

# Translational data validate OBX-115 mechanism of action: Impact of dosing on clinical outcome in advanced melanoma

Gino K In,<sup>1</sup> Jason A Chesney,<sup>2</sup> Alexander N Shoushtari,<sup>3</sup> Justin T Moyers,<sup>4</sup> Yazan Samhuri,<sup>5</sup> Tirrell T Johnson,<sup>6</sup> Rodabe N Amaria,<sup>7</sup> Giridharan Ramsingh,<sup>8</sup> Camille Renard,<sup>8</sup> Bulent Arman Aksoy,<sup>8</sup> Rachel Burga,<sup>8</sup> Mercay Reuter,<sup>8</sup> Prakash Prabhakar,<sup>8</sup> Lauren McLaughlin,<sup>8</sup> Allison Betof Warner,<sup>8</sup>  
1. Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; 2. UofL Health – Brown Cancer Center, Louisville, KY, USA; 3. Memorial Sloan Kettering Cancer Center, New York, NY, USA; 4. The Angeles Clinic and Research Institute, A Cedars-Sinai Affiliate, Los Angeles, CA, USA; 5. Allegheny Health Network Cancer Institute, Pittsburgh, PA, USA; 6. Orlando Health Cancer Institute, Orlando, FL, USA; 7. The University of Texas MD Anderson Cancer Center, Houston, TX, USA; 8. Obsidian Therapeutics, Cambridge, MA, USA; 9. Stanford University School of Medicine, Stanford, CA, USA

## Introduction

- OBX-115 tumor-infiltrating lymphocytes (TIL) express membrane-bound IL15 (mIL15) regulated by the FDA-approved small-molecule drug acetazolamide (ACZ)
- mIL15 abrogates the need for IL2 and results in a differentiated safety profile and promising efficacy in advanced melanoma<sup>1,2</sup>
- Translational data from a phase 1 single-center study demonstrated ACZ-driven OBX-115 TIL expansion, and tumor infiltration and validated the proposed OBX-115 mechanism of action<sup>3</sup>
- Herein, we report translational data from patients with advanced melanoma treated with escalating dose levels of OBX-115 and ACZ in the multicenter Agni-01 phase 1/2 study to further validate OBX-115 mechanism of action

## Methods

### Figure 1. Single-arm Phase 1/2 Agni-01 Study Design (NCT06060613)

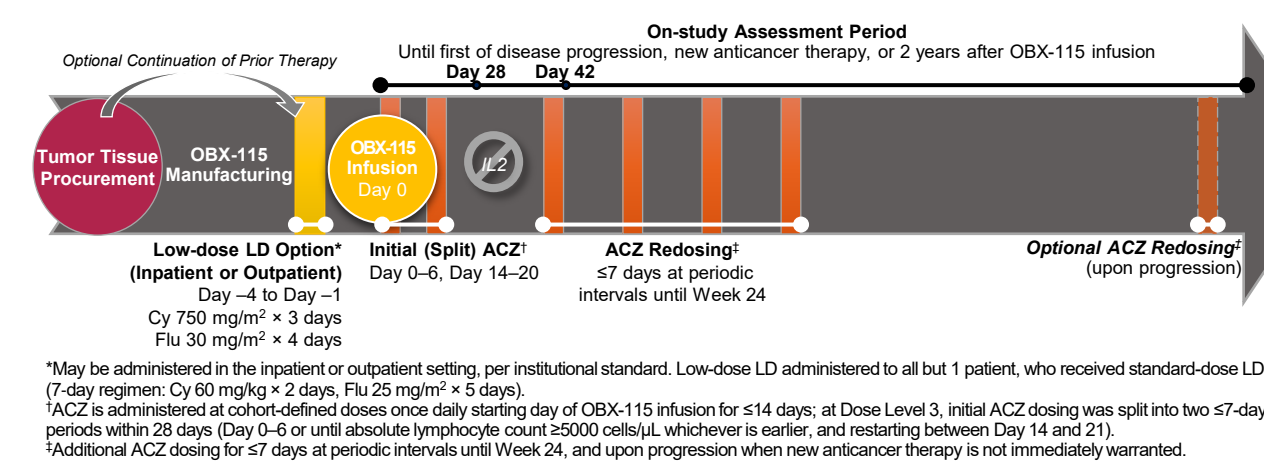


Figure 1. Agni-01 trial design and clinical results were previously described.<sup>2</sup> After tumor tissue procurement, patients could receive optional continuation of prior therapy during OBX-115 manufacturing. Patients then received lymphodepletion (standard- or low-dose options) followed by OBX-115 infusion (Day 0) and ≤14 days of orally administered ACZ (at cohort-defined doses and dosing schemes; Figure 2) or until absolute lymphocyte count was ≥5000 cells/μL, whichever was earlier. ACZ was redosed for ≤7 days at periodic intervals and upon progression when new anticancer therapy was not immediately warranted.

