LB144

OBX-115 membrane-bound IL15 (mbIL15)-expressing engineered tumor-infiltrating lymphocytes (TIL) overcome target cell immune evasion related to major histocompatibility complex (MHC) silencing through both direct killing and transactivation of NK cells in patient-derived autologous non-small cell lung cancer (NSCLC) models

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Introduction

- Non-engineered TIL cell therapy has demonstrated antitumor activity in metastatic NSCLC [1,2]
- MHC loss remains a key mechanism of resistance to TIL cell therapy and other immunotherapies in NSCLC [3]
- Tumoricidal CD8+ TIL have demonstrated MHC-independent cytotoxicity through lymphotoxin/LTBR in melanoma and are enriched in patients responsive to TIL cell therapy [4]
- OBX-115 engineered TIL express mbIL15 under pharmacologic regulation using the FDA-approved small-molecule drug acetazolamide (ACZ; **Figure 1**)
- We hypothesized that OBX-115 would retain cytotoxicity and reactivity following MHC loss in NSCLC due to both LTBR-mediated cytotoxicity and mbIL15-mediated natural killer (NK) cell transactivation

Figure 1. OBX-115 Mechanism of Action

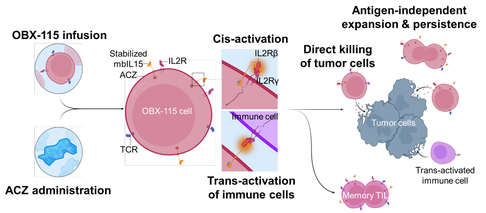

Figure 1. OBX-115 acts through ACZ-dependent cis-activation, which drives antigen-independent expansion and persistence, and transactivation, which can activate other immune cells including NK cells.

Methods

- OBX-115 TIL were generated from NSCLC tumor samples as previously described (**Figure 2**) [5]
- Autologous NK cells were expanded from donor-matched peripheral blood mononuclear cells (PBMC) using IL2 and irradiated feeder cells expressing IL21 and 4-1BBL
- Autologous patient-derived cell lines (PDc) were developed using EpiCult or DermaCult media and genetically modified to knock-out (KO) the B2M locus using a CRISPR-Cas9 system. B2M KO was confirmed using flow cytometry and Sanger sequencing
- For the transactivation assay, OBX-115 cells were rested overnight with or without ACZ. The OBX-115 cells were then either inactivated by mitomycin C before adding to NK cells or directly incubated with autologous NK cells for another 24 hrs to allow transactivation. The transactivated NK cells were assessed by pSTAT5 flow staining. The effector cells including NK cells or NK/OBX-115 cell mixture were then added to the dye-labeled PDc to assess functionality by quantitation of IFNγ secretion and cytotoxicity as assessed by live-cell imaging of Caspase 3/7-positive cells

Results

Figure 2. OBX-115 manufacturing process generates CD8+ enriched, minimally exhausted TIL cell therapy product

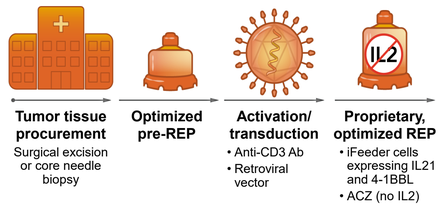

Figure 3. Generating autologous PDc models for MHC loss via CRISPR KO of B2M locus

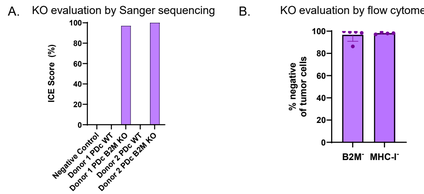

Figure 3. (A) Validation of PDc B2M KO by Sanger sequencing, evaluated by Inference of CRISPR Edits (ICE). (B) Evaluation of B2M KO through flow cytometry, shown as the average and standard deviation of B2M (n=5) and MHC-negative populations (n=4).

