

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 IL-2R α 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 IL-2R $\beta\gamma$ receptor. Early clinical data from several “not α ” IL-2R $\beta\gamma$ 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 potentially expand NK cells (~7-13X) while eliciting only modest CD8⁺ T cell expansion (~3X) in patients.³⁻⁵

NK cells have been shown to drive the toxicity of an IL-2R $\beta\gamma$ 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 AB248, a CD8-targeted IL-2R $\beta\gamma$ agonist, and demonstrate an improved profile compared to untargeted IL-2 pathway agonists.

Overview of *cis*-targeting

To design AB248, 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

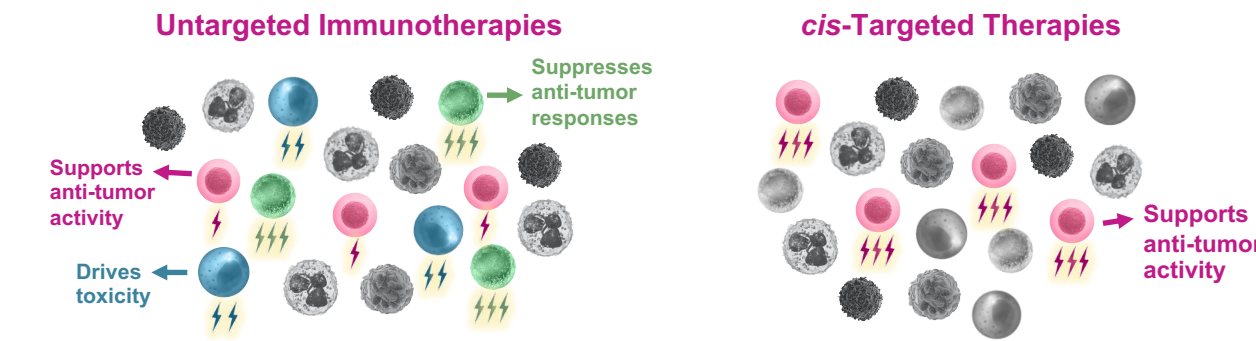
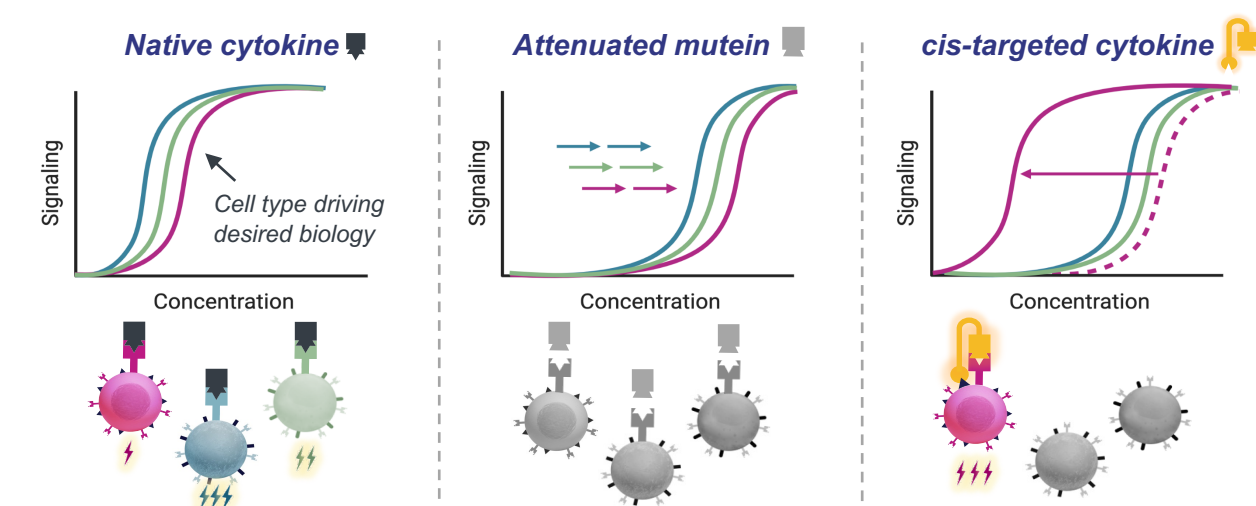


