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Tumor-infiltrating lymphocytes (TIL) engineered with membrane-bound IL15 (cytoTIL15™ cells) exhibit pharmacologically regulatable signaling in cis and trans

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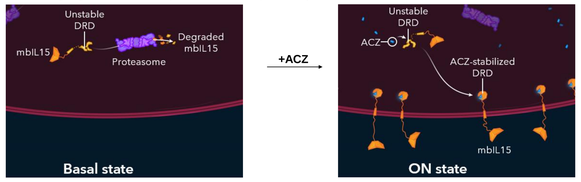
Introduction

- We have previously shown the successful engineering of membrane-bound IL15 (mbIL15)-modified tumor-infiltrating lymphocytes (TIL; cytoTIL15™ cells) from solid tumors such as melanoma, which are IL2-independent, and exhibit enhanced persistence and anti-tumor activity compared with non-engineered TIL[1,2] (clinical candidate OBX-115: NCT05470283)
- mbIL15 expression is regulated by a drug-responsive domain (DRD) and induced upon delivery of the FDA-approved small-molecule ligand acetazolamide (ACZ), and was designed to engage the IL2Rβ and IL2Rγ receptors in both cis and in trans



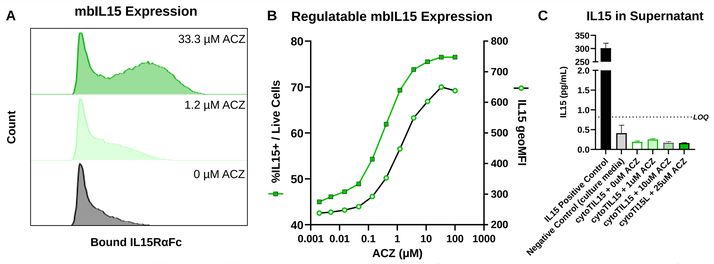
- Natural killer (NK) cells are innate lymphocytes that are potently responsive to soluble IL15 and are capable of exerting MHC-independent cytotoxicity. In this study, we sought to examine the potential of cytoTIL15 cells to transactivate NK cells and potentiate NK cell proliferation and effector function in vitro

Methods

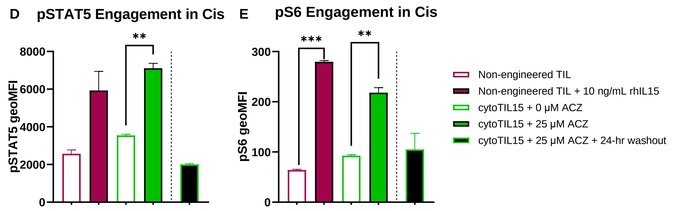
- cytoTIL15 cells contain a regulatable mbIL15 construct consisting of IL15 tethered by a long flexible GS-linker to the cell membrane without any shedding domains, and was designed such that IL15 was predicted to engage IL2Rβ and IL2Rγ receptors in cis and trans, without interfering with binding of IL15Rα
- To test this hypothesis, cytoTIL15 cells and non-engineered TIL (dependent on IL2) were expanded with feeder cells for 14 days in a proprietary rapid-expansion protocol (REP) process, after which cells were immediately assessed for expression of mbIL15 and downstream signaling by flow cytometry, or co-cultured with allogeneic healthy donor NK cells with and without transwell inserts
- Functional effects of cytoTIL15 were compared with those of soluble recombinant human IL15 (rhIL15)
  - Transactivation was assessed by phosphoflow of TIL and NK co-cultures with/without transwell inserts
  - Proliferation of NK cells was quantified by cell counting and fluorescent incorporation

Results

Figure 1. cytoTIL15 cells demonstrate robust ACZ-dependent regulation of mbIL15 in cis



