Selective activation of CD8⁺ T cells by a CD8-targeted IL-2 results in enhanced anti-tumor efficacy and safety

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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 clinical potential. Insights into the role of IL2Rα in the pathogenesis of VLS sparked the development of second-generation IL-2 molecules referred to as "not-α" IL-2s, which signal through the IL2Rβγ receptor. Early clinical data from several "not α" IL2Rβγ 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 both regulatory T cells (~2X) and NK cells (~7-12X) while eliciting only modest CD8⁺ T cell expansion (~3X) in patients.[3-5]

NK cells have been shown to drive the toxicity of an IL2Rβγ 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 efficacy of many IL-2 and IL-15-based therapies, while CD8⁺ T cells have been shown to be critical for efficacy.[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 the development of a CD8-targeted IL2Rβγ agonist and demonstrate potent anti-tumor efficacy with an improved therapeutic index compared to untargeted IL-2 pathway agonists.

Overview of *cis*-targeting

To design molecules with CD8-restricted IL-2 signaling, we employed an approach called *cis*-targeting (**Figure 1**). To generate an optimal *cis*-targeted cytokine, first the potency of the cytokine must be reduced to decrease signaling to its cognate receptor. Then activity is selectively rescued via avidity provided by a targeting domain. This domain recognizes an antigen expressed on the surface of the desired cell type. (**Figure 2**)

Figure 1: Overview of *cis*-targeting

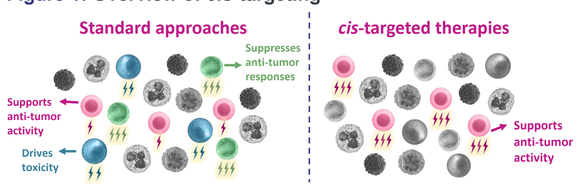
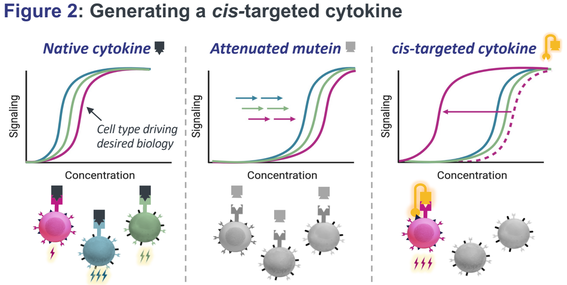


Figure 2: Generating a *cis*-targeted cytokine

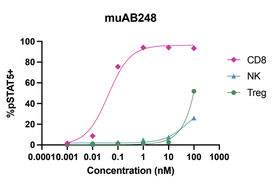


Methods

- Mouse phospho-STAT5 (pSTAT5) assays were performed by incubating the indicated concentrations of cytokine molecules for 30 minutes with single cell suspensions derived from mouse splenocytes, fixed using 4% PFA, washed, permeabilized with MeOH, and stained for flow cytometry.
- Tumor efficacy studies were performed by injecting $0.5x10^6$ B16F10 or $1.5x10^6$ MC-38 cells subcutaneously on the right 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) was injected intraperitoneally (i.p.) 2 days prior to treatment or 350 µg of anti-CD8 (clone 53-5.8) was injected i.p. 3 days and 1 day prior to treatment, and weekly thereafter.
- In murine pharmacodynamic studies, tumors and blood were harvested 5 days after treatment and processed for flow cytometry.
- For cynomolgus monkey studies, CD8-targeted IL-2 was dosed intravenously and peripheral blood was taken at the indicated time points for hematology and flow cytometry assessment.
- Human pSTAT5 assays were 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 then permeabilized with MeOH, stained intracellularly, and run on the flow cytometer.

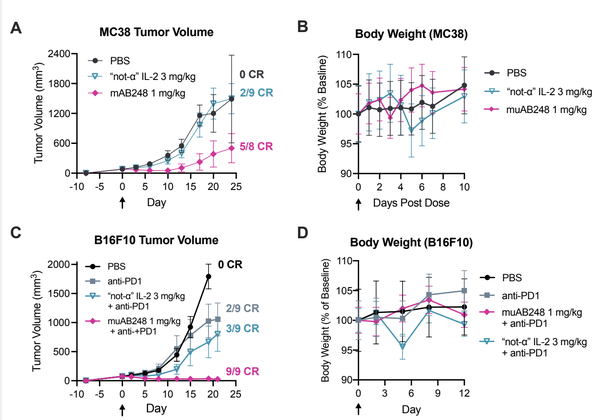
Results

Figure 3: A CD8-targeted IL-2 molecule achieves selective signaling on murine CD8⁺ T cells



A CD8-selective IL-2 for murine CD8⁺ T cells, muAB248, was generated as described in **Figure 2**. The in vitro selectivity profile of muAB248 on murine splenocytes is shown above, as assessed by phospho-STAT5 (pSTAT5) staining following in vitro stimulation.

