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OBX-115 TIL from non-small cell lung cancer (NSCLC) are enriched for putative tumor-reactive, stem-like T cells with enhanced tumor cytotoxicity: Results from multimodal phenotypic analysis

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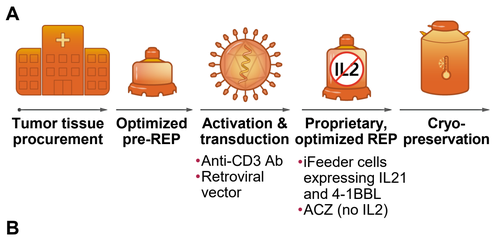
Introduction

- Clinical response to tumor-infiltrating lymphocyte (TIL) cell therapy is associated with presence of tumor-reactive clonotypes in the infusion product[1,2]
- Addition of 4-1BB agonist in the pre-rapid expansion protocol (pre-REP) phase of non-engineered TIL manufacturing has been shown to enhance TIL expansion and enrich for putative tumor-specific clones[3]
- OBX-115 TIL are engineered with regulatable, membrane-bound interleukin 15 (mbIL15), a cytokine that supports expansion of memory CD8+ T cells,[4] manufactured using a process that includes 4-1BB agonism in both pre-REP and REP[5]
- TIL engineering and REP process modifications may drive further enrichment of tumor-reactive clonotypes
- We sought to determine the phenotype and tumor reactivity of OBX-115 TIL in NSCLC relative to a conventional, IL2-based non-engineered TIL expanded using irradiated PBMC feeders

Methods

- TIL were generated from paired NSCLC tumor samples using either the OBX-115 process or a conventional IL2-based non-engineered TIL process
- Tumor digests were sequenced using combined single-cell RNA & T-cell receptor (TCR) sequencing. Using a validated tumor-reactive TIL gene-expression profile,[6,7] we computationally predicted putative tumor-reactive clonotypes from the tumor digest T cells
- Bulk TCR Vbeta sequencing was then used to track the dynamics of these putative tumor-reactive clonotypes throughout TIL expansion
- TIL were phenotyped via flow cytometry and assessed for functional reactivity against autologous tumor digests or patient tumor-derived cell lines (PDc) in 3D co-cultures

Figure 1. Overview of OBX-115 manufacturing process and donor cohort



B

Donor	Patient Age	Stage	Pre-treatment	Histology
1	65	T3N2	cis/peme/pembro ×3	LCNEC
2	62	T3N0	cis/pacli/nivo ×2	LCNEC
3	64	T1N0	n/a	LUAD
4	71	T3N2	carbo/peme/nivo ×3	LUAD
5	66	T3N1	carbo/abraxane/pembro ×3	LUSC
6	76	IIB	n/a	LUAD
7	47	IIB vs IIIA	cis/alimta/opdivo ×3	LUAD
8	72	T1cN0M0	n/a	LUAD
9	63	T1cN0M0	n/a	LUAD

Figure 1. (A) Schematic representation of the OBX-115 manufacturing process. **(B)** Donor characteristics of NSCLC tumors included.

Results

Figure 2. OBX-115 process generates higher pre-REP yield, with a more consistent, CD8+ T-cell–enriched composition

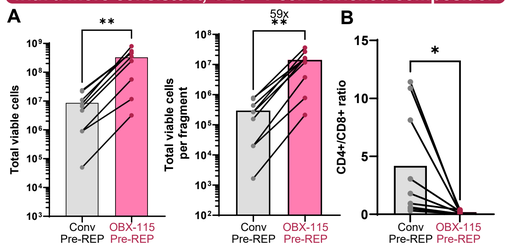


Figure 2. (A) The OBX-115 process led to increased total pre-REP TIL expansion and fragment-normalized expansion relative to the conventional, high-dose IL2-based process. **(B)** Pre-REP TIL generated using the OBX-115 process showed a more consistent, CD8+ enriched composition. Paired t-test, n=9, *p<0.05, **p<0.01.

Figure 3. OBX-115 process enriches for CD8+ and CD39-CD69- "stem-like" T cells

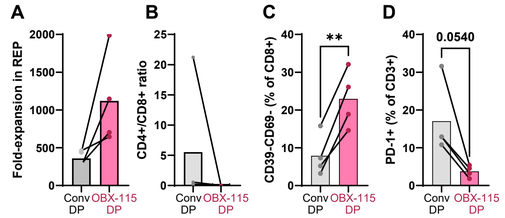


Figure 3. (A) Robust expansion of OBX-115 drug product (DP) in REP. **(B)** OBX-115 DP were enriched for CD8+ cells. **(C)** OBX-115 DP showed a significantly higher proportion of CD39-CD69- "stem-like" phenotype. **(D)** OBX-115 DP showed a trend toward a lower proportion of PD-1+ cells. Paired t-test, n=4, **p<0.01.