Figure 2: Generating a *cis*-targeted cytokine



Methods

- Binding to human PBMCs was assessed following a 2 hour incubation at 4°C via flow cytometry.
- 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 with MeOH, stained intracellularly, and run on the flow cytometer.
- To perform immunotoxicity assays, PBMCs were stimulated for 24 hours in the presence of IL-2, “not α ” IL-2, or AB248. For some conditions, CD56⁺ NK cells were depleted using a commercially available depletion kit. Supernatant cytokine production was measured by MSD.
- For TCR stimulation studies, human CD8⁺ T cells were cultured in the presence of AB248 either on anti-CD3 coated plates or in absence of anti-CD3 stimulation. After 48 hours, supernatant was isolated and analyzed via MSD.
- For gene expression analysis, human CD8⁺ T cells were sorted into naive, central memory, effector memory and effector subsets, then cultured in the presence of IL-2 or AB248. After 24 hours, cells were analyzed via RNAseq.
- Cynomolgus monkeys were dosed with 0.5 mg/kg of “not α ” IL-2 (n=2) or AB248 (n=3) intravenously and immune cell counts were assessed in peripheral blood following treatment.

Results

Figure 3: Overview of AB248’s molecular design

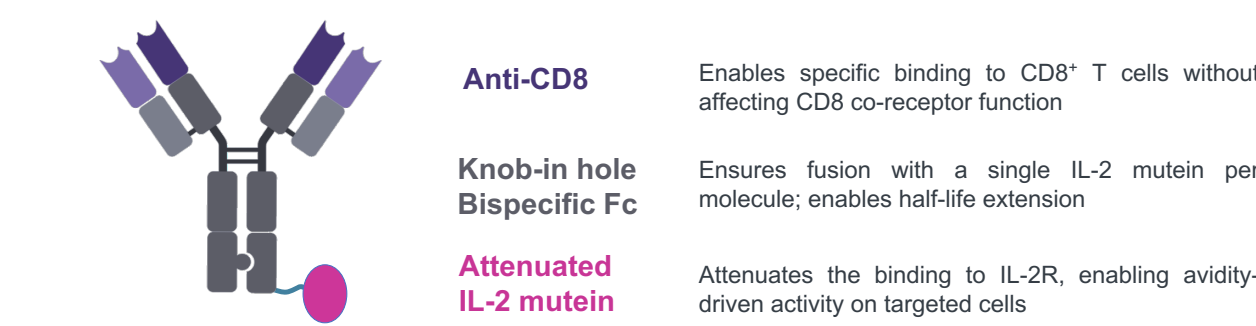
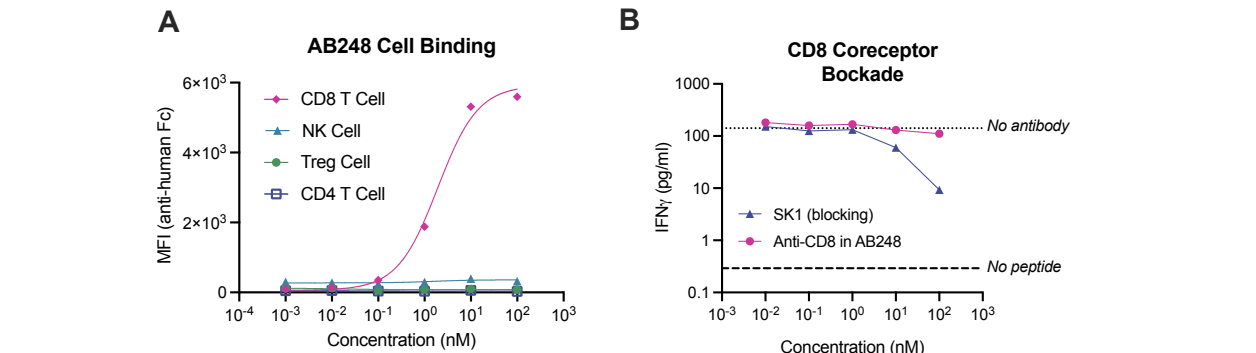
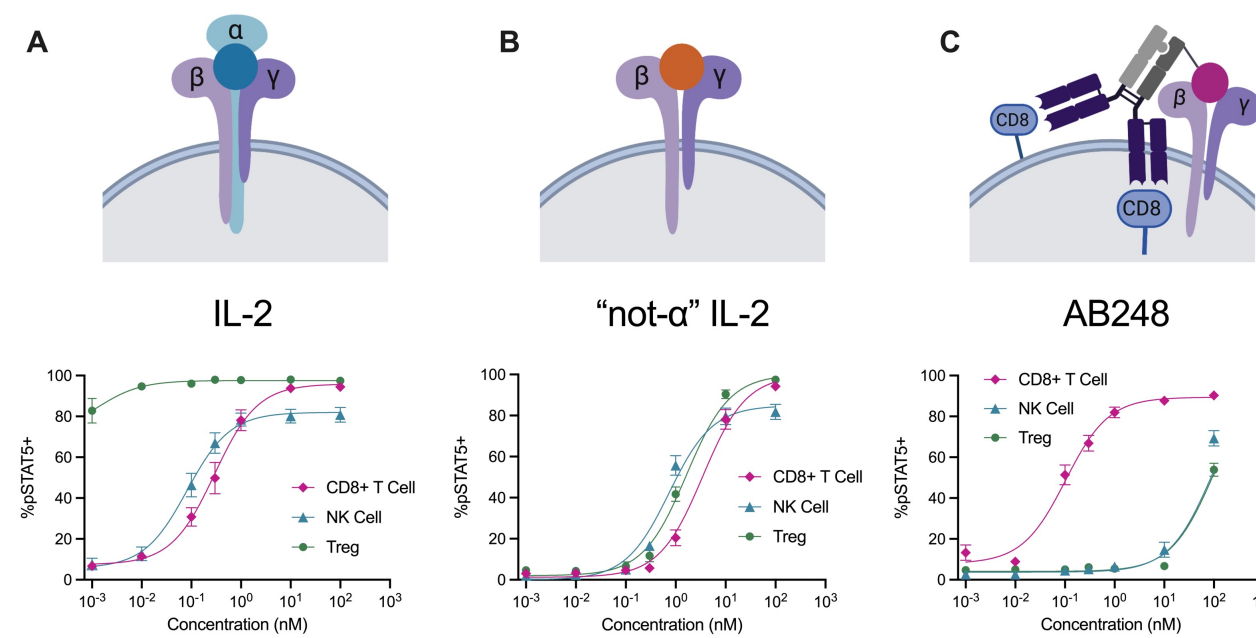


Figure 4: AB248 binds selectively to CD8⁺ T cells; binding does not interfere with CD8 co-receptor function



