Superior anti-tumor activity of IL-12–engineered TIL (IOV-5001) in a simulated tumor microenvironment

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Background

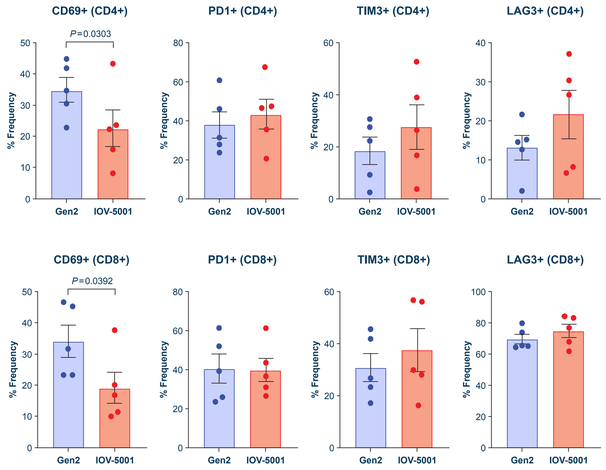
- Lifileucel is an autologous tumor-infiltrating lymphocyte (TIL) cell immunotherapy that was recently approved by the FDA for the treatment of advanced melanoma.[1] Lifileucel is manufactured with Iovance's proprietary 22-day Gen 2 manufacturing process. Iovance is currently working on developing new assets to further enhance the efficacy of the TIL product beyond lifileucel
- IL-12–producing TIL has shown clinical benefit; however, circulating IL-12–related AEs limited further development[2]
- IOV-5001 is a next-generation cytokine-engineered autologous TIL drug product (DP). It represents a novel strategy to enhance anti-tumor cytotoxic activity through the inducible expression of cell surface-tethered IL-12 and constitutive expression of a tethered growth factor.[3,4] Meanwhile, the culture media during rapid expansion protocol (REP) was optimized[4]
- The current study assessed the phenotype and improved anti-tumor activity of IOV-5001 in a metabolic tumor microenvironment (TME) simulation that mimics metabolic disorder and immune suppression

Methods

- Manufacturing of IOV-5001:
  - Tumors of various histologies, including non-small cell lung cancer, melanoma, breast, head and neck, and endometrial cancer, were fragmented and cultured in an IL-2 containing media. Then, TIL were harvested and followed by transduction with a lentivirus vector containing a gene encoding membrane-tethered IL-12 (TeIL-12) driven by an NFAT (nuclear factor of activated T cells) promoter, and a membrane-tethered growth factor driven by an EF-1α (elongation factor 1 alpha) promoter. After gene transduction, TIL cells were subsequently expanded with a REP in an optimized media
- Characterization of IOV-5001:
  - The phenotype of IOV-5001 was characterized by flow cytometry
  - Gene expression profiling and T-cell receptor (TCR) repertoire were analyzed by next-generation sequencing
  - In vitro anti-tumor activity was investigated in a metabolic TME simulation

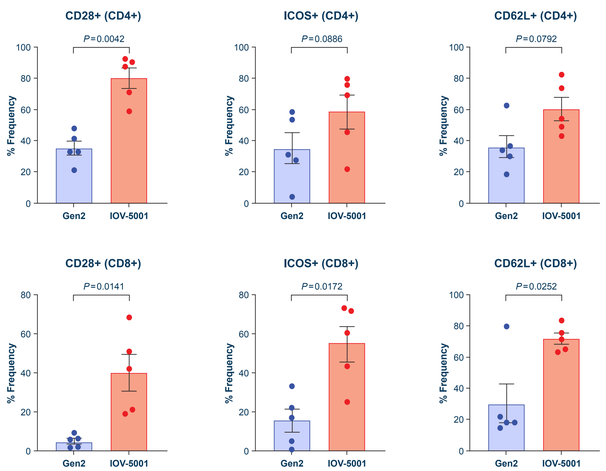
Results

Figure 1. Reduced expression of CD69 and no difference in the expression of PD-1, TIM3, and LAG3



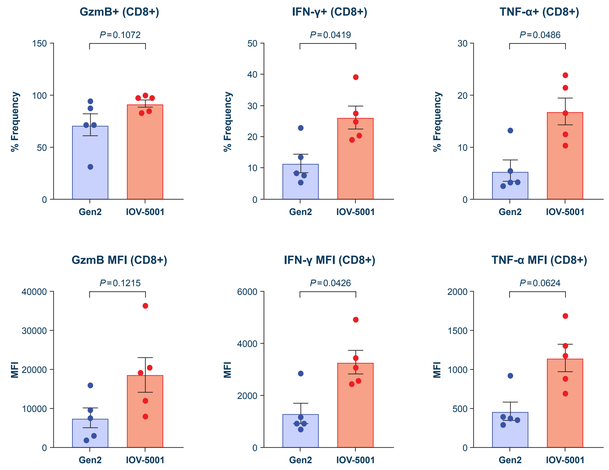
Statistical analysis was performed using a paired t-test.

