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

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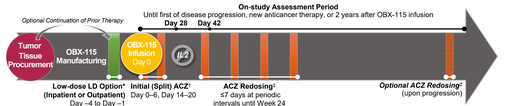
Introduction

- OBX-115 tumor-infiltrating lymphocytes (TIL) express membrane-bound IL15 (mbIL15) regulated by the FDA-approved small-molecule drug acetazolamide (ACZ)
- mbIL15 abrogates the need for IL2 and results in a differentiated safety profile and promising efficacy in advanced melanoma[1,2]
- Translational data from a phase 1 single-center study demonstrated ACZ-driven OBX-115 TIL expansion, persistence, and tumor infiltration and validated the proposed OBX-115 mechanism of action[3]
- 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)



*May be administered in the inpatient or outpatient setting, per institutional standard. Low-dose LD administered to all but 1 patient, who received standard-dose LD (7-day regimen: Cy 60 mg/kg × 2 days, Flu 25 mg/m² × 5 days).
†ACZ is administered at cohort-defined doses once daily starting day of OBX-115 infusion for ≤14 days; at Dose Level 3, initial ACZ dosing was split into two ≤7-day periods within 28 days (Day 0–6 or until absolute lymphocyte count ≥5000 cells/µL whichever is earlier, and restarting between Day 14 and 21).
‡Additional ACZ dosing for ≤7 days at periodic intervals until Week 24, and upon progression when new anticancer therapy is not immediately warranted.

Figure 1. Agni-01 trial design and clinical results were previously described.[2] 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

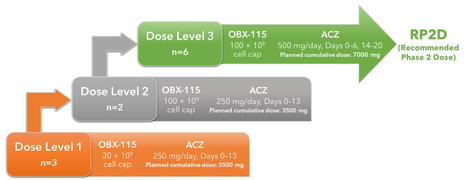


Figure 2. Patients were treated with escalating doses of OBX-115 and ACZ. On Day 0, OBX-115 was infused at doses of ≤30 ×10^9 (DL1) or ≤100 ×10^9 (DL2, DL3) cells. Post-infusion, patients received oral ACZ daily (≤14 days, Days 0–13) at 250 mg (DL1, DL2) or 500 mg (DL3; Days 0–6, 14–20). ACZ was redosed 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 mbIL15 transgene to distinguish OBX-115 cells from non-transduced cells

Results

- The 11 patients included in the ASCO 2025 analysis[2] 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, ×10^9 cells	Planned Cumulative ACZ Initial Dose (First 28 days), mg	Mean (SD) Cumulative ACZ Initial Dose (First 28 days), mg
All Patients (N=11)	59.5 (27.2)	5409	4432
Dose Level 3 / RP2D (n=6) OBX-115 cap 100×10^9 cells, 500 mg ACZ	69.5 (22.7)	7000	6125
Dose Level 2 (n=2) OBX-115 cap 100×10^9 cells, 250 mg ACZ	79.6 (7.1)	3500	2000
Dose Level 1 (n=3) OBX-115 cap 30×10^9 cells, 250 mg ACZ	26.3 (6.5)	3500	2667

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

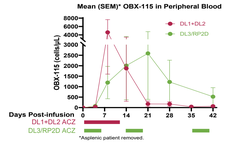


Figure 3. In DL1 and DL2, initial ACZ dosing was received for ≤14 days, from Day 0–13, leading to an initial peak, followed by early drop and limited persistence of OBX-115. In DL3/RP2D, split dosing was introduced, in which initial ACZ dosing was received for a total of ≤14 days, from Day 0–6, with 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

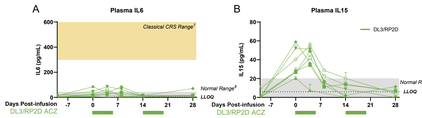


Figure 4. Analysis of plasma cytokine levels revealed that even at the higher ACZ dosing of DL3/RP2D, leading to increased mbIL15 expression on OBX-115 cells, IL6 levels of individual patients remained low and were far below those seen with classical cytokine release syndrome (CRS) following OBX-115 infusion and ACZ dosing (**A**).[7] 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

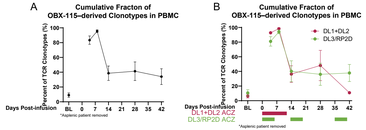


Figure 5. Peripheral blood mononuclear cell (PBMC) TCR sequencing (TCRseq) showed persistence of OBX-115–derived clonotypes. (**A**) After an initial peak at around Day 7 post-infusion, OBX-115–derived TCR clonotypes persisted in PB at an elevated post-Baseline steady-state through Day 42. (**B**) Day 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

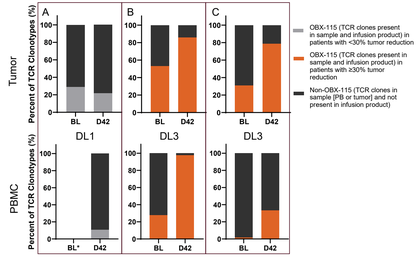


Figure 6. Baseline and post-infusion (Day 42) TCR clonotypes were assessed in tumor and PB. 3 patients had Baseline and post-infusion tumor biopsy samples; PBMC samples for these patients are provided for comparison. (**A**) 1 of these 3 patients received DL1 and achieved <30% tumor reduction; this patient's 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).