### Figure 2. Protocol-defined Dose-escalation Strategy

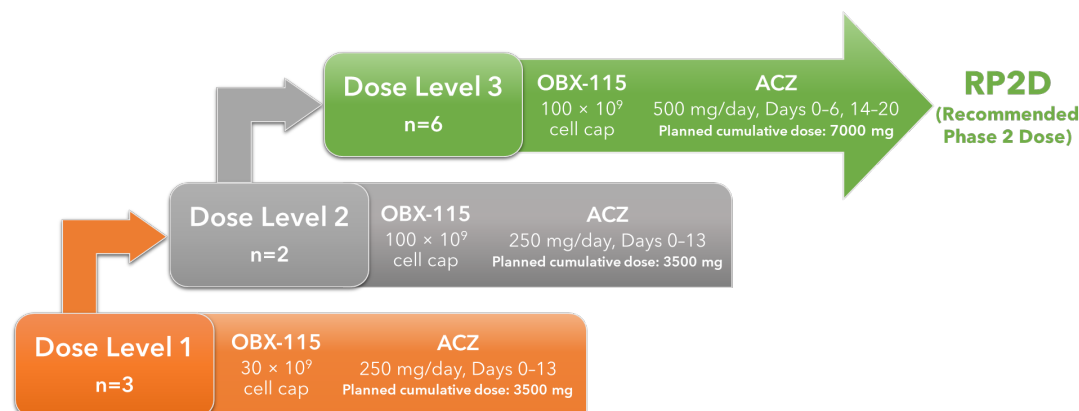


Figure 2. Patients were treated with escalating doses of OBX-115 and ACZ. On Day 0, OBX-115 was infused at doses of ≤30 × 10<sup>9</sup> (DL1) or ≤100 × 10<sup>9</sup> (DL2, DL3) cells. Post-infusion, patients received oral ACZ daily (≤14 days, Days 0–13) at 250 mg (DL1, DL2) or 500 mg (DL3; Days 0–6, 14–20). ACZ was redosed as an outpatient (≤7 days) Q6W starting ~Day 35.

## Translational Analyses

- Peripheral blood (PB), tumor tissue, and serum samples were collected at Baseline (pre-lymphodepletion) and subsequent timepoints for longitudinal analysis. Samples were assessed as follows:
  - OBX-115 DNA by droplet digital polymerase chain reaction (ddPCR): PB and tumor tissue
  - T-cell receptor (TCR) repertoire by RepSeq+ platform (iRepertoire): PB and tumor tissue
  - Phenotype by spectral flow cytometry: PB
  - Cytokines by median fluorescence intensity using multiplex bead-based immunoassay Luminex: Serum
- Day 42 formalin-fixed paraffin-embedded tumor biopsy samples were QC'ed and sectioned by Lanterne Dx; middle sections were then prepared for RNAscope-based profiling by ACD Bio (Biotechne). A custom probe was designed against a unique sequence within the mIL15 transgene to distinguish OBX-115 cells from non-transduced cells

## Results

- The 11 patients included in the ASCO 2025 analysis<sup>2</sup> are included in this analysis (DL1, n=3; DL2, n=2; DL3/RP2D, n=6)
  - Patients had advanced and substantially pre-treated disease
  - ORR was 67% (4/6), with 2 CRs (33%) in 6 patients receiving the recommended phase 2 dose (RP2D)
- Among all 11 patients, the mean initial ACZ dose in the first 28 days was 4432 mg (Table 1); DL3/RP2D received the highest cumulative ACZ initial dose (mean, 6125 mg) of all dose groups

Table 1. OBX-115 and ACZ Dosing, by Dose Level

	Mean (SD) OBX-115 Infused Dose, ×10 <sup>9</sup> cells	Planned Cumulative ACZ Initial Dose (First 28 days), mg	Mean (SD) Cumulative ACZ Initial Dose (First 28 days), mg
<b>All Patients (N=11)</b>	59.5 (27.2)	5409	4432
<b>Dose Level 3 / RP2D (n=6)</b> OBX-115 cap 100×10 <sup>9</sup> cells, 500 mg ACZ	69.5 (22.7)	7000	6125
<b>Dose Level 2 (n=2)</b> OBX-115 cap 100×10 <sup>9</sup> cells, 250 mg ACZ	79.6 (7.1)	3500	2000
<b>Dose Level 1 (n=3)</b> OBX-115 cap 30×10 <sup>9</sup> cells, 250 mg ACZ	26.3 (6.5)	3500	2667

