IOV-5001, autologous tumor-infiltrating lymphocytes armored with inducible membrane-tethered interleukin-12, shows enhanced antitumor efficacy with an improved cellular state

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Background

- Lifileucel (manufactured using Gen 2 process) is an autologous tumor-infiltrating lymphocyte (TIL) immunotherapy that was recently approved by the United States Food and Drug Administration for the treatment of metastatic melanoma.[1] Iovance is currently developing next-generation TIL immunotherapies to further enhance efficacy
- TIL engineered to express secreted interleukin (IL)-12 has previously shown clinical benefit in patients with melanoma, but further development was terminated because of IL-12–driven immune-related adverse events[2]
- IOV-5001 is a next-generation TIL immunotherapy engineered to express nuclear factor of activated T-cells (NFAT)–inducible membrane-tethered IL-12 (TeIL12) upon antigen engagement and a constitutive membrane-tethered growth factor. NFAT-driven TeIL12 provides an enhanced safety profile by limiting systemic exposure to inflammatory cytokines by restricting expression within tumors upon antigen engagement. No shedding of IL-12 was detected in circulation in an in vivo patient-derived xenograft (PDX) model.[3] Additionally, cell culture optimization (CCO) through media formulation was designed to boost the functionality of IOV-5001
- The current study evaluated the function of IOV-5001 in a metabolically suppressive tumor microenvironment and the gene expression profile that provides support for augmented function in IOV-5001 TIL

Methods

- TIL were derived from solid tumor tissues, cultured, and transduced with a lentiviral vector encoding inducible TeIL12 bicistronically with a growth factor. IOV-5001 TIL were compared to Gen 2 TIL throughout the study
- Antitumor activity of IOV-5001 was tested in vitro via a tumor antigen-directed T-cell receptor (TCR) killing assay or against an autologous patient-derived tumor organoid (PDTO)
- Transcriptional profiling (bulk and single-cell) was used to characterize the molecular features of IOV-5001

Results

Regulation of TeIL12 and Signaling After Stimulation

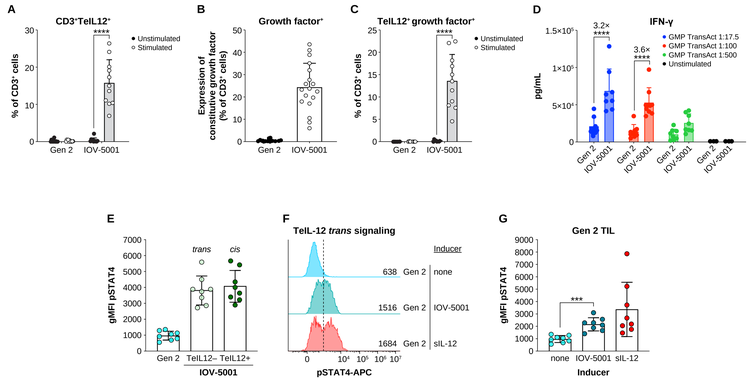

Figure 1. Regulation of TeIL12 and signaling after stimulation. (**A**) Regulated expression of TeIL12 after overnight stimulation (n=11). (**B**) Expression of growth factor in IOV-5001 (n=18). (**C**) Double positive expression of TeIL12 and growth factor after stimulation (n=11). (**D**) Induction of IFN-γ after overnight stimulation with various concentrations of GMP TransAct. Fold increase is shown above (n=8). (**E**) TeIL12 signaling *in trans* and *in cis* measured by pSTAT4 flow cytometry within IOV-5001 TIL after stimulation (n=8). (**F-G**) Representative histogram (**F**) and summary data (**G**) showing pSTAT4 expression within Gen 2 TIL alone or co-cultured with activated IOV-5001 TIL (n=8). Data are presented as mean ± SD. Statistical tests: two-way ANOVA with Sidak's multiple comparison test (**A, C, D**); one-way ANOVA with Tukey's post hoc test (**G**). ****P*<0.001. *****P*<0.0001.