Figure 4. OBX-115 TIL show ACZ-dependent, MHC-independent IFNγ secretion when co-cultured with autologous PDc

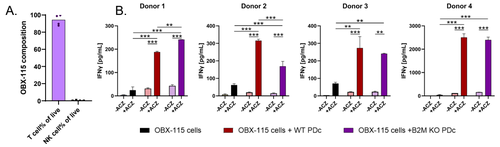

Figure 4. (A) OBX-115 cells showed a T-cell–predominant composition with minimal NK cells (<2%) in all donors tested (n=5). (B) OBX-115 cells showed ACZ-dependent IFNγ secretion upon co-culture with autologous PDc that was either not blocked or partially blocked by B2M KO. ANOVA; **P<0.01, ***P<0.005.

Figure 5. OBX-115 TIL show ACZ-dependent, MHC-independent cytotoxicity against autologous PDc

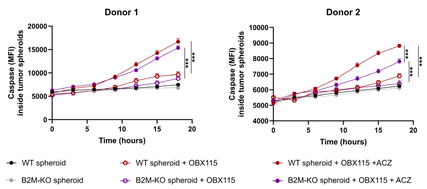

Figure 5. OBX-115 TIL showed ACZ-dependent cytotoxicity against autologous PDc in a 3D spheroid co-culture that was either not blocked or partially blocked by B2M KO. ANOVA; ***P<0.005.

Figure 6. MHC-independent cytotoxicity can be partially blocked by LTBR blockade

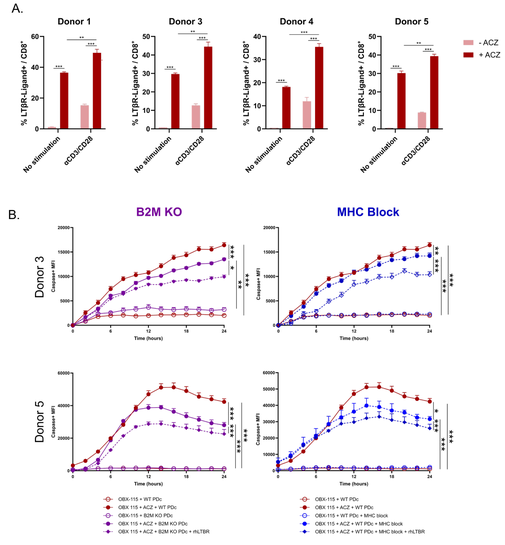

Figure 6. (A) Staining of LTBR ligand expression by recombinant LTBR on OBX-115 ± ACZ treatment and ± activation by aCD3/CD28. (B) OBX-115–elicited MHC-independent cytotoxicity could be partially blocked using recombinant LTBR, indicating lymphotoxin/LTBR–mediated cytotoxicity. ANOVA; *P<0.05, **P<0.01, ***P<0.005.

Figure 7. OBX-115 transactivates autologous NK cells

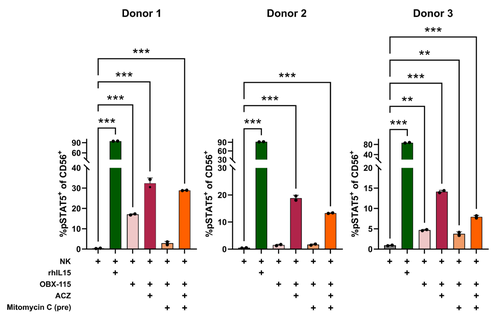

Figure 7. OBX-115 could transactivate autologous NK cells, as evidenced by increased phosphorylated STAT5 signaling detected in NK cells when co-cultured with OBX-115 cells in the presence of ACZ. OBX-115 cells when pre-treated with mitomycin-C could still transactivate autologous NK cells through mbIL15. ANOVA; **P<0.01, ***P<0.005; comparison shown against NK-cell alone controls.