### Figure 3. OBX-115 Expansion in PB Responds to ACZ Dosing

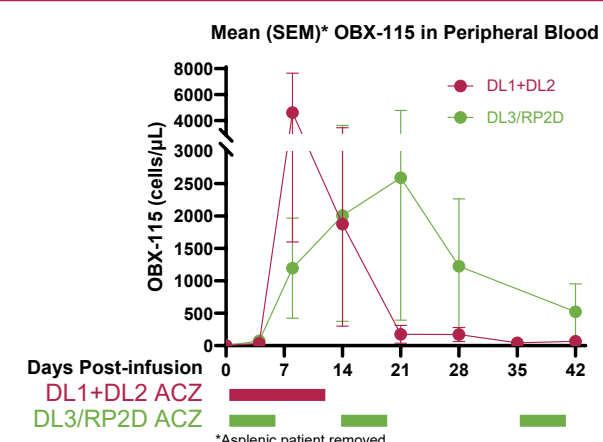


Figure 3. In DL1 and DL2, initial ACZ dosing was received for ≤14 days, from Day 0–13, leading to an initial peak, followed by early drop and limited persistence of OBX-115. In DL3/RP2D, split dosing was introduced, in which initial ACZ dosing was received for a total of ≤14 days, from Day 0–6, with at least 7 days of rest before resuming dosing on Day 14–20. The split dosing regimen allowed patients to receive a higher cumulative ACZ dose within the first 28 days, and led to a differentiated OBX-115 expansion and persistence profile, marked by increased persistence in PB from Day 21–42.

### Figure 4. Plasma Levels of IL6 and IL15 Remain Low After Infusion

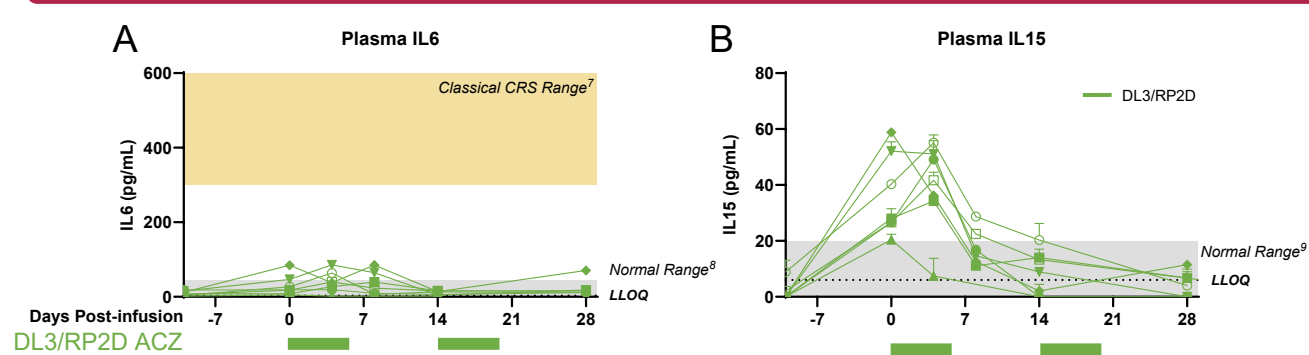


Figure 4. Analysis of plasma cytokine levels revealed that even at the higher ACZ dosing of DL3/RP2D, leading to increased mIL15 expression on OBX-115 cells, IL6 levels of individual patients remained low and were far below those seen with classical cytokine release syndrome (CRS) following OBX-115 infusion and ACZ dosing (A).<sup>7</sup> IL15 levels were elevated at Day 0 (pre-infusion) due to intrinsic IL15 production in response to lymphodepletion, but were not further elevated post-infusion (after Day 0), suggesting minimal IL15 shedding from OBX-115 (B).

