The CD8⁺ T cell selectivity of AB248 is essential for optimal anti-tumor activity and safety in nonclinical models

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Introduction

High-dose (HD) IL-2 induces durable clinical responses in a subset of cancer patients, but severe toxicity, including vascular leak syndrome (VLS), limits its therapeutic window. Insights into the role of IL-2Rα in the pathogenesis of VLS sparked the development of second-generation IL-2 and IL-15-based molecules referred to as not-α IL-2 and IL-15 variants, which signal through the IL-2Rβγ receptor. Early clinical data from several not-α IL-2Rβγ agonists in melanoma and renal cell carcinoma suggest lower objective responses rates compared to historical data for HD IL-2; moreover, dosing of these compounds is limited by toxicity.[1,2] Importantly, not-α IL-2s expand regulatory T cells (~2X) and potently expand NK cells (~7-13X) while eliciting only modest CD8⁺ T cell expansion (~2-3X) in patients.[3-5]

NK cells have been shown to drive the toxicity of an IL-2Rβγ agonist in preclinical models: body weight loss, liver toxicity, and hypothermia seen after treatment were all shown to be NK cell-dependent.[6] Furthermore, NK cells have been shown to be dispensable for the anti-tumor activity of many IL-2 and IL-15-based therapies, while CD8⁺ T cells have been shown to be critical for anti-tumor activity.[7-9] We therefore hypothesized that maximizing the activity of IL-2 on CD8⁺ T cells, while limiting its activity on immunosuppressive Tregs, NK cells, and other IL-2-responsive populations would result in improved on-target pharmacology, antitumor immunity, and tolerability. Here, we describe AB248, a CD8-targeted IL-2Rβγ agonist, and demonstrate an improved profile compared to untargeted IL-2 pathway agonists.

Cis-targeting enables cell-type selectivity

To design AB248, an approach called *cis*-targeting was employed (**Figure 1**). To generate an optimal *cis*-targeted cytokine, the potency of the cytokine is reduced to decrease signaling to its cognate receptor. Then activity is selectively rescued via avidity provided by a targeting domain recognizing an antigen expressed on the surface of the desired cell type. In the case of AB248, an IL-2 mutein with abrogated IL-2Rα binding and reduced IL-2Rβγ affinity was fused to an anti-CD8 targeting antibody (**Figure 2**). This design enables over 500-fold preference for CD8+ T cells (**Figure 3**).

Figure 1: *Cis*-targeting may address the problematic pleiotropy associated with wild type cytokines

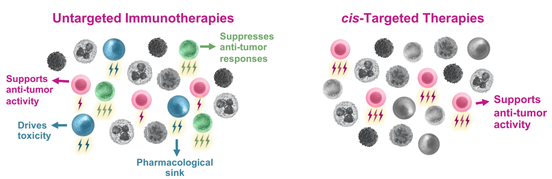
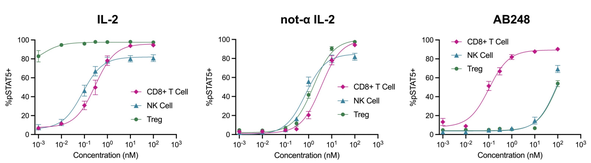


Figure 2: Overview of AB248's molecular design

Anti-CD8	Enables specific binding to CD8⁺ T cells without affecting CD8 co-receptor function
Engineered Bispecific Fc	Ensures fusion with a single IL-2 mutein; enables half-life extension; avoids FcγR interactions
Attenuated IL-2 mutein	Attenuates the binding to IL-2R, enabling avidity-driven activity on targeted cells

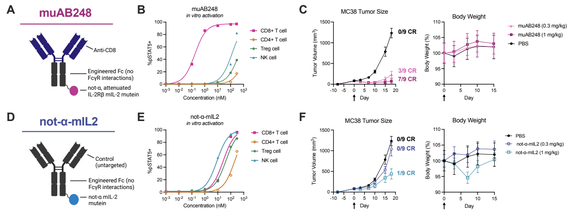
Figure 3: AB248 drives CD8⁺ T cell-selective IL-2 signaling with over 500-fold preference