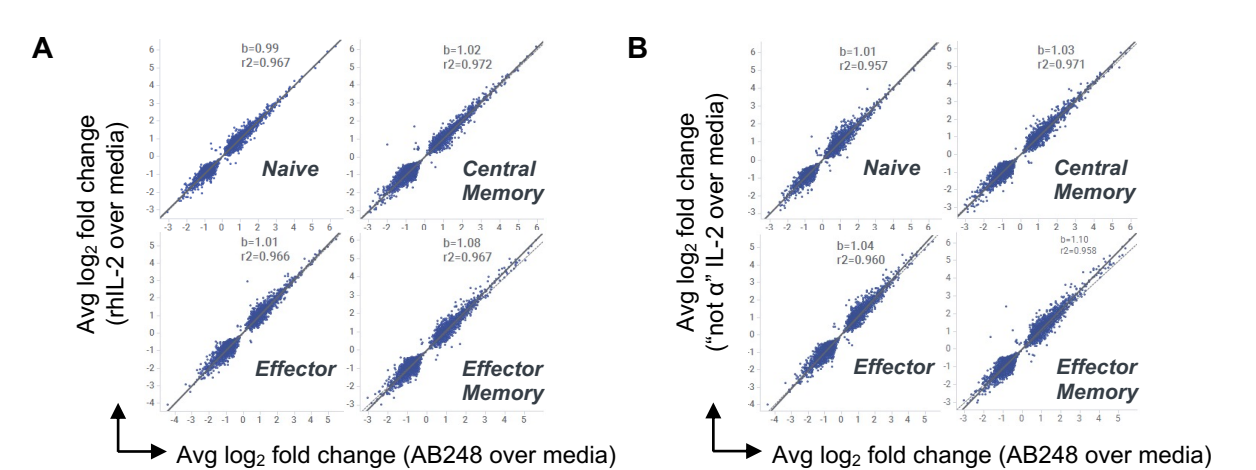
Binding of AB248 to human PBMCs was assessed by flow cytometry. Shown is binding of AB248 to CD8⁺ T, NK cells, Treg and CD4⁺ T cells (A). CD8⁺ T cells recognizing CMV pp65 were cultured in the presence of pp65 peptide-loaded targets. Co-culture was performed with the indicated concentrations of antibodies for 24 hours followed by an IFN γ ELISA on supernatant to quantify antigen recognition (B).

Figure 5: AB248 drives CD8⁺ T cell-selective IL-2 signaling with approximately 1000-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)

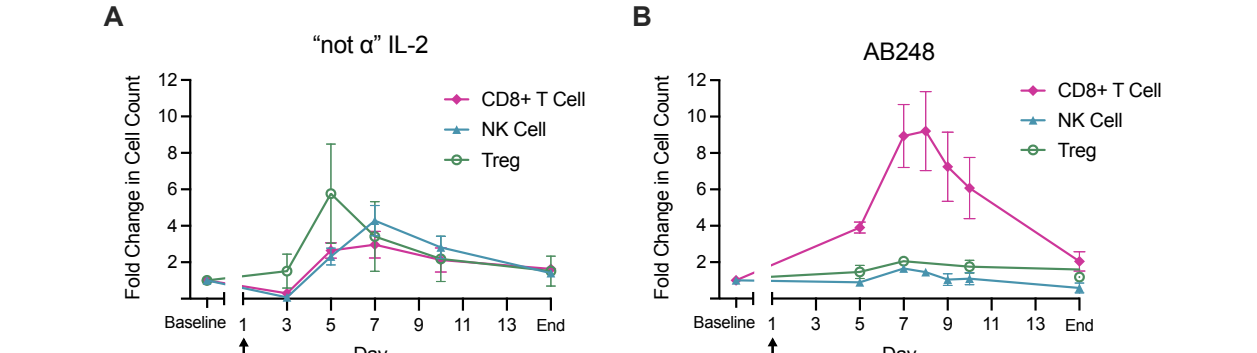
Figure 6: AB248 induces the expected IL-2 gene signature with high fidelity on human CD8⁺ T cells



Human CD8⁺ T cells from 3 donors were sorted into naive, central memory, effector memory and effector subsets, and then cultured in the presence of IL-2, “not α ” IL-2, or AB248 at approximately EC99. After 24 hours, cells were analyzed via RNAseq. Plotted is gene expression over media control, with each differentially expressed gene represented by a point.

