Preclinical Activity and Manufacturing Feasibility of Genetically Modified *PDCD-1* Knockout (KO) Tumor-Infiltrating Lymphocyte (TIL) Cell Therapy

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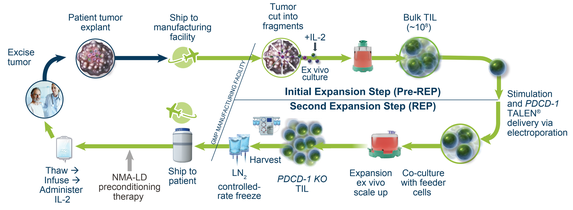
Background

- Adoptive cell therapy using autologous tumor-infiltrating lymphocytes (TIL; lifileucel, LN-145) has demonstrated encouraging efficacy and safety in both the post-immune checkpoint inhibitor (ICI) and ICI-naïve settings in patients with advanced solid tumors
  - One-time lifileucel TIL cell therapy achieved durable responses in the post-ICI setting in patients with advanced/unresectable melanoma,[1,2] with an objective response rate (ORR) of 36% and duration of response (DOR) not reached after 33.1 months of follow-up[2]
  - In ICI-naïve patients with advanced melanoma, early-line combination of lifileucel plus pembrolizumab resulted in a 60% ORR, with a 30% complete response rate[3]
- Although effective, anti–programmed cell death protein (PD)-1 ICI therapy is limited by poor penetration into the tumor, internalization, and endocytic clearance[4-8]; by contrast, TIL are actively transported into the tumor, can move independently based on chemoattraction, and are not cleared or internalized[9]
- Administration of anti–PD-1 antibodies is associated with immune-related adverse events (AEs) due to non-specific upregulation of immune pathways through the systemic activation and proliferation of T cells[10]
- IOV-4001, a *PDCD-1* knockout (KO) TIL cell therapy, may enhance the efficacy of TIL cell therapy and abrogate the need for systemic anti–PD-1 therapy, while avoiding short- and long-term systemic AEs associated with anti–PD-1/PD-L1 therapy
  - Transcription activator-like effector endonucleases (TALEN®) are hybrid molecules composed of a DNA-binding domain and the FokI nuclease. Combination of 2 TALEN® arms directed at the *PDCD-1* gene encoding PD-1 mediates DNA double-strand breaks, leading to gene disruption and PD-1 inactivation[11-13]
  - A process has been established for the generation of TALEN®-mediated *PDCD-1* KO TIL and their expansion to therapeutically relevant numbers with robust effector function and phenotypic markers indicative of functional TIL (TALEN® gene-editing technology is licensed from Cellectis)[14]
- Here, we describe IOV-4001 (1) in vivo preclinical activity, (2) clinical-scale manufacturing process development, and (3) phenotypic and functional characterization

Methods

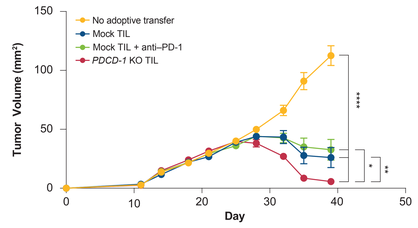
- **Manufacturing**:
  - A 22-day clinical-scale IOV-4001 manufacturing process was established, including pre-rapid expansion protocol (pre-REP), activation, electroporation, resting, and REP for the generation of *PDCD-1* KO TIL (developed with TALEN® gene-editing technology in collaboration with Cellectis, Paris, France)
  - Development runs were generated in non-Good Manufacturing Practice (GMP) scale at Iovance Process Development (Tampa, FL)
  - Manufacturing runs were generated in GMP manufacturing scale at the contract manufacturing organization (CMO)