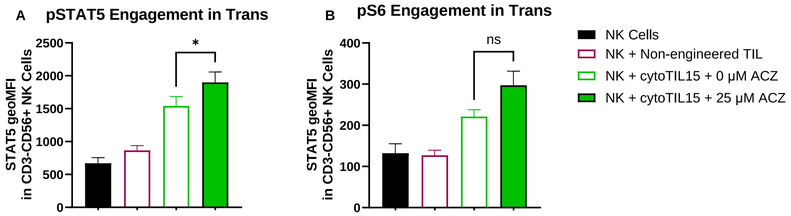
- After expansion in REP with ACZ, cryopreserved cytoTIL15 cells and non-engineered TIL were cultured with 0–100 µM ACZ for 24 hours, after which mbIL15 expression was examined with an IL15RαFc detection reagent (n=3 melanoma TIL donors; **Figure 1A**)
- ACZ can regulate expression of mbIL15 (EC_{50}=0.51 µM ACZ); non-engineered TIL did not express mbIL15 in the presence or absence of ACZ (**Figure 1B**)
- cytoTIL15 cells cultured with ranging ACZ for 3d produce basal IL15 in cell supernatant (LOQ = 0.82 pg/mL; **Figure 1C**)



- Consistent with expression, the activity of IL15 acting in cis in cytoTIL15 cells was dependent on ACZ dosing, as measured by potentiation of downstream signal transduction
- Phosphorylation of signal transducer and activator of transcription 5 (STAT5; at Y694) was ACZ-dependent (EC_{90}=3.43 µM ACZ) and bioactivity was similar to that of non-engineered TIL cultured with 10 ng/mL rhIL15 (**Figure 1D**)
- Phosphorylation of ribosomal protein S6 (S6; at S235/236) was ACZ-dependent (EC_{90}=4.32 µM ACZ) and bioactivity was similar to that of non-engineered TIL cultured with 10 ng/mL rhIL15 (**Figure 1E**)
- cytoTIL15 cells cultured in the absence of ACZ demonstrate significantly decreased IL15 bioactivity, as the average geoMFI for pSTAT5=3541 with 0 µM ACZ vs. 7110 with 25 µM ACZ (p=0.006), and the average geoMFI for pS6=92.6 with 0 µM ACZ vs. 218 with 25 µM ACZ (p=0.007); statistics performed via student's t-test with **p<0.005, ***p<0.0005
- Once ACZ was removed, pSTAT5 and pS6 levels returned to baseline within 24h, highlighting the tunability of our regulated mbIL15 construct in cytoTIL15 cells