Figure 4. OBX-115 process expands distinct TCR repertoire compared to conventional process

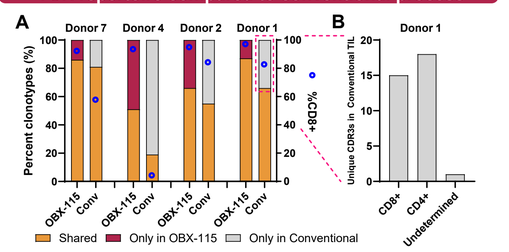


Figure 4. Bulk TCR sequencing of TCR-Vβ revealed differences in the top 100 TCR clonotypes in OBX-115 DP compared to conventional DP. **(A)** Among the top 100 clonotypes present in either DP, manufacturing of OBX-115 TIL led to distinct TCR repertoire as compared to conventional TIL, which could be partially attributed to the higher fraction of CD4 T cells in the conventional TIL. **(B)** Bulk TCR sequencing on sorted CD4+ vs CD8+ TIL from conventional TIL DP validates that differences between OBX-115 and conventional TIL clonotypes are partially driven by CD4+/CD8+ composition.

Figure 5. scRNAseq-based tumor-reactive TCR score

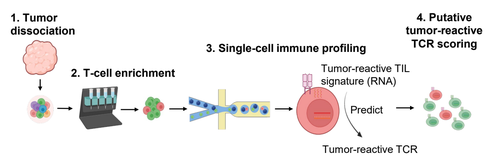


Figure 5. Schematic representation of the workflow used to generate putative tumor-reactive TCR scores from paired single-cell TCR/RNA sequencing of tumor-digest TIL.

Figure 6. TR30 and NeoTCR8 scores show concordant detection of putative tumor-reactive clones enriched for genes associated with effector function

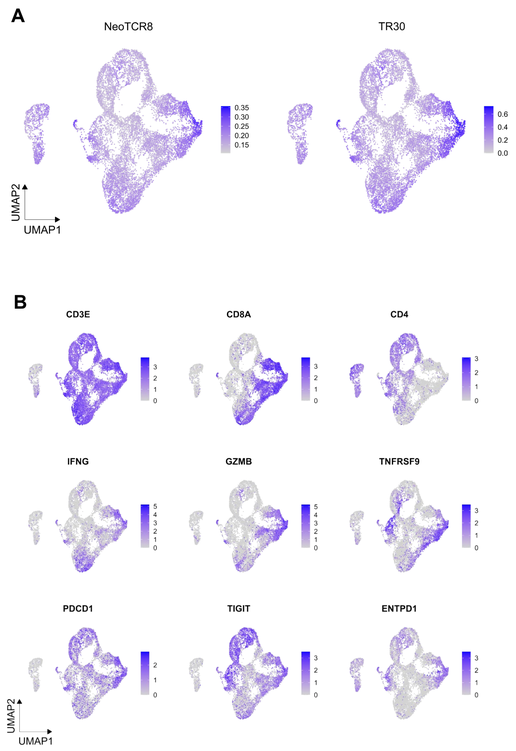


Figure 6. Scoring of putative tumor-reactive TCRs using NeoTCR8 and TR30 signatures. **(A)** Combined UMAP plots of single-cell RNA-seq data from NSCLC donors (n=3) overlayed with NeoTCR8 and TR30 scores. **(B)** Tumor-reactive CD8+ clones clustered closely with the commonly screened tumor-reactive T-cell signatures including functional (*IFNG*, *GZMB*) and activation/exhaustion genes (*PDCD1*, *TIGIT*, *ENTPD1*).

Figure 7. OBX-115 DP is enriched for putative tumor-reactive TCRs compared to conventional DP