### Figure 5. OBX-115-derived TCR Clonotypes Persist in PB

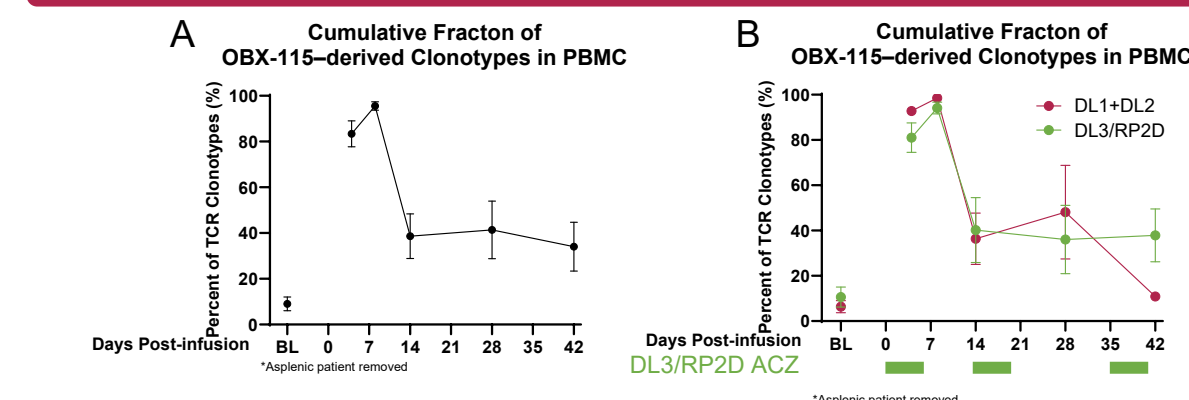


Figure 5. Peripheral blood mononuclear cell (PBMC) TCR sequencing (TCRseq) showed persistence of OBX-115-derived clonotypes. (A) After an initial peak at around Day 7 post-infusion, OBX-115-derived TCR clonotypes persisted in PB at an elevated post-Baseline steady-state through Day 42. (B) Day 42 persistence of OBX-115-derived clonotypes was higher for patients receiving DL3/RP2D than for those receiving DL1 or DL2.