The in vitro activation profiles on human peripheral blood immune cells of IL-2, a not-α IL-2, and AB248 are shown above, as assessed by pSTAT5 staining following in vitro stimulation. (n = 10 donors)

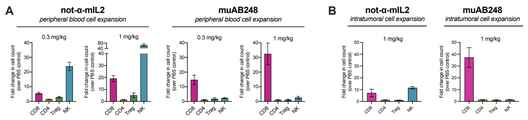
Results with muAB248 in Mice

Figure 4: AB248's murine surrogate drives potent anti-tumor activity without body weight loss in mice, unlike a representative not-α IL-2



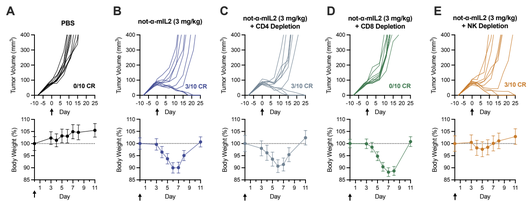
Structure of murine molecules (A, D) and in vitro pSTAT5 activity (B, E). MC38 tumor-bearing mice were treated with the indicated doses intravenously once 8 days following tumor injection; shown are tumor volume and body weight (C, F).

Figure 5: muAB248 demonstrates strong and selective CD8⁺ T cell expansion in blood and tumors, while not-α IL-2 shows an NK cell bias



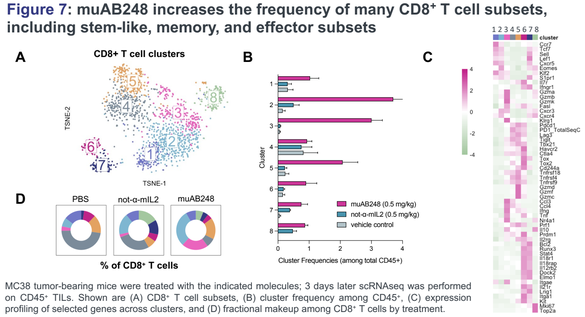
Naïve mice (A) or MC38 tumor-bearing mice (B) were treated i.v. with the indicated molecules and peripheral blood (A) or tumors (B) were analyzed for immune cell counts via flow cytometry.

Figure 6: Antitumor activity of a representative not-α IL-2 depends on CD8⁺ T cells and not NK cells; NK cells drive toxicity-induced body weight loss



MC38 tumor-bearing mice were treated i.v. with a representative not-α IL-2 (not-α-mIL2) at 3 mg/kg. In some groups, mice were depleted of the indicated cell types beginning 2 days prior to therapy and continuing twice weekly thereafter.

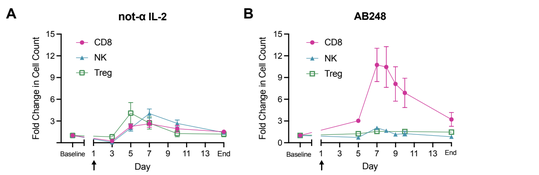
Figure 7: muAB248 increases the frequency of many CD8⁺ T cell subsets, including stem-like, memory, and effector subsets



MC38 tumor-bearing mice were treated with the indicated molecules; 3 days later scRNAseq was performed on CD45⁺ TILs. Shown are (A) CD8⁺ T cell subsets, (B) cluster frequency among CD45⁺, (C) expression profiling of selected genes across clusters, and (D) fractional makeup among CD8⁺ T cells by treatment.