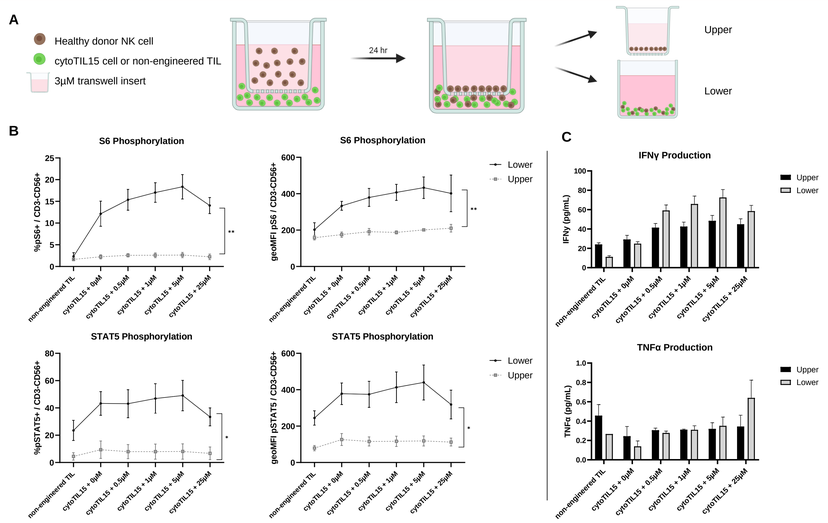
Results

Figure 2. mbIL15 can induce NK cell signaling in trans



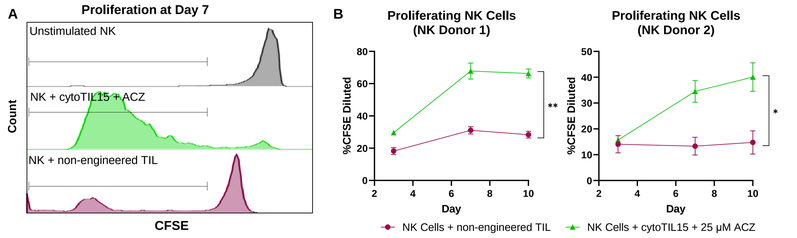
- Non-engineered TIL and cytoTIL15 cells were thawed and rested with 0–100 µM ACZ for 24 hours; concurrently, allogeneic NK cells derived from healthy donor peripheral blood mononuclear cells (PBMCs) were thawed and rested in cytokine-free conditions for 24 hours. After rest, TIL and NK cells were co-cultured at 1:1 and 5:1 ratios for an additional 24 hours
- Co-culture of NK cells with cytoTIL15 cells, but not non-engineered TIL, led to increased IL15 signaling in trans, with phosphorylation of STAT5 (**Figure 2A**) and S6 (**Figure 2B**) increasing in an ACZ-dependent manner (average geoMFI of pSTAT5=1541 with 0 µM ACZ vs. 2034 with 25 µM ACZ, p = 0.04; average geoMFI of pS6=221 with 0 µM ACZ vs. 297 with 25 µM ACZ, p=0.11; n=3 NK donors evaluated against n=5 TIL donors); statistical analyses performed via student's t-test with *p<0.05

Figure 3. Transactivation by cytoTIL15 cells occurs preferentially with direct cell:cell contact



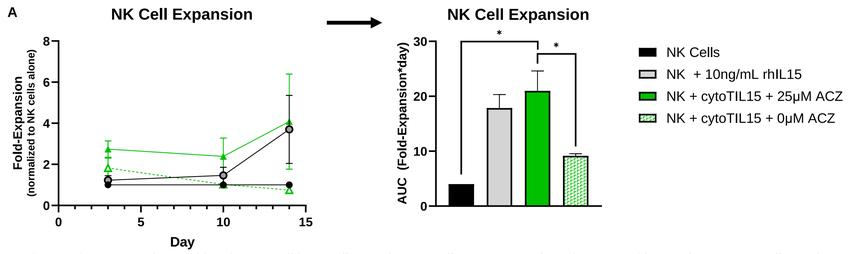
- Non-engineered TIL and cytoTIL15 cells were thawed and rested with 0–100 µM ACZ for 24 hours; concurrently, allogeneic NK cells derived from healthy donor PBMCs were thawed and rested in cytokine-free conditions for 24 hours. After rest, TIL and NK cells were co-cultured at a 1:1 ratio with TIL seeded into the culture well (Lower) and NK cells seeded atop a 0.1–3 µM transwell insert (Upper) for an additional 24 hours, after which cell suspensions and supernatant were evaluated from each compartment independently
- NK cells migrated into the bottom chamber in response to the IL15 gradient established by the presence of cytoTIL15 cells (**Figure 3A**)
- IL15-mediated bioactivity occurred at a greater magnitude when direct TIL:NK contact was achieved (%pS6 p=0.007, geoMFI p=0.005, %pSTAT5 p=0.035, geoMFI p=0.025; statistical analyses performed via ANOVA with *p<0.05, **p<0.005, **Figure 3B**)
- Production of effector cytokines IFNγ and TNFα was correspondingly greater from NK cells in direct contact with cytoTIL15 cells vs. separated by a transwell membrane, and cytokine production was ACZ-dependent (**Figure 3C**)

Figure 4. Transactivation of NK cells with mbIL15 expressed on TIL promotes proliferation

