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

Patrick Innamarato, Nathan Gilbert, Marcus Machin, Mohammed Alkhouli, Tim Erickson, Judy Fang, Nino Mtchedlidze, Gerard Sapena, Brian Gastman, Joe Dean, Behzad Damirchi, Joe Yglesias, Rongsu Qi, Sean Hall, Hequn Yin, Yongliang Zhang Iovance Biotherapeutics, Inc., San Carlos, CA, USA

# Background

- Lifileucel is an autologous tumor-infiltrating lymphocyte (TIL) cell immunotherapy that was recently approved by the FDA for the treatment of advanced melanoma.<sup>1</sup> Lifileucel is manufactured with lovance's proprietary 22-day Gen 2 manufacturing process. lovance 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<sup>2</sup>
- 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.<sup>3,4</sup> Meanwhile, the culture media during rapid expansion protocol (REP) was optimized<sup>4</sup>

## Results

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



Gen2 IOV-5001

Gen2 IOV-5001

Statistical analysis was performed using a paired t-test.

Gen2 IOV-5001

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

Gen2 IOV-5001



Statistical analysis was performed using a paired t-test.

• 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

### **Figure 3**. Increased expression of T-cell cytotoxicity-related components: GzmB, IFN- $\gamma$ , and TNF- $\alpha$ (CD8+ T cells)



Statistical analysis was performed using a paired t-test.

Note: CD4+ T cells displayed increased expression of GzmB, IFN- $\gamma$ , and TNF- $\alpha$  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.

### 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 membranetethered IL-12 (TelL-12) driven by an NFAT (nuclear factor of activated T cells) promoter, and a membrane-tethered growth factor driven by an EF-1 $\alpha$  (elongation factor 1 alpha) promoter. After gene transduction, TIL cells were subsequently expanded with a REP in an optimized media

#### **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).





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.





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

#### **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  $\mu$ M adenosine, 5 ng/mL TGF $\beta$ 1) 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

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#### **Abbreviations**

ANOVA, analysis of variance; Ctrl, control; DP, drug product; GSEA, Gene Set Enrichment Analysis; GzmB, Granzyme B; EF-1α, elongation factor 1α; HPAC, human pancreatic cancer; ICOS, inducible costimulator; IFN-y, 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; TelL-12, membranetethered 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- $\alpha$ , tumor necrosis factor alpha.

#### Disclosures

PI, NG, MM, MA, TE, JF, NM, GS, BG, JD, BD, JY, RQ, SH, HY, and YZ are employees of lovance and may own stock.

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