatibility complex; NK, natural killer; PBMC, peripheral blood mononuclear cell; RCC, renal cell carcinoma; REP, rapid expansion protocol; TCM, central memory T-cells; TCR, T-cell receptor; TEM, effector memory T-cells; TIL, tumor-infiltrating lymphocytes; TN, naïve T-cells; TVC, total viable cells.

Disclosures

- All authors meet the criteria for authorship set forth by the International Committee of Medical Journal Editors
- KO, CH, AW, AN, and AV are employees of Iovance and may have stock options

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