Figure 2. IOV-5001 TIL increased expression of CD28, ICOS, and CD62L



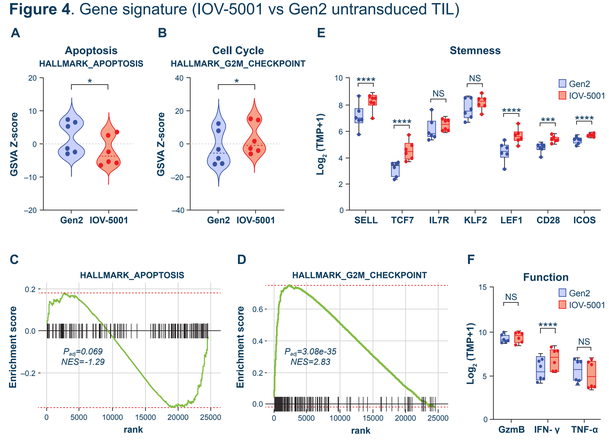
Statistical analysis was performed using a paired t-test.

Figure 3. Increased expression of T-cell cytotoxicity-related components: GzmB, IFN-γ, and TNF-α (CD8+ T cells)



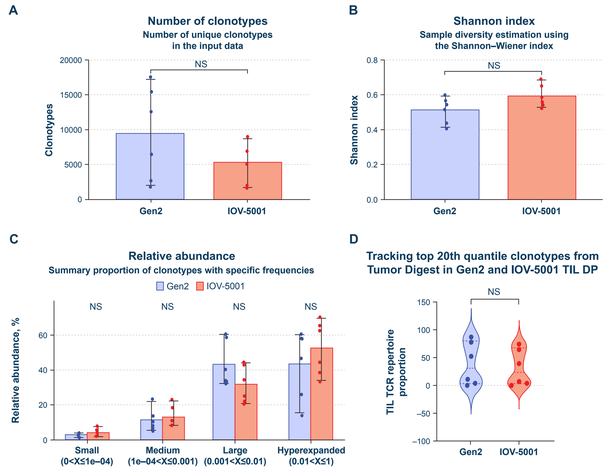
Statistical analysis was performed using a paired t-test.
Note: CD4+ T cells displayed increased expression of GzmB, IFN-γ, and TNF-α expression although did not achieve statistical significance.

Figure 4. Gene signature (IOV-5001 vs Gen2 untransduced TIL)



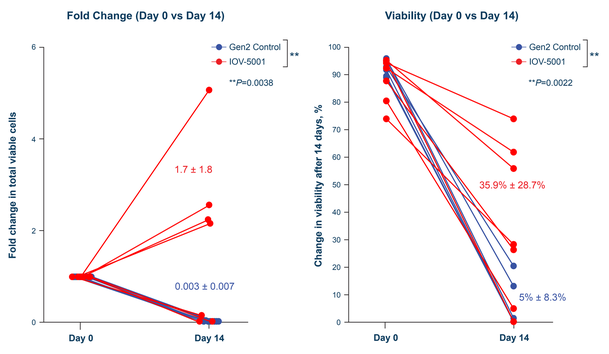
Bulk RNA-seq TPM gene expression matrix was used as input for GSVA quantification of (**A**) apoptosis and (**B**) cell cycle (G2M Checkpoint) Hallmark gene signatures. *P* values based on Wilcoxon matched-pairs signed rank test **P*<0.05 (n=6). Ranked gene list from DESeq2 differential gene expression analysis with Hallmark gene signatures was used as input for Gene Set Enrichment Analysis (GSEA). Shown are enrichment plots of (**C**) apoptosis and (**D**) cell cycle (G2/M checkpoint) gene sets. Individual gene expression boxplot visualizations of (**E**) T-cell stemness-associated and (**F**) functional genes; ****P*<0.0005; *****P*<0.0001.