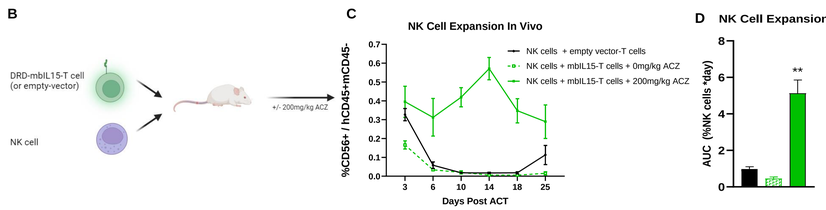


- After 24 hours rest in cytokine-free conditions, allogeneic NK cells were co-cultured at 1:1 with TIL (cytoTIL15 cells and non-engineered TIL) generated from 3 donors ± 25 µM ACZ; NK cells were pre-labeled CFSE to quantitate proliferation over 10 days, and proliferating cells were identified as those with CFSE dilution (**Figure 4A**)
- The frequency of proliferating cells was greatest from co-cultures of NK cells with cytoTIL15 cells supported by ACZ (p<0.02; **Figure 4B**); statistical analyses performed via ANOVA with *p<0.05, **p<0.005

Figure 5. mbIL15 acting in trans can promote NK cell expansion in vitro and in vivo



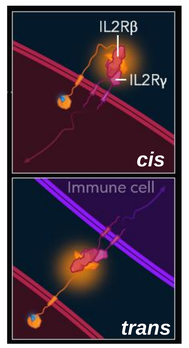
- After 24 hours rest in cytokine-free conditions, allogeneic NK cells were co-cultured at 1:1 with TIL (cytoTIL15 cells and non-engineered TIL) generated from 3 donors ± 25 µM ACZ; NK cells were cultured with 10 ng/mL rhIL15 as a control. Cell counts were obtained and expansion in co-cultures was normalized to expansion of NK cells alone; statistical analyses performed via student's t-test with *p<0.05
- NK cell expansion over 14 days in vitro was greatest in co-cultures containing cytoTIL15 cells supported by ACZ (**Figure 5A**)



- T cells from healthy donor PBMCs were transduced to express regulatable mbIL15 and were co-infused into non–tumor-bearing immunodeficient NSG mice with NK cells derived from the same donor PBMC. Animals were dosed with 200 mg/kg ACZ daily and peripheral blood was obtained at Days 3, 6, 10, 14, 18, and 25 post-adoptive cell transfer (ACT; **Figure 5B**)
- The frequency of NK cells was tracked via flow cytometry, with NK cells identified as human CD56+ cells out of a population of human CD45+ mouse CD45- cells (**Figure 5C**); statistical analyses performed via student's t-test with **p<0.005
- NK cells responded to mbIL15 transactivation and achieved greater expansion and accumulation in an ACZ-dependent manner (AUC with control empty-vector T cells=0.97, AUC with mbIL15 in the absence of ACZ=0.45, AUC with ACZ=5.13, p<0.0014; **Figure 5D**)

Conclusions

- cytoTIL15 cells demonstrate regulatable, **ACZ-dependent functionality of IL15 in cis**, as measured by downstream phosphorylation of STAT5 and S6 in engineered TIL
- cytoTIL15 cells can potentiate **IL15-induced trans-activation** of bystander immune cells
- The transactivation of bystander NK cells is more robust when mediated by transactivation occurring with **direct cell:cell contact**, as separation with a transwell insert leads to lower levels of IL15 engagement and cytokine production
- mbIL15 acting in trans can robustly support the **proliferation and expansion** of bystander NK cells in vitro and in vivo



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2. Burga R, et al. Digital spatial profiling and antigen-dependent phenotypic analysis of IL15-engineered tumor-infiltrating lymphocytes (cytoTIL15 therapy) in an allogeneic melanoma PDX model. Presented at SITC 37th Annual Meeting 2022 (abstract 390).

Abbreviations

ACT, adoptive cell transfer; ACZ, acetazolamide; AUC, area under the curve; D, day; IL2, interleukin 2; IL15, interleukin 15; mbIL15, membrane-bound IL15; MHC, major histocompatibility complex; NK, natural killer; PBMC, peripheral blood mononuclear cells; REP, rapid-expansion protocol; rhIL15, recombinant human iL15; S6, ribosomal protein S6; STAT, signal transducer and activator of transcription 5; TIL, tumor-infiltrating lymphocytes; Treg, regulatory T cell.

Disclosures

- All authors are fulltime employees and shareholders of Obsidian Therapeutics, Inc. (Cambridge, MA, USA)

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