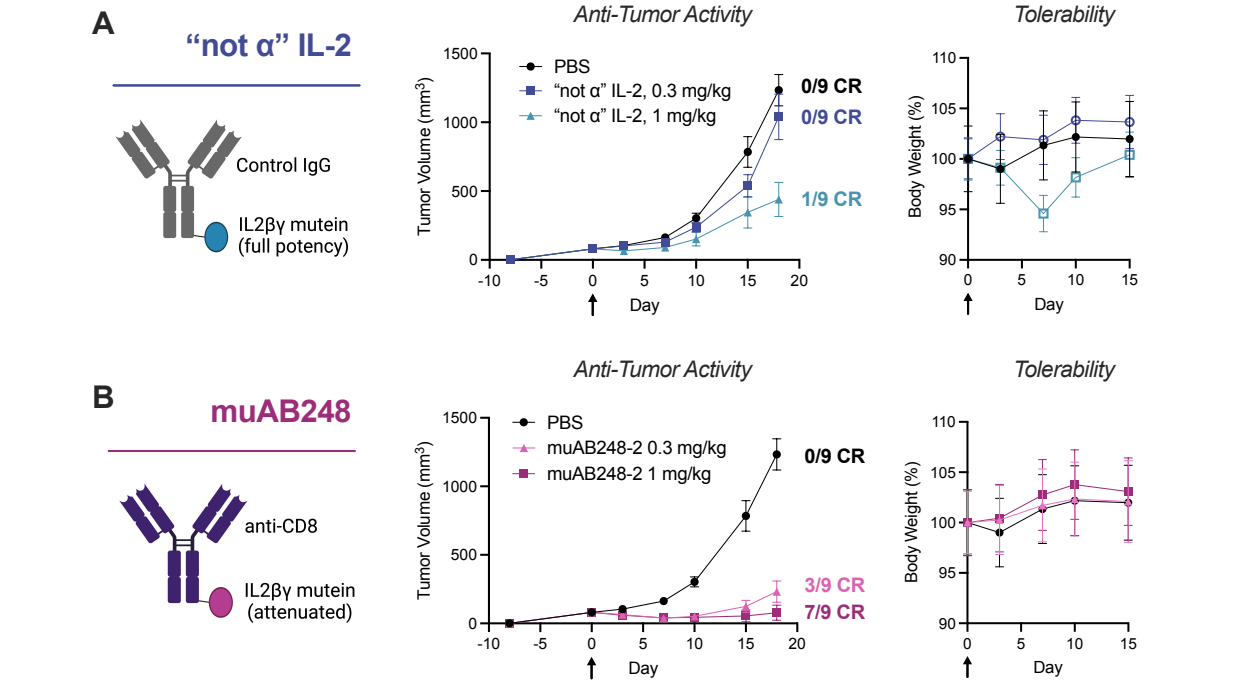
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Figure 7: AB248 drives strong and selective CD8⁺ T cell expansion in non-human primates