Figure 5. IOV-5001 TIL shows comparable proportion of expanded TCR clonotypes from baseline tumor digest compared with Gen2 TIL



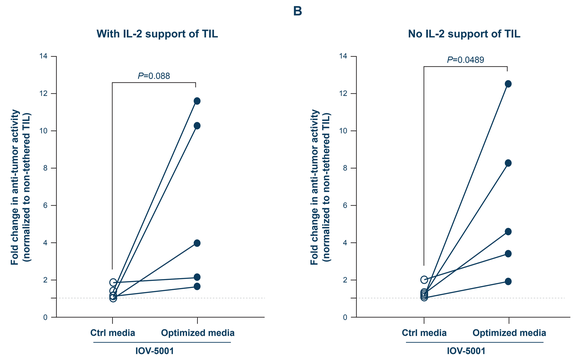
Bulk TCR-seq was performed on matched baseline tumor digest and TIL from Gen2 and IOV-5001 groups. (**A**) Number of unique clonotypes, (**B**) Shannon TCR repertoire diversity, and (**C**) clonotype proportion distribution show no difference between Gen2 and IOV-5001 groups. Expanded clonotypes from the baseline tumor digest of each sample were identified and their proportions in the final TIL drug product quantified. *P* values based on Wilcoxon rank sum test in R (n=6). Visualized is a violin plot tracking the (**D**) top 20th quantile proportion clonotypes. *P* value based on Wilcoxon matched-pairs signed rank test in GraphPad Prism (n=6).

Figure 6. IOV-5001 exhibits increased survival and persistence in vitro in the absence of IL-2



Cell proliferation and viability of Gen2 IOV-5001 TIL after 14 days in vitro. (**A**) Fold change in the number of Gen2 vs IOV-5001 TIL, and (**B**) cell viability were assessed 14 days after in vitro culture in IL-2–absent culture medium. Data for each group are presented as mean ± SD. Statistical analysis on Day 14 was performed using two-way ANOVA with Šídák's multiple comparisons test.

Figure 7. Efficacy of IOV-5001 with or without IL-2 support in a metabolic TME simulation



In vitro activity of IOV-5001 TIL. Assessment of tumor killing of human pancreatic adenocarcinoma cells (HPAC) that express high levels of mutant KRAS (G12D) by IOV-5001 transduced with KRAS (G12D)-specific TCR measured by Incucyte over 4 days in a metabolic TME simulation (1.5 mM glucose, 20 mM lactic acid, 100 μM adenosine, 5 ng/mL TGFβ1) with (**A**) or without (**B**) IL-2 (30 IU/mL) support. n = 7, biological replicates. Statistical analyses were made by repeated measures, one-way ANOVA with post-hoc Tukey's multiple comparisons test.

Conclusions

- **IOV-5001 exhibits a favorable T-cell phenotype characterized by increased T-cell stemness and functionality markers**
- **IOV-5001 shows a comparable TCR repertoire to Gen2 TIL**
- **IOV-5001 exhibits superior anti-tumor activity in a metabolic TME simulation**
- **IOV-5001 warrants further development in clinical trials to assess its potential for increased efficacy against solid tumors**

References

1. AMTAGVI (lifileucel). https://www.amtagvi.com/. Accessed August 2024.
2. Zhang L, et al. *Clin Cancer Res*. 2015;21:2278–2288.
3. Zhang Y, et al. SITC annual meeting 2023. *J Immunother Cancer*. 2023:403.
4. Innamarato P, et al. ESMO congress 2024. *Ann Oncol*. 2024:1032p.

Abbreviations

ANOVA, analysis of variance; Ctrl, control; DP, drug product; EF-1α, elongation factor 1α; GSEA, Gene Set Enrichment Analysis; GzmB, Granzyme B; HPAC, human pancreatic cancer; ICOS, inducible costimulator; IFN-γ, interferon gamma; IL-2, interleukin-2; LAG3, lymphocyte activation gene 3 protein; MFI, mean fluorescence intensity; NES, normalized enrichment score; NFAT, nuclear factor of activated T cells; NS, not significant; PD-1, programmed cell death protein 1; REP, rapid expansion protocol; TCR, T-cell receptor; TeIL-12, membrane-tethered nuclear factor of activated T cells–interleukin-12; TIL, tumor-infiltrating lymphocyte; TIM3, T-cell immunoglobulin domain and mucin domain protein 3; TME, tumor microenvironment; TNF-α, tumor necrosis factor alpha.

Disclosures

PI, NG, MM, MA, JF, GS, JD, BD, JY, RQ, JC, HY, and SH are employees of Iovance and may own stock.

Acknowledgments

- This study was sponsored by Iovance Biotherapeutics, Inc. (San Carlos, CA, USA)
- Editorial support was provided by Peloton Advantage, LLC, an OPEN Health company, and funded by Iovance