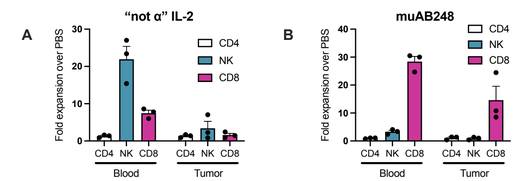
Figure 4: muAB248 drives superior anti-tumor activity compared to "not α" IL-2 as monotherapy or in combination with anti-PD1; no body weight loss observed with muAB248



B16F10 or MC-38 tumors were established in C57BL6 mice for 8 days and then treated intravenously with the indicated therapies. Tumor volumes (A, C) and body weights (B, D) were recorded over time.

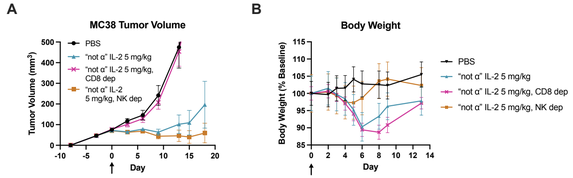
Results

Figure 5: muAB248 drives selective expansion of CD8⁺ T cells in the periphery and tumor, while a "not α" IL-2 largely drives NK cell expansion



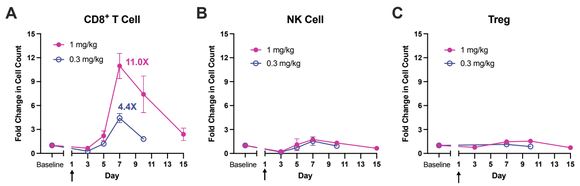
B16F10 bearing C57BL6 mice were dosed subcutaneously with 1 mg/kg of either muAB248 or control "not-α" IL-2 alongside anti-PD1. Peripheral blood and tumors were taken 5 days after treatment, and immune cell frequencies were quantified using flow cytometry. Plotted here is the fold change in absolute cell count over PBS treated mice for "not-α" IL-2 (A) and muAB248 (B).

Figure 6: Body weight loss induced by "not α" IL-2 depends on NK cells, but efficacy depends on CD8⁺ T cells



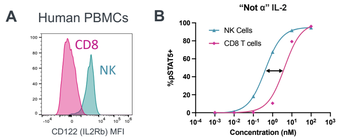
MC-38 bearing C57BL6/J mice were depleted of NK cells or CD8⁺ T cells prior to intravenous dosing with 5 mg/kg of "not-α" IL-2. Tumor volume (A) and body weights (B) were measured over time.

Figure 7: CD8-targeted IL-2 potently and selectively expands CD8⁺ T cells in cynomolgus monkeys



Cynomolgus monkeys were dosed intravenously with 0.3 or 1 mg/kg of a CD8-targeted IL-2 tool molecule (cyAB248). Absolute counts of CD8⁺ T cells, NK cells, and Tregs were measured using flow cytometry and hematology (n=2 per dose level per molecule).

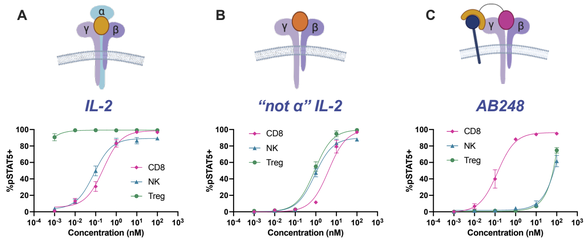
Figure 8: NK cells are disproportionately activated by "not-α" IL-2



Human NK cells express higher levels of IL-2Rβ than CD8⁺ T cells, in agreement with published literature[10] (A). This translates to an increased potency on NK cells over to CD8⁺ T cells as assessed by a pSTAT5 assay on human cells for a representative "not-α" IL-2 (B).

Results

Figure 9: AB248 is a CD8⁺ T cell selective IL-2



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.

Conclusions

- ***Cis*-targeting is an approach that allows for selective signaling on chosen cell types.**
- **Restricting the activity of IL-2 to CD8⁺ T cells results in improved anti-tumor efficacy and therapeutic index as compared to an un-targeted "not α" IL-2 comparator.**
  - A CD8-selective IL-2 molecule (muAB248) demonstrates potent anti-tumor activity, driving a majority of complete responses in established MC-38 tumors with a single dose and outperforming a "not α" IL-2 comparator.
  - muAB248 shows marked synergy with anti-PD1 against established B16F10 tumors, resulting in robust tumor control in all mice following a single dose.
  - No body weight loss is seen in mice at efficacious doses of muAB248.
  - NK cells drive toxicity-induced body weight loss in mice following treatment with a "not α" IL-2, but are dispensable for efficacy. In contrast, CD8⁺ T cells are essential for efficacy and do not contribute to body weight loss.
- **CD8-selective IL-2 safely expands CD8+ T cells in cynomolgus monkeys without significant pharmacodynamic effects on other IL2Rβγ-expressing cell types.**
- **AB248 is a CD8-targeted IL-2 molecule in development for the treatment of solid tumors.**

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