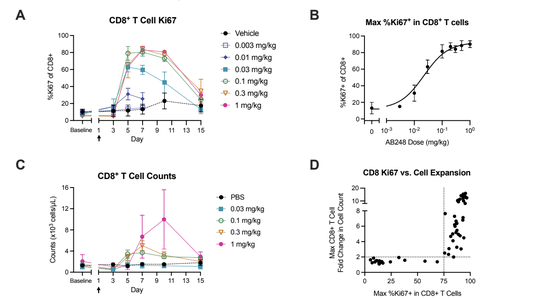
Results with AB248 in Non-Human Primates

Figure 8: AB248 drives strong and selective CD8⁺ T cell expansion in non-human primates



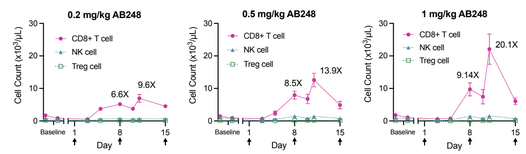
Cynomolgus monkeys were dosed with 0.5 mg/kg of a representative not-α IL-2 or AB248 i.v. on day 1 and immune cell counts were assessed in peripheral blood following treatment. Shown is fold expansion in absolute cell count of CD8⁺ T cells, NK cells, and Tregs over baseline for a representative not-α IL-2 (A) or AB248 (B).

Figure 9: AB248 demonstrates robust, dose-dependent pharmacology on CD8⁺ T cells in non-human primates



Cynomolgus monkeys were dosed i.v. with AB248 at the indicated doses and CD8⁺ T cell counts and Ki67 expression in CD8⁺ T cells was assessed. Panels (B) and (D) represent aggregated data after a single dose of AB248 across multiple studies.

Figure 10: AB248 safely and selectively expands CD8⁺ T cells over 20-fold in non-human primates

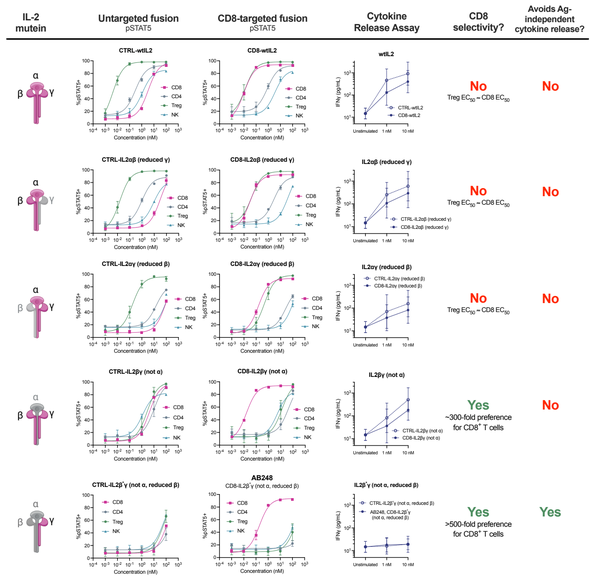


Dose Level	CD8+ T cell Expansion	Adverse Clin. Path. Findings	Adverse Histopath. Findings	Comments
vehicle	1.4X	none	none	Soft stool (1 animal)
0.2 mg/kg	**9.6X**	none	none	No clinical observations
0.5 mg/kg	**13.9X**	none	none	Generally well tolerated, with occasional transient soft stool or diarrhea, predominantly following the first dose
1 mg/kg	**20.1X**	none	none	Generally well tolerated, with transient diarrhea and occasional vomiting, predominantly following the first dose

In a GLP study, cynomolgus monkeys were dosed i.v. with AB248 at the indicated doses weekly for one month. Pharmacodynamics were assessed in peripheral blood following the first two doses. AB248 was generally well tolerated, without adverse histopathology or clinical pathology findings at any dose level, including no evidence of vascular leak syndrome, hypotension, fever, cytokine release syndrome, eosinophilia, or liver toxicity.