### Figure 6. Patient-level Differences in OBX-115-derived TCR Clonotype Remodeling in Tumor and PBMC

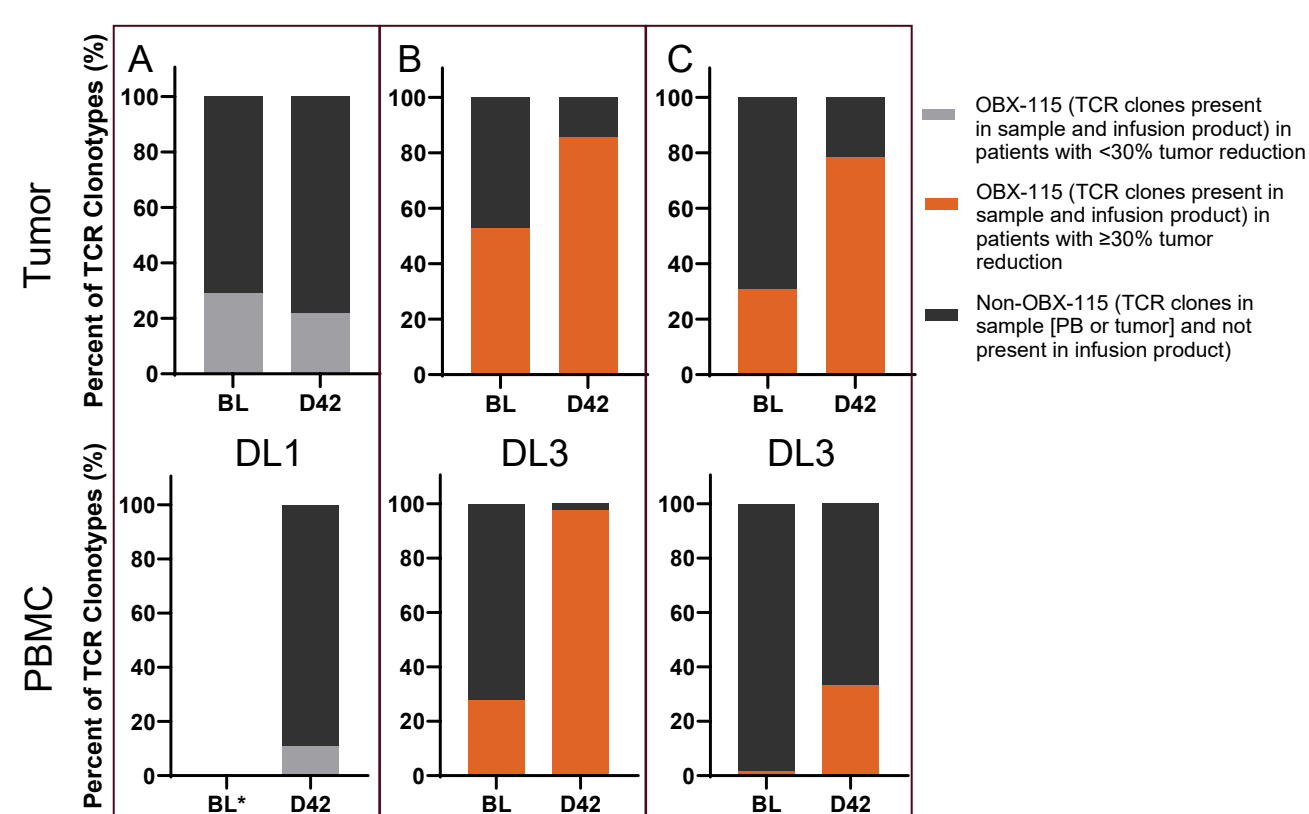


Figure 6. Baseline and post-infusion (Day 42) TCR clonotypes were assessed in tumor and PB. 3 patients had Baseline and post-infusion tumor biopsy samples; PBMC samples for these patients are provided for comparison. (A) 1 of these 3 patients received DL1 and achieved <30% tumor reduction; this patient's tumor did not demonstrate an increase in the proportion of OBX-115-derived TCR clonotypes post-infusion relative to Baseline (Baseline PBMC was not available for comparison; light gray). (B and C) 2 of these 3 patients received OBX-115 and ACZ at DL3/RP2D and achieved ≥30% tumor reduction; these patients' tumors and PBMC demonstrated TCR clonotype remodeling post-infusion (orange).

## Results (cont.)

### Figure 7. Infused CD8+ T<sub>EM</sub> Expand in PB

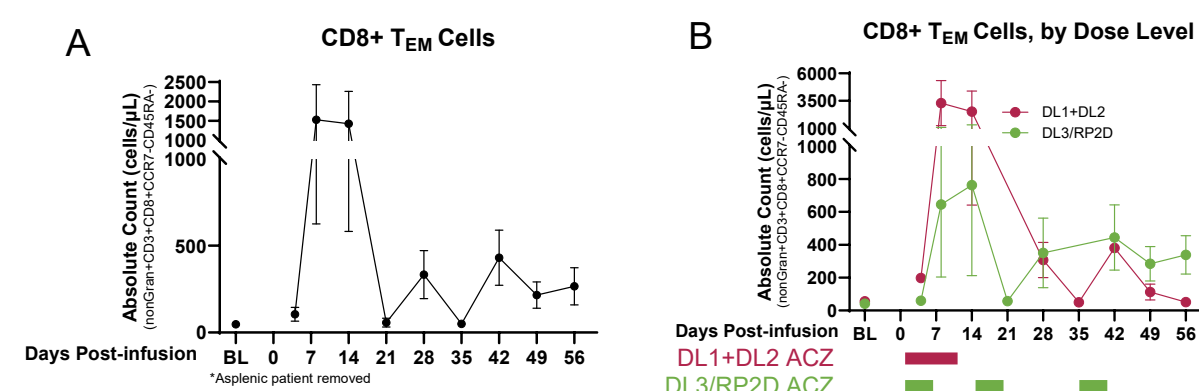


Figure 7. Infused OBX-115 cells are predominately CD8+.<sup>3</sup> (A) Spectral flow cytometry demonstrated robust peripheral expansion of infused CD8+ effector memory T cells (T<sub>EM</sub>). (B) The uninterrupted initial ACZ dosing in DL1/DL2 led to a larger early CD8+ T<sub>EM</sub> cell peak than with DL3/RP2D, but DL3/RP2D maintained better persistence at Day 56.

### Figure 8. NK-like T Cells Demonstrate Peripheral Surge

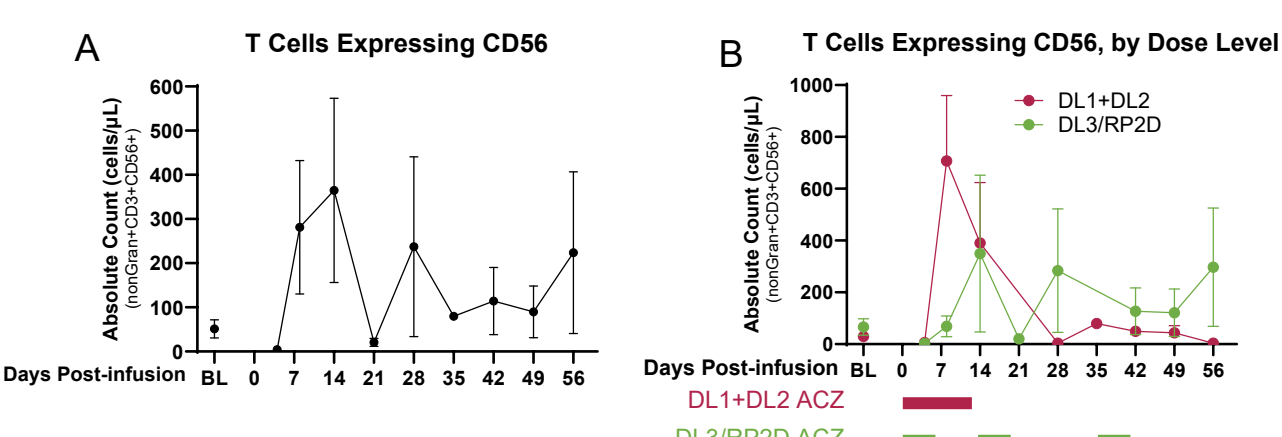


Figure 8. NK-like T cells are present in the OBX-115 infusion product<sup>3</sup> likely due to the novel manufacturing process utilizing 4-1BB agonist and IL15. (A) Spectral flow cytometry demonstrated a surge of natural killer (NK)-like T cells post-infusion. (B) DL1+DL2 demonstrated a larger early peak than with DL3/RP2D, but DL3/RP2D appeared to maintain better persistence.

### Figure 9. Low PD-1 Expression is Observed on Peripheral T Cells

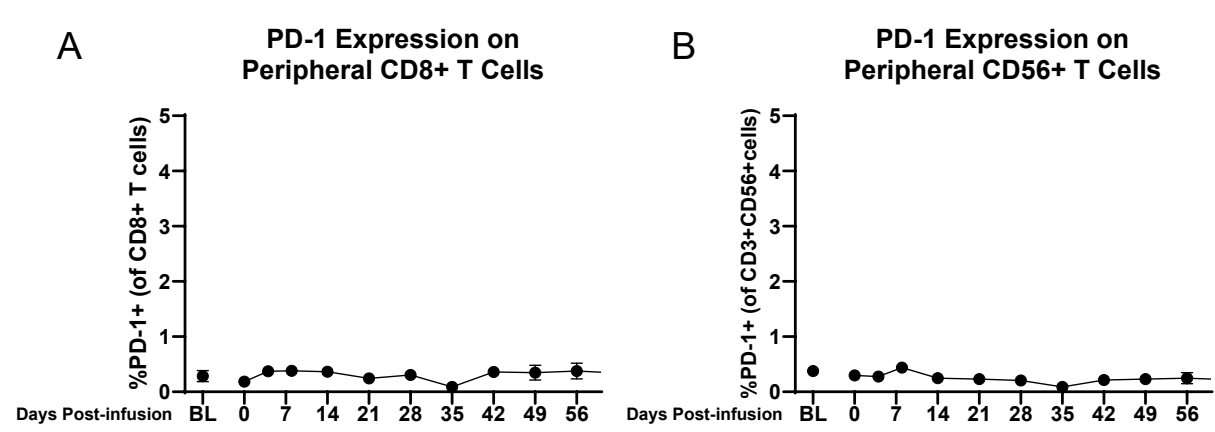


Figure 9. Spectral flow cytometry demonstrated consistently very low (<1%) expression of PD-1 in both CD8+ (A) and CD3+CD56+ (B) cells through Day 56, suggesting that PD-1-mediated resistance mechanisms were not triggered in response to OBX-115 infusion in either cell type.