- **In Vivo Antitumor Activity:**
  - Mice expressing human interleukin-2 (hIL-2) under the control of a cytomegalovirus promoter (hIL-2 NOG)[15] were engrafted with melanoma tumor cells and received adoptive transfer of either **(A)** autologous *PDCD-1* KO TIL, **(B)** mock TIL (TIL electroporated without TALEN®), **(C)** mock TIL + anti–PD-1 antibody, or **(D)** no adoptive transfer of TIL; (n=14 each)
  - Tumor size was measured twice per week for 39 days
- **Product Release:**
  - Final *PDCD-1* KO TIL product was characterized for:
    - Total viable cells (TVC) 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 ($CD45^+CD3^+$ phenotype), assayed by immunofluorescence staining and flow cytometry
    - Potency (interferon-γ [IFNγ] release), assayed by ELISA using the Quantikine® IFNγ ELISA kit (R&D Systems, Minneapolis, MN, USA)
  - *PDCD-1* KO efficiency was evaluated based on PD-1 expression of *PDCD-1* KO TIL compared to mock TIL by flow cytometry
- **Phenotype:**
  - Final harvested *PDCD-1* KO TIL products were assayed for extended phenotypic markers using 2 multicolor flow cytometry panels to characterize TIL purity, identity, memory subset, activation, and exhaustion status
- **Characterization:**
  - **IL-2 Assay:** To assess the safety of the *PDCD-1* KO TIL product, the in vitro proliferative capacity of the final product in the absence of IL-2 was assessed over a period of 28 days
  - **Karyotyping Assay:** Cytogenetic examinations were performed by NeoGenomics Laboratories (Fort Myers, FL, USA). Briefly, cryopreserved *PDCD-1* KO TIL samples were rested and activated to harvest the metaphase cells for G-banding cytogenetic analysis. Three replicates of mitotic cells were analyzed, fixed, and stained to perform G-banding
- **Statistical Analysis:**
  - Unpaired Student t-test was used to analyze differences in phenotype, and Wilcoxon rank-sum test was used to detect differences in mouse in vivo studies; $p < 0.05$ was considered statistically significant

Results

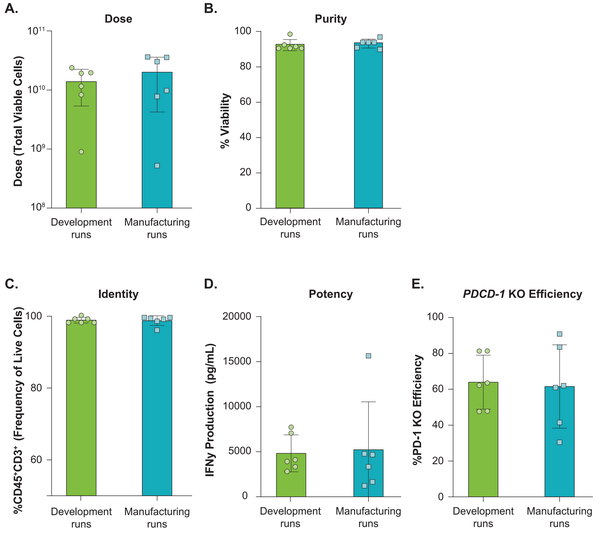
Figure 1. *PDCD-1* KO TIL Show Increased Antitumor Activity



To assess the in vivo efficacy of *PDCD-1* KO TIL, hIL-2 NOG mice (n=14 per treatment group) engrafted with melanoma tumor cells were adoptively transferred with *PDCD-1* KO or mock TIL. Anti–PD-1 antibody treatment combined with mock TIL was included as a control for PD-1/PD-L1 blockade. Statistical significance is denoted by *$p < 0.05$, **$p < 0.01$, and ****$p < 0.0001$.