IOV-5001 Exhibits Enhanced Killing and Antitumor Cytokine Production in a Metabolically Suppressive Tumor Microenvironment

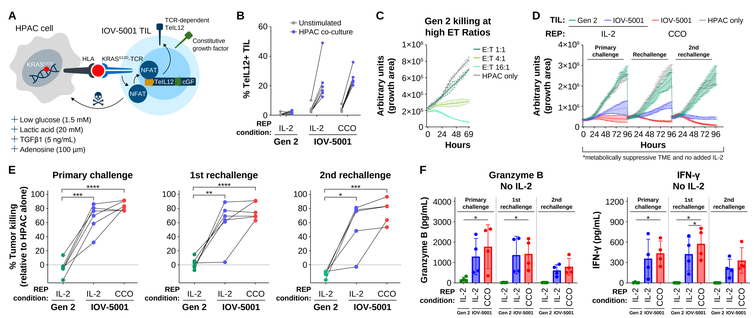

Figure 2. TeIL12 expression enhances the antitumor function of TIL in the absence of IL-2 in an in vitro TCR antigen-directed model. (**A**) Schematic of induction of membrane-bound IL-12 (TeIL12) in TIL that constitutively express a second membrane-bound growth factor (IOV-5001) in a TCR–activation-dependent manner. HPAC cells express the KRAS G12D neoantigen (HPAC^{G12D}), targeted by TIL transduced with KRAS-TCR triggers NFAT-driven TeIL12 and subsequent cytotoxicity. (**B**) Gen 2 and IOV-5001 TIL transduced with the KRAS TCR were assessed for TeIL12 at REP harvest, or 24 h after co-culture with HPAC^{G12D} cells. (**C**) Representative plot of cytotoxicity of HPAC^{G12D} cells by Gen 2 KRAS-TCR+ TIL at various E:T ratios to identify optimal tumor growth control using Incucyte. (**D**) Representative plot of cytotoxicity of HPAC^{G12D} cells over 3 challenges. IOV-5001 KRAS-TCR+ TIL expanded using an optimized REP media (●, IOV-5001+CCO) or standard REP media (●, IOV-5001 Gen 2) were compared with control KRAS-TCR+ TIL (●, Gen 2). TIL:HPAC cells were cultured at a 2:1 E:T ratio without exogenous IL-2 support in media consisting of 1.5 mM glucose, 20 mM lactic acid, 5 ng/mL TGFβ1, and 100 μm adenosine. (**E**) Percent HPAC^{G12D} killing by various TIL. (**F**) Supernatants were collected 24 h after initiating the coculture with the HPAC^{G12D} cells without IL-2 and tested for levels of Granzyme B and IFN-γ using the ELLA automated ELISA system. n = 6, biological donors in technical replicates of three (lung cancer, n=2; endometrial cancer, n=1; pancreatic cancer, n=2; colorectal cancer, n=1). Statistical analyses were made by mixed-model (**E**) or two-way ANOVA (**F**) with post hoc Tukey's multiple comparisons test. **P*<0.05. ***P*<0.01. ****P*<0.001. *****P*<0.0001.

IOV-5001 TIL Demonstrate Enhanced Killing Against Patient-Derived Tumor Organoids

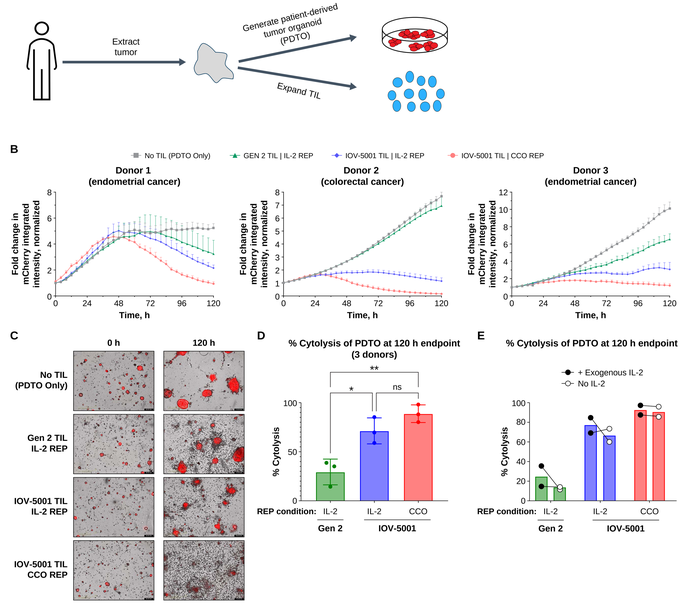

Figure 3. IOV-5001 demonstrate enhanced killing against PDTO. (**A**) Schematic showing generating PDTO to assess TIL function. (**B**) Functional assessment of TIL displayed as change in fluorescent signal intensity of PDTOs transduced with an mCherry reporter gene to quantitatively assess PDTO growth. TIL were added at 0 h. E:T ratio was optimized for each donor. Data are normalized to signal intensity per well of the PDTO at the 0 h timepoint. (**C**) Representative images of PDTO cultures from donor 1 at 0 h and 120 h timepoints showing an enhancement in controlling tumor growth when cultured with IOV-5001. (**D**) Percent cytolysis at the 120 h endpoint in the presence of IL-2 (n=3). (**E**) Paired data of the percent cytolysis of PDTOs with or without exogenous IL-2 support. Data are presented as mean ± SEM. Statistical tests: one-way ANOVA with Tukey's post hoc test. **P*<0.05. ***P*<0.01.

Transcriptomic Profile of IOV-5001

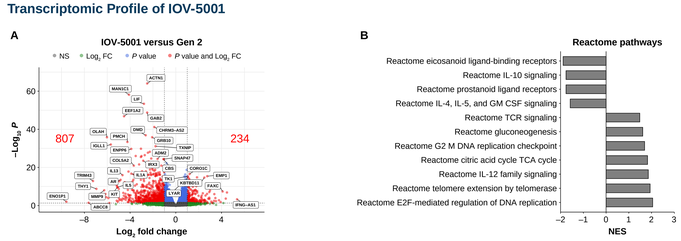

Figure 4. Bulk transcriptome profiling of Gen 2 and IOV-5001 TIL. (**A**) Volcano plot visualization of DESeq2 DEG analysis results. Ranked gene list from DESeq2 DEG analysis with Reactome gene signatures was used as input for GSEA. Top enriched pathways from (**B**) Reactome as bar plot visualization of NES. n=6, biological donors.