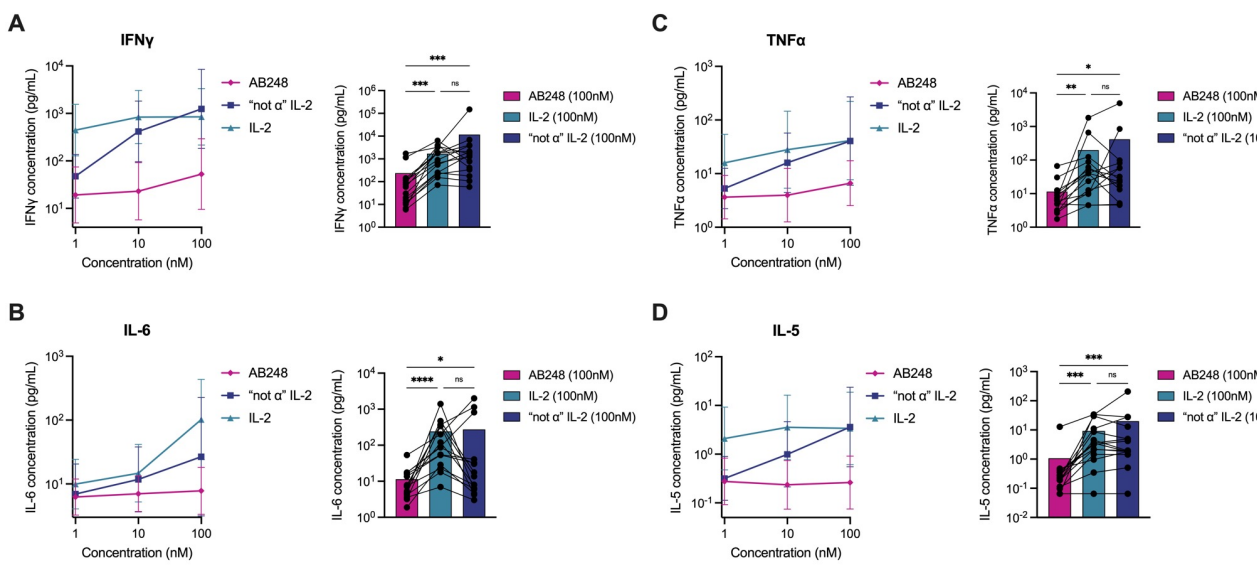
Cynomolgus monkeys were dosed with 0.5 mg/kg of “not α ” IL-2 or AB248 intravenously on day 1 and immune cell counts were assessed in peripheral blood following treatment. Shown is fold expansion in absolute cell count of CD8⁺ T cells, NK cells, and Tregs over baseline for “not α ” IL-2 (A) or AB248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

Figure 8: CD8-targeted IL-2 demonstrates superior efficacy and tolerability compared to a representative “not α ” IL-2 in MC38 model



C57BL/6 mice were injected with 1.5×10⁶ MC38 cells and treated 8 days later once with 0.3 or 1 mg/kg of “not α ” IL-2 (A), or muAB248 (B). Shown are tumor volume and body weight over time, with the number of complete responses (CR) designated for each treatment.

Figure 10: AB248 demonstrates significantly lower cytokine release from human PBMCs compared to IL-2 and “not α ” IL-2



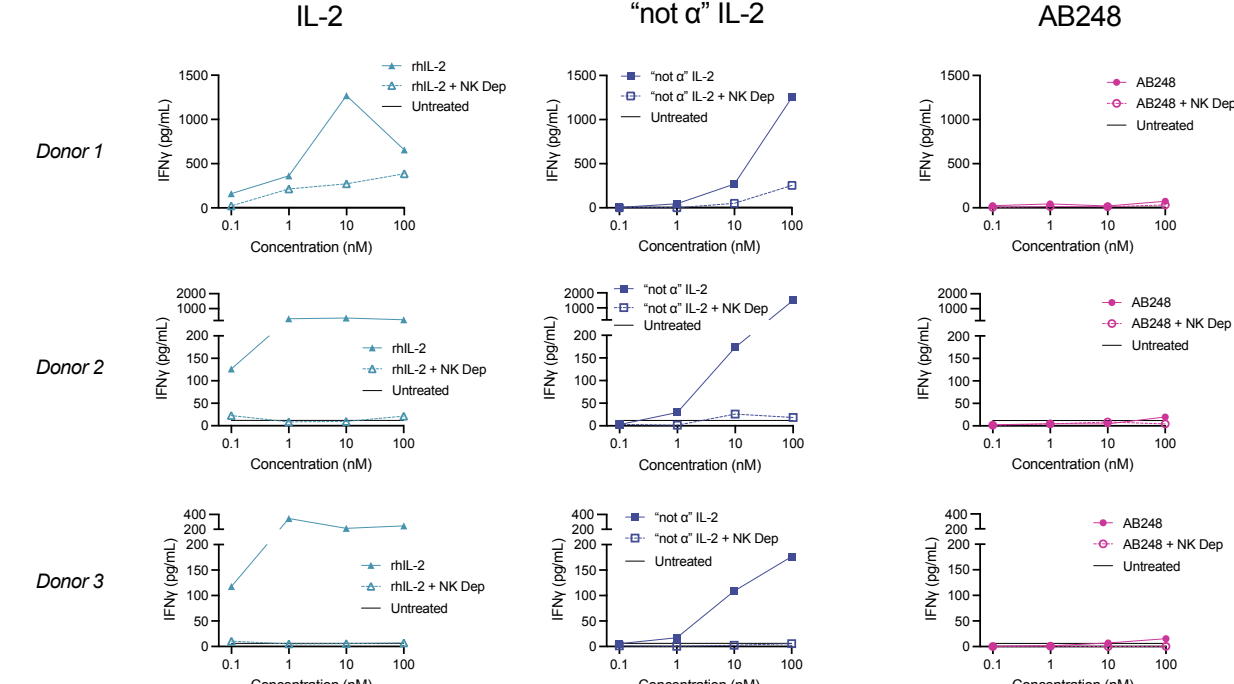
Human PBMCs were cultured for 24 hours in the presence of AB248, IL-2, or “not α ” at the indicated concentrations, and supernatant was analyzed for cytokines via MSD. Shown are IFN γ (A), TNF α (B), IL-6 (C), and IL-5 (D). M. The 100 nM values for each donor are also represented as bar plots with lines connecting individual donors. (n=15)