- Efficiency of *PDCD-1* KO for the autologous melanoma TIL used in the patient-derived xenograft (PDX) model was 75%, as assessed by flow cytometry
- Enhanced in vivo antitumor activity was observed in *PDCD-1* KO TIL-treated mice relative to mice treated with mock TIL alone or mock TIL + anti–PD-1 antibody

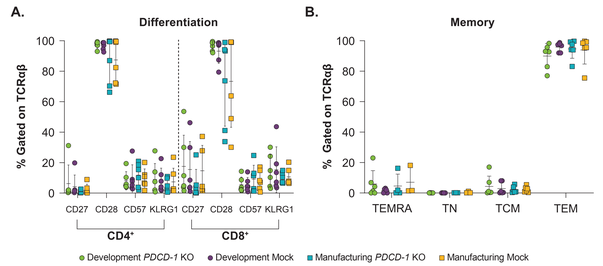
Figure 2. Viable Cell Dose, Purity, Identity, Potency, and *PDCD-1* KO Efficiency of TIL Product



Values are displayed as mean ± SD.

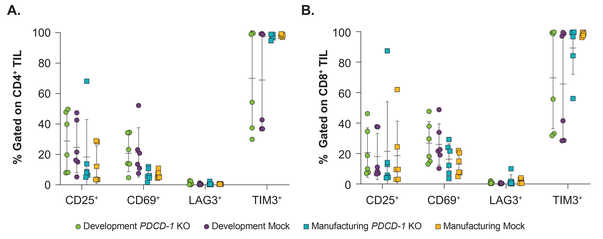
- All *PDCD-1* KO TIL products met the release criteria for dose, purity, identity, potency, and *PDCD-1* KO efficiency. No statistically significant differences were observed between the development and manufacturing runs
  - Both development and manufacturing runs produced final *PDCD-1* KO TIL products of comparable dose **(A)** and viability **(B)**
  - The median (range) identity (%$CD45^+CD3^+$) of the final TIL product in development and manufacturing runs was 98.5% (98%–100%) and 98.7% (96%–99%), respectively **(C)**
  - Median IFNγ release in development and manufacturing runs of the final *PDCD-1* KO TIL product was 4015 pg/mL and 4725 pg/mL, respectively **(D)**
  - Median (range) *PDCD-1* KO efficiency in development and manufacturing runs was 63% (48%–81%) and 62% (31%–91%), respectively **(E)**
  - *PDCD-1* KO TIL products were comparable to mock TIL in terms of growth, purity, identity, and potency
  - As shown in previous studies,[14] dose, purity, identity, and potency results were comparable between mock and *PDCD-1* KO in development runs (data not shown)

Figure 3. TIL Differentiation and Memory



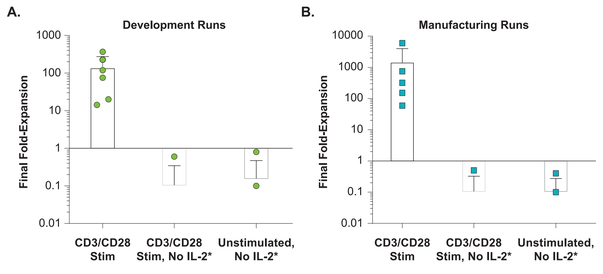
- The CD28 marker was highly expressed in both *PDCD-1* KO and mock TIL in development and manufacturing samples, whereas other markers such as CD27, CD57, and KLRG1 were expressed at low levels **(A)**
- Naïve (TN), central memory (TCM), effector memory (TEM), and effector memory RA^+(TEMRA) T cell subsets were defined using CD45RA and CCR7 expression. A majority of the TIL lots displayed predominantly effector memory phenotype **(B)**
- No statistically significant differences in TIL differentiation markers or memory phenotype were observed between *PDCD-1* KO and mock TIL in the development and manufacturing runs

Figure 4. Expression of Activation- and Inhibitory-Related Markers on *PDCD-1* KO TIL



- Multicolor flow cytometry was used to characterize TIL activation and inhibitory receptor expression on $CD4^+$ **(A)** and $CD8^+$ TIL **(B)**
- No statistically significant differences in marker expression were observed between *PDCD-1* KO and mock TIL in the development and manufacturing runs

Figure 5. IL-2–Independent Proliferation Assay of *PDCD-1* KO TIL Products



*Cell count data < lower limit of quantitation on the NC200 cell counter. Green circles and blue squares represent individual samples within the development and manufacturing runs, respectively, and values are displayed as mean + SD.