Results with AB248 on Human Cells

Figure 11: Proper IL-2 mutein design is essential for CD8⁺ T cell selectivity and avoidance of antigen-independent cytokine release



Untargeted IL-2 muteins (Fc fusions) or anti-CD8 antibody fusions were generated, and pSTAT5 assays or cytokine release assays were run on human peripheral blood cells. CD8⁺ T cell selectivity of CD8-targeted fusions and propensity for antigen-independent cytokine release was assessed.

Conclusions

- **AB248 is a CD8⁺ T cell-selective IL-2 designed for improved efficacy and safety**
  - *Cis*-targeting enables over 500-fold selectivity for CD8⁺ T cells over other cell types
- **In mouse models, restricting the activity of IL-2 to CD8⁺ T cells improves anti-tumor activity and tolerability as compared to an untargeted not-α IL-2**
  - CD8-selectivity allows muAB248 to maximize pharmacology on CD8⁺ T cells and avoid the large IL-2R⁺ sink, improving anti-tumor activity
  - Selectivity for CD8⁺ T cells also avoids NK cell driven toxicity and Treg-mediated immunosuppression
- **High dimensional analysis of mouse tumor immune infiltrate after treatment demonstrates an increase in many CD8⁺ T cell subclusters by muAB248, distinct from not-α IL-2**
  - Increases observed in stem-like, effector, and memory CD8⁺ T cell subsets
- **In non-human primates, AB248 exhibits a differentiated pharmacodynamic profile, selectively expanding CD8⁺ T cells by over 20-fold**
  - Potent pharmacology on CD8⁺ T cells was observed without any adverse histopathology or clinical pathology findings
  - AB248 was tolerated at doses that exceed mouse efficacious doses
- **Cytokine release assays with human PBMCs show low propensity for inflammatory cytokine production with AB248 compared to a variety of untargeted and CD8-targeted IL-2 mutein variants**
  - Proper mutein design enables AB248 to achieve both CD8⁺ T cell selectivity as well as low cytokine release assay signal
- **Collectively, these data support the development of AB248 as an investigational therapy for the treatment of solid tumors**

Methods

- The human pSTAT5 assay was performed by incubating cells with the indicated concentrations of cytokine molecules for 25 minutes. Cells were placed on ice, surface stained, washed, and then fixed. Cells were permeabilized, stained intracellularly, and run on the flow cytometer.
- Tumor studies were performed by injecting 1.5x10⁶ MC38 cells subcutaneously on the flank of C57BL6J mice. After 8 days, mice were randomized to receive the indicated treatments, and tumor volume and body weight were tracked over time. In depletions studies, 200 µg of anti-NK1.1 (clone PK136), 350 µg of anti-CD8 (clone 53-5.8), or 250 µg of anti-CD4 (clone GK1.5) were injected i.p. 2 days prior to treatment, on treatment day, and twice weekly thereafter.
- The single cell RNA sequencing study was performed by injecting MC38 cells subcutaneously of C57BL6J mice. After 14 days, mice were randomized to receive the indicated treatment. Tumors were harvested on day 3 post treatment and viable CD45⁺ cells were sorted for sequencing. Single-cell emulsions were obtained using the 10X Genomics Chromium X Controller and libraries were prepared using the Next GEM Single Cell 5' HT v2 kit with feature barcoding and TCR amplification. To identify CD8⁺ sub-populations, principal component analysis was performed on highly variable genes and the first 50 principal components were used for nearest neighbor calculations and Louvain clustering. Two-dimensional tSNE visualization of CD8⁺ cells was also based on PCA of highly variable genes.
- Cynomolgus monkeys were dosed with the indicated dose of AB248 intravenously and immune cells were analyzed in peripheral blood following treatment. In the GLP study, comprehensive toxicity assessment was undertaken, including clinical pathology and histopathology.
- To perform cytokine release assays, PBMCs were stimulated for 24 hours in the presence of the indicated cytokine fusion molecules. Supernatant cytokine production was measured by MSD.

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