References

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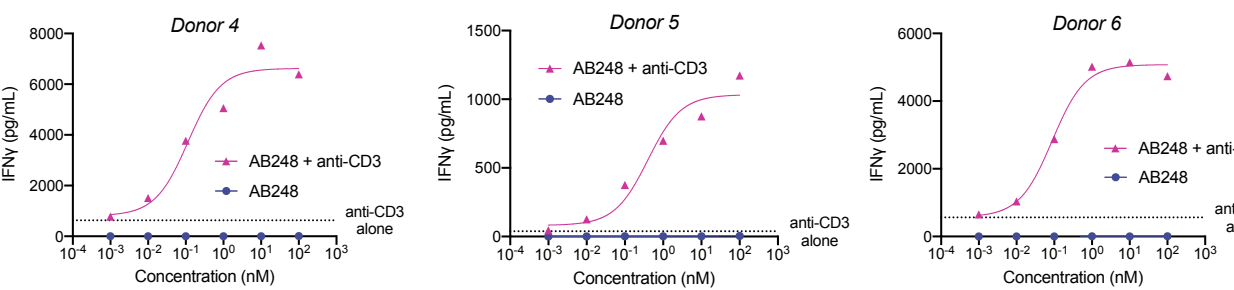
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Figure 11: NK cells mediate IFN γ production from human PBMCs after IL-2 and “not α ” IL-2 exposure



Human PBMCs from 3 donors were stimulated as described in Figure 10 either with or without NK cell depletion via CD56 prior to culture initiation. Shown is IFN γ in supernatant for each donor.

Figure 12: AB248 alone does not drive IFN γ production by CD8⁺ T cells; AB248 augments IFN γ production by CD8⁺ T cells also receiving T cell receptor (TCR) activation



Human CD8⁺ T cells from 3 donors were isolated and stimulated in the presence or absence of anti-CD3 with the indicated concentration of AB248. Supernatant was assessed for IFN γ .

Conclusions

- AB248 is a human CD8-selective IL-2 designed for improved efficacy and safety
 - *Cis*-targeting approach enables approximately 1000-fold selectivity for CD8⁺ T cells over other cell types
- AB248 binds selectively to CD8⁺ T cells by targeting CD8, and binding does not alter CD8 co-receptor function
- The expected IL-2 gene signature is observed following stimulation of CD8⁺ T cells in vitro with AB248
- AB248 shows strong and selective CD8