- Using an IL-2–independent proliferation assay, it was demonstrated that none of the *PDCD-1* KO TIL products underwent malignant transformation following TALEN®-mediated genome editing
  - None of the stimulated (anti-CD3/CD28) or unstimulated samples were proliferative in the absence of IL-2 **(A & B)**
  - All samples cultured in the presence of IL-2 showed proliferation **(A & B)**

Table 1. Summary of Karyotyping Results From *PDCD-1* KO TIL Products

Sample ID	G-Banding Results	
	Mock	*PDCD-1* KO
1	Normal female: 46[a] XX^b, [20][c]	Normal female: 46[a], XX^b, [20][c]
2	Normal male: 46[a], XY^b, [20][c]	Normal male: 46[a], XY^b, [20][c]
3	Normal female: 46[a], XX^b, [20][c]	Normal female: 46[a], XX^b, [20][c]
4	Normal male: 46[a], XY^b, [20][c]	Normal male: 46[a], XY^b, [20][c]
5	Normal male: 46[a], XY^b, [20][c]	Normal male: 46[a], XY^b, [20][c]
6	Normal female: 46[a], XX^b, [20][c]	Normal female: 46[a], XX^b, [20][c]

[a]Number of chromosomes.
[b]Sex complement.
[c]Number of analyzed metaphase cells.

- No clonal chromosomal abnormalities were observed in G-banding analysis, indicating that there was no genotoxicity following TALEN®-mediated genome editing at *PDCD-1*
  - All samples analyzed by G-banding produced sufficient metaphases for a full study; normal G-banding patterns were observed

Conclusions

- The in vivo antitumor activity of *PDCD-1* KO TIL was superior to that of mock TIL (electroporated without TALEN®) in the presence or absence of anti–PD-1, suggesting that endogenous PD-1 inhibition may confer a functional advantage to TIL
- *PDCD-1* KO TIL clinical-scale manufacturing was feasible, and the TIL product quality attributes and phenotype were acceptable
  - TIL attributes in all development and manufacturing runs were comparable and met the product release criteria
  - None of the *PDCD-1* KO TIL products underwent malignant transformation following TALEN®-mediated genome editing as determined by IL-2–independent proliferation assay
  - No TALEN®-induced clonal chromosomal abnormalities were identified by G-banding
  - Importantly, lack of complete *PDCD-1* KO in the TIL product may spare other PD-1–dependent in vivo cellular functions
- Together, these data support clinical investigation of IOV-4001, an autologous *PDCD-1* KO TIL cell therapy, as a potential therapeutic option in patients with advanced solid tumors
  - A clinical study of IOV-4001 in patients with metastatic melanoma and advanced NSCLC is expected to begin in 2022

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Abbreviations

CMO, contract manufacturing organization; DAPI, 4',6-diamidino-2-phenylindole; DOR, duration of response; GMP, Good Manufacturing Practice; hIL-2, human interleukin-2; ICI, immune checkpoint inhibitor; IFNγ, interferon-γ; IL-2, interleukin-2; LAG3, lymphocyte-activation gene 3; KO, knockout; LN_2, liquid nitrogen; ORR, objective response rate; PD-1, programmed cell death protein-1; PDX, patient-derived xenograft; REP, rapid expansion protocol; Stim, stimulated; TALEN®, transcription activator-like effector endonucleases; TCM, central memory T 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; TVC, total viable cells.

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Disclosures

- AN, AV, AW, CH, VG, KO, MM, SW, MJ, and RC are employees of Iovance Biotherapeutics and have stock options
- JB has no financial disclosures