Figure 8. OBX-115–transactivated NK cells elicit increased IFNγ secretion toward MHC-deficient K562 cells

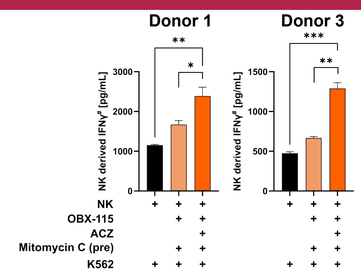

Figure 8. ACZ pretreated and mitomycin-C–inactivated OBX-115 cells could transactivate autologous NK cells and the transactivated NK cells could elicit increased IFNγ release upon co-culture with MHC-deficient K562 cells when compared with non-transactivated NK cells or NK cells preincubated with OBX-115 cells without the presence of ACZ. #NK derived IFNγ is calculated by subtracting IFNγ levels detected in TIL/K562 co-cultures from IFNγ levels detected in TIL/NK/K526 tri-cultures with matched E:T ratios and treatment conditions. ANOVA; *P<0.05, **P<0.01, ***P<0.005.

Figure 9. OBX-115 transactivates NK cells in the presence of ACZ and retains cytotoxicity toward autologous B2M KO PDc

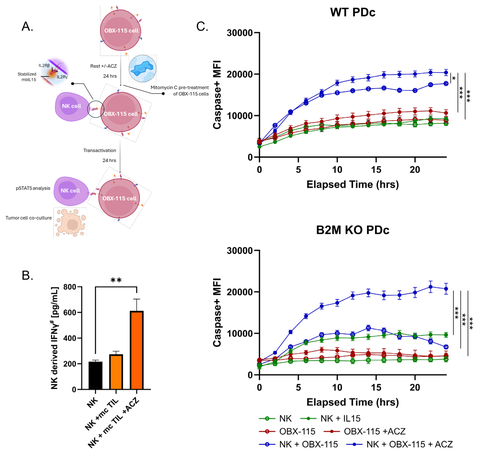

Figure 9. (A) Experimental set up for OBX-115 TIL/NK transactivation assay. (B) OBX-115 TIL transactivated NK cells elicit increased IFNγ secretion toward autologous PDc with B2M KO. (B) OBX-115 TIL, when mixed with autologous NK cells, could retain cytotoxicity toward B2M KO PDc through ACZ-dependent transactivation (data from Donor 3 shown). #NK derived IFNγ is calculated by subtracting IFNγ levels detected in TIL/PDc co-cultures from IFNγ levels detected in TIL/NK/PDc tri-cultures with matched E:T ratios and treatment conditions. ANOVA; *P<0.05, **P<0.01, ***P<0.005.

Conclusions

- OBX-115 TIL retain **ACZ-dependent cytotoxicity** and **IFNγ secretion** against **MHC-deficient** autologous tumor cell line
- ACZ-dependent cytotoxicity could be partially blocked by recombinant LTBR, indicating **MHC-independent cytotoxicity** is partially mediated by LTBR signaling
- OBX-115 could **transactivate NK cells** in an ACZ-dependent manner, which could **elicit IFNγ secretion** toward MHC-deficient K562 cells
- **OBX-115** and **NK cells** together can elicit **effective killing against** both **MHC-competent and -deficient** autologous tumor cells
- In addition to MHC-dependent OBX-115 cytotoxicity, **MHC-independent cytotoxicity** mediated by LTBR signaling and **transactivated endogenous NK cells** in the presence of ACZ potentially **overcomes a common antigen escape mechanism in NSCLC**

References

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2. Creelan BC et al. *Nat Med* 2021;27:1410-8.
3. Wang C et al. *Nat Cancer* 2025;6:801-19.
4. Xie H et al. SITC 2026 (Abstract 371).
5. Bott M et al. SITC 2024 (Abstract 444).

Abbreviations

ACZ, acetazolamide; B2M, Beta-2 macroglobulin; DP, drug product; , inference of CRISPR edits; KO, knock-out; LTBR, lymphotoxin beta receptor; mbIL15, membrane-bound IL15; MHC, major histocompatibility complex; NK, nature killer; NSCLC, non-small cell lung cancer; PDc, patient-derived cell line; REP, rapid expansion protocol; TIL, tumor-infiltrating lymphocyte.

Disclosures

AJS 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, ZA, DKS, GR** are employees of and hold stock equity in Obsidian Therapeutics (Cambridge, MA, USA).

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