Figure 7. Infused CD8+ T_{EM} Expand in PB

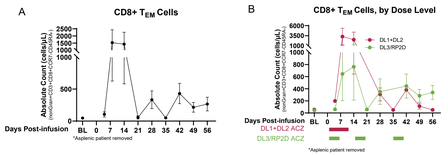


Figure 7. Infused OBX-115 cells are predominately CD8+.[3] (**A**) Spectral flow cytometry demonstrated robust peripheral expansion of infused CD8+ effector memory T cells (T_{EM}). (**B**) The uninterrupted initial ACZ dosing in DL1/DL2 led to a larger early CD8+ T_{EM} cell peak than with DL3/RP2D,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

Figure 8. NK-like T Cells Demonstrate Peripheral Surge

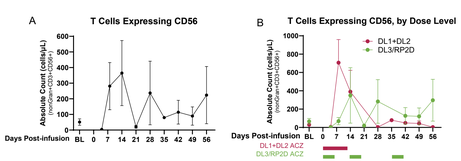


Figure 8. NK-like T cells are present in the OBX-115 infusion product[3] likely due to the novel manufacturing process utilizing 4-1BB agonism and IL15. (**A**) Spectral flow cytometry demonstrated a surge of natural killer (NK)-like T cells post-infusion. (**B**) DL1+DL2 demonstrated a larger early 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

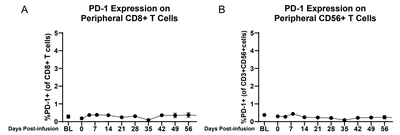


Figure 9. Spectral flow cytometry demonstrated consistently very low (<1%) expression of PD-1 in both CD8+ (**A**) and CD3+CD56+ (**B**) cells through Day 56, suggesting that PD-1–mediated resistance mechanisms were not triggered in response to OBX-115 infusion in either cell type.

Figure 10. Endogenous NK Cells Expand Robustly Post-infusion

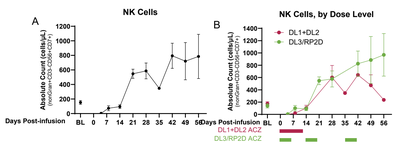


Figure 10. NK cells, which are not present in the OBX-115 infusion product,[3] were reduced during lymphodepletion and (**A**) expanded 4-fold post-infusion, with a delayed peak compared to T-cells and NK-like T cells, suggesting a mechanism for trans-presentation of OBX-115–derived mbIL15. (**B**) At DL3/RP2D, ACZ-responsive expansion of OBX-115 increased mbIL15 available for trans-presentation to expand endogenous NK cells.

Figure 11. RNAscope-based Probing Confirms Engraftment of CD8+CD56+ mbIL15+ OBX-115 TIL in Day 42 Tumor Biopsy

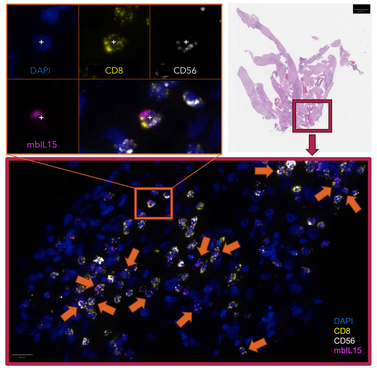


Figure 11. Using RNAscope, mbIL15+ cells were identified at the single-cell level in post-infusion tumor biopsy, confirming engraftment of OBX-115 cells. CD8+mbIL15+ and CD8+CD56+mbIL15+ cells were detected, suggesting a potential mechanism for NK-like T cells expressing regulatable mbIL15 in addition to the purported mechanism using mbIL15-expressing cytotoxic T cells.

Figure 12. ctDNA Reduction in All Patients at Day 14

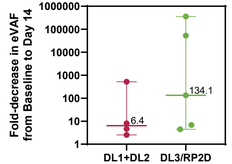


Figure 12. ctDNA eVAF declined by Day 14 in all evaluable patients, and was undetectable in 2 patients receiving DL3/RP2D. Median eVAF fold-decrease from Baseline was higher in DL3/RP2D than DL1+DL2 (134.1 vs 6.4).

Conclusions

- **Cytokine signal**, typically with IL2 for non-engineered TIL, has been shown to be **critical for clinical efficacy of TIL**[6]
- 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**
  - **Greater ctDNA fold-reduction**, suggestive of **antitumor activity**, in DL3/RP2D than in DL1+DL2
- 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)

References

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Abbreviations

ACZ, acetazolamide; BL, Baseline; CRS, cytokine release syndrome; ctDNA, circulating tumor DNA; Cy, cyclophosphamide; D, day; ddPCR, droplet digital polymerase chain reaction; DL, dose level; eVAF, estimated variant allele frequency; Flu, fludarabine; IL, interleukin; LD, lymphodepletion; LLOQ, lower limit of assay quantitation; mbIL15, 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_{EM}, effector memory T cells; TIL, tumor-infiltrating lymphocyte.

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Disclosures

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