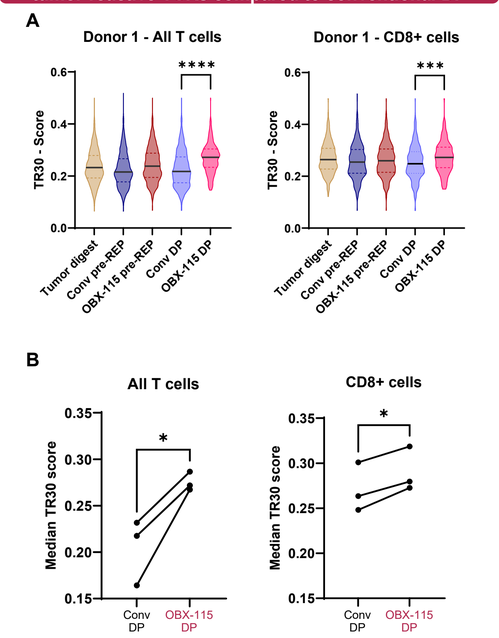


Figure 7. TR30 gene signature revealed greater enrichment for tumor-reactive TCRs in the OBX-115 DP compared to conventional DP across three donors. **(A)** Mapping TR30-scored putative tumor-reactive TCR clones from the tumor digest of Donor 1 onto its respective pre-REP TIL and subsequent DP revealed a greater median number of tumor-reactive TCRs in the OBX-115 DP compared to the conventional DP (Mann-Whitney U test, ****p<0.0001, *p<0.05). **(B)** Across three donors, OBX-115 DP had a significantly higher median TR30 score relative to conventional DP, which increased when gated on CD8+ T cells (paired t-test, n=3, *p<0.05). **(C)** There was also a trend toward higher median NeoTCR8 score when comparing OBX-115 DP to conventional DP.

Figure 9. OBX-115 TIL exert superior functional tumor reactivity/cytotoxicity relative to conventional TIL

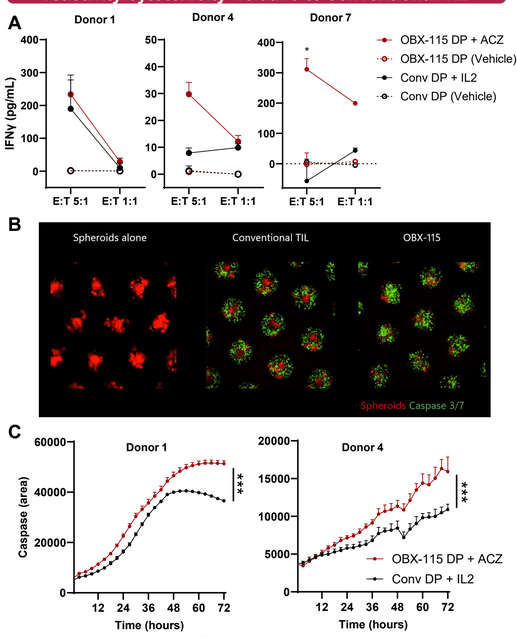


Figure 9. (A) OBX-115 showed similar or increased IFNγ secretion as compared with conventional TIL in co-culture assays with autologous tumor digests. IFNγ release was calculated by deducting secretion from TIL-tumor digest co-cultures from TIL-alone conditions. **(B)** Representative images from TIL co-cultures with autologous tumor spheroids showed greater cytotoxicity of OBX-115 TIL against autologous PDC lines than conventional TIL. **(C)** Quantification of caspase 3/7 signal within spheroids in co-cultures (paired t-test, *p<0.05, ***p<0.001).

Conclusions

- The OBX-115 process generated a **greater number of pre-REP TIL** per tumor fragment vs conventional process, indicative of **manufacturing success**
- Post-REP, OBX-115 TIL displayed a **higher proportion of CD39-CD69- "stem-like" progenitor cells** and **a trend toward lower proportion of PD-1+ cells** relative to conventional TIL
- OBX-115 was **enriched for putative tumor-reactive TCR clonotypes** compared to conventional TIL, even when sorted for CD8+ cells
- Moreover, OBX-115 demonstrated **superior functional tumor reactivity** relative to conventional TIL, marked by increased cytotoxicity against autologous tumor cell lines in 3D co-cultures and similar or higher IFNγ secretion upon co-culture with autologous tumor digests
- Together, these favorable attributes support **further investigation of OBX-115 in patients with metastatic NSCLC** (NCT06060613)

Abbreviations

DP, drug product; mbIL15, membrane-bound IL15; LCNEC, large cells neuroendocrine carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; NSCLC, non-small cell lung cancer; PDc, patient-derived cell line; REP, rapid expansion protocol; TCR, T-cell receptor; TIL, tumor-infiltrating lymphocyte.

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Disclosures

Adam J Schoenfeld reports consulting or advisory roles with Johnson & Johnson/Janssen, KSQ Therapeutics, Perceptive Advisors, Heat Biologics, Bristol-Myers Squibb, Enara Bio, Umoja Biopharma, Oppenheimer, Iovance Biotherapeutics, Lyell Immunopharma, Merck, Immunocore, Legend Biotech, Amgen, and Prelude Therapeutics; travel, accommodations, or expenses from Iovance Biotherapeutics and Instil Bio; research funding from GlaxoSmithKline, Merck, Bristol-Myers Squibb, Iovance Biotherapeutics, Achilles Therapeutics, Amgen, PACT Pharma, Harpoon Therapeutics, and Instil Bio; and other relationship with Merck, Bristol-Myers Squibb, Iovance Biotherapeutics, PACT Pharma, Achilles Therapeutics, GlaxoSmithKline, Harpoon Therapeutics, Amgen, and Instil Bio. AVO and ZA are employees of Obsidian Therapeutics, Inc. (Cambridge, MA, USA).

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