### Figure 10. Endogenous NK Cells Expand Robustly Post-infusion

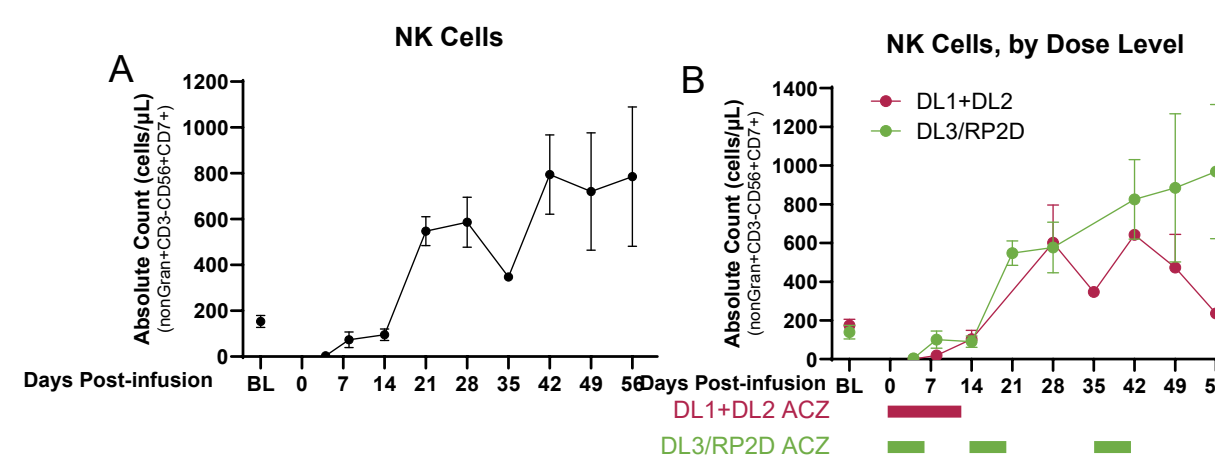


Figure 10. NK cells, which are not present in the OBX-115 infusion product,<sup>3</sup> were reduced during lymphodepletion and (A) expanded 4-fold post-infusion, with a delayed peak compared to T-cells and NK-like T cells, suggesting a mechanism for trans-presentation of OBX-115-derived mIL15. (B) At DL3/RP2D, ACZ-responsive expansion of OBX-115 increased mIL15 available for trans-presentation to expand endogenous NK cells.

## References

- Amaria RN et al, ASCO 2024 (Abstract 9515).
- Chesney JA et al, ASCO 2025 (Abstract 9517).
- Amaria RN et al, ASCO 2025 (Abstract 9519).
- Kluger H et al. J Immunother Cancer 2020;8(1).
- Kluger H et al. J Immunother Cancer 2023;11(3).
- Dudley ME et al. J Immunother 2002;25(3).
- Pabst T et al. Exp Hematol. 2020;88:7–14.
- Said E et al. J Med Virol. 2021 Jun;93(6):3915–3924.
- Lamana A et al. Eur Cytokine Netw. 2010
- Sep;21(3):186–94.