Single-cell transcriptomic profile of IOV-5001

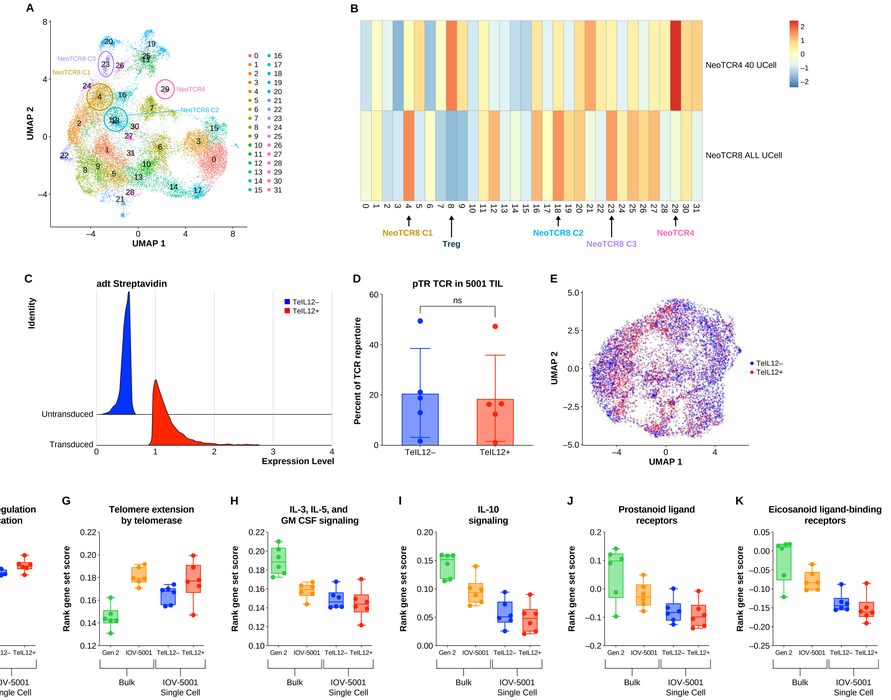

Figure 5. Single-cell transcriptome profiling of Pre-REP TIL with matching Gen 2 and IOV-5001 TIL DP single-cell analysis. Integrated UMAP visualizations of pre-REP TIL (**A**) single-cell clusters. (**B**) Heatmap visualization of NeoTCR8 and NeoTCR4 z-score normalized gene expression levels in single-cell clusters with annotation of pTR clusters. TeIL12+ cells labeled with streptavidin DNA oligo tagged secondary antibody staining was used to distinguish TeIL12+ from TeIL12− cells (in the IOV-5001 TIL DP). (**C**) Ridge plot visualization of upper and lower quartile threshold definitions for TeIL12+ vs TeIL12− cells in IOV-5001 TIL DP. (**D**) TCR clones from day 11 NeoTCR8 and NeoTCR4 pTR T-cell clusters as well as top clonotypes from tumor digest bulk TCR-seq were tracked to the IOV-5001 TIL DP and their proportion in the DP quantified. No difference in proportion of pTR TCRs between TeIL12+ vs TeIL12 TIL− was observed, suggesting an unbiased transduction. Wilcoxon matched-pairs signed rank stats test used to determine no significant difference. Integrated UMAP visualizations of IOV-5001 TIL DP subset on CD8+ T cells annotating (**E**) TeIL12+ vs TeIL12− cells and showing slight differences in distribution of transduced vs untransduced cells suggesting transduced cells exhibit an altered phenotype. (**F-K**) Rank-based gene set quantification of expression data from Bulk RNA-seq as well as IOV-5001 TIL DP scRNA-seq visualized as box plots for Reactome pathways. n=6, biological donors. Data are presented as mean values ± SD.

Conclusions

- IOV-5001 exhibits tight regulation of TeIL12 that boosts the expression of IFN-γ only upon stimulation
- The secretion of IFN-γ is lacking in resting IOV-5001 TIL, highlighting the absence of leaky TeIL12 activity and IL-12 shedding[3]
- TeIL12 demonstrates activity *in cis* and *in trans*, highlighting the capability of antigen-engaged IOV-5001 to activate neighboring TIL within the tumor microenvironment
- IOV-5001 repeatedly kills target cells with consistent production of antitumor cytokines within a metabolically suppressive tumor microenvironment
- IOV-5001 exhibits enhanced killing against autologous three-dimensional PDTOs
- Gene-expression profiling provides mechanistic evidence supporting the enhanced function of IOV-5001
- The enhanced molecular and functional characteristics highlight IOV-5001 as a promising product for improving TIL efficacy in solid tumors and support the advancement of IOV-5001 into human clinical trials for eventual registration

Acknowledgments

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Abbreviations

ANOVA, analysis of variance; CCO, cell culture optimization; cGF, constitutive growth factor; DEG, differential gene expression; DP, drug product; E:F, effector to target; ELISA, enzyme-linked immunosorbent assay; GM CSF, granulocyte-macrophage colony-stimulating factor; GSEA, Gene Set Enrichment Analysis; HPAC, human pancreatic adenocarcinoma cells; IL, interleukin; IFN, interferon; NES, Normalized Enrichment Score; NFAT, nuclear factor of activated T cells; ns, not significant; PDTO, patient-derived tumor organoids; pre-REP, pre-rapid expansion protocol; pTR, putative tumor reactive; RNA-seq, RNA sequencing; scRNA-seq, single-cell RNA sequencing; SD, standard deviation; SEM, standard error of the mean; TCA, tricarboxylic acid; TCR, T-cell receptor; TCR-seq, T-cell receptor sequencing; TeIL12, membrane-tethered IL-12; TIL, tumor-infiltrating lymphocyte; UMAP, Uniform Manifold Approximation and Projection

References

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3. Innamarato P, et al. *Ann Oncol*. 2024;35(suppl 2):S674-S711. 1032P. ESMO 2024 Congress.

Disclosures

- Patrick Innamarato, Joe Dean, Mohammed Alkhouli, Marcus Machin, Nathan Gilbert, Sadie Johnson, Gerard Sapena, Nino Mtchedlidze, Behzad Damirchi, Robyn Oldham, Andrew Yuhas, Judy Fang, Rongsu Qi, Hequn Yin, Sean Hall: Employees of Iovance Biotherapeutics, Inc., San Carlos, CA
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