### Figure 11. RNAscope-based Probing Confirms Engraftment of CD8+CD56+ mIL15+ OBX-115 TIL in Day 42 Tumor Biopsy

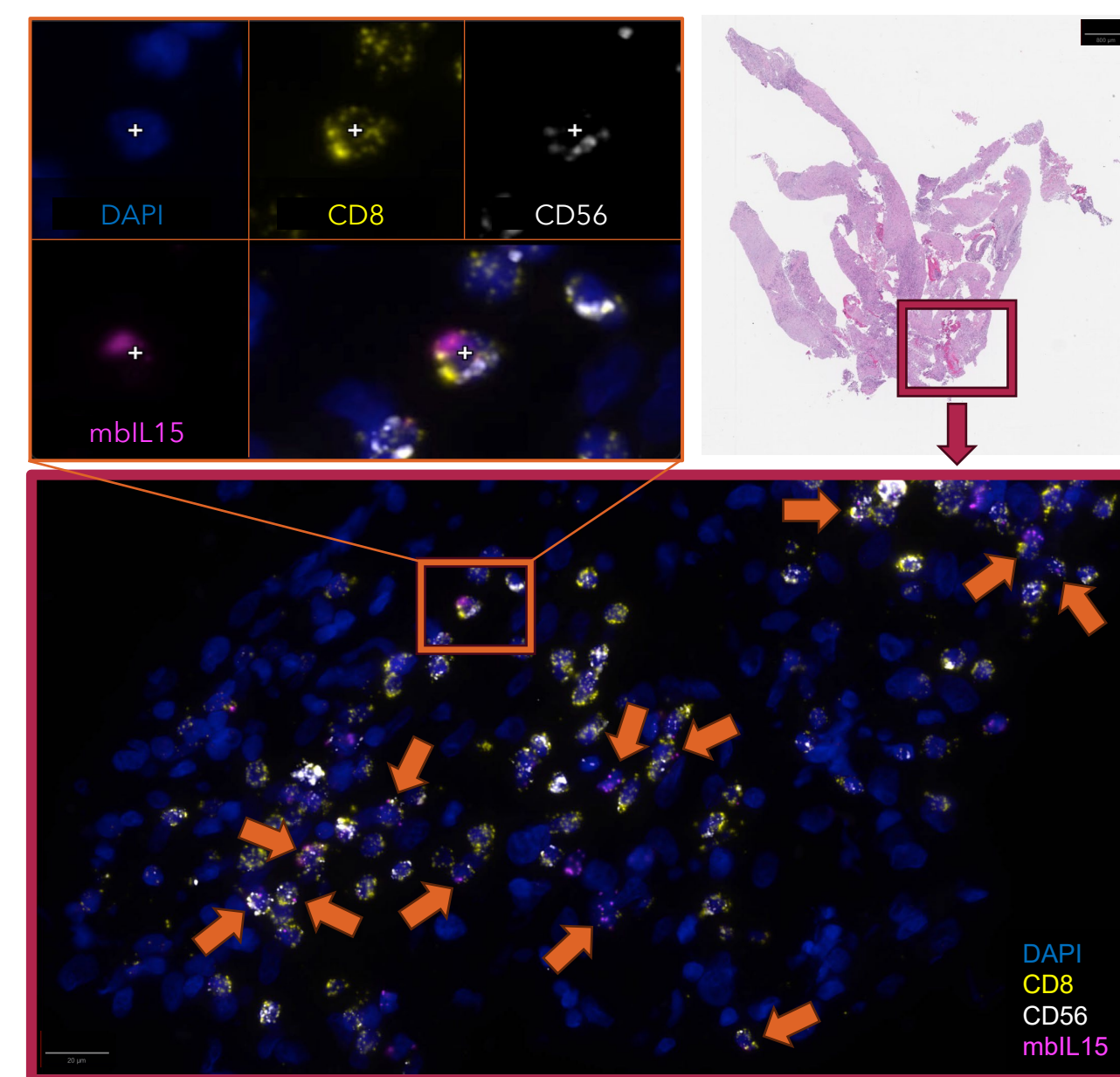


Figure 11. Using RNAscope, mIL15+ cells were identified at the single-cell level in post-infusion tumor biopsy, confirming engraftment of OBX-115 cells. CD8+mIL15+ and CD8+CD56+mIL15+ cells were detected, suggesting a potential mechanism for NK-like T cells expressing regulatable mIL15 in addition to the purported mechanism using mIL15-expressing cytotoxic T cells.

## Conclusions

- Cytokine signal, typically with IL2 for non-engineered TIL, has been shown to be **critical for clinical efficacy of TIL**<sup>6</sup>
- In the current study, **DL3/RP2D optimized OBX-115 and ACZ dosing**, leading to:
  - Expansion, persistence, and infiltration of OBX-115 into tumors
  - PBMC and tumor TCR repertoire remodeling
  - Cis- and trans-immune cell activation with CD8+ T cell-, NK cell-, and NK-like T cell-mediated innate and adaptive immune response
- The regulatable and membrane-bound delivery of IL15 enables a potentially **safer, regulatable, cytokine delivery mechanism** for TIL cell therapy; further, **elimination of IL2 may expand patient safety and eligibility for TIL cell therapy**
- Phase 2 enrollment is ongoing in advanced melanoma and non-small cell lung cancer (NSCLC)

## Abbreviations

ACZ, acetazolamide; BL, Baseline; CRS, cytokine release syndrome; Cy, cyclophosphamide; D, day; ddPCR, droplet digital polymerase chain reaction; DL, dose level; Flu, fludarabine; IL, interleukin; LD, lymphodepletion; LLOQ, lower limit of assay quantitation; mIL15, membrane-bound interleukin 15; NK, natural killer; NSCLC, non-small cell lung cancer; PB, peripheral blood; PBMC, peripheral blood mononuclear cells; PD-1, programmed cell death protein-1; Q6W, every 6 weeks; RP2D, recommended phase 2 dose; TCR, T-cell receptor; T<sub>EM</sub>, effector memory T cells; TIL, tumor-infiltrating lymphocyte.

## Acknowledgments

- The authors thank the patients, their families, and study personnel who have participated in the study
- This study is funded by Obsidian Therapeutics, Inc. (Cambridge, MA, USA)
- Spectral flow cytometry and analysis were provided by Teiko Bio (Salt Lake City, UT)
- Editorial assistance was provided by Amanda Kelly (Obsidian) and funded by Obsidian

## Disclosures

GKI reports institutional research support from Regeneron, Array, Idera, Replimune, Xencor, Instil Bio, Pfizer, and Checkmate; consulting fees from Sanofi and Pfizer; honoraria from BMS, Merck, Regeneron, Array, Castle, Sanofi, Replimune, and Pfizer; and advisory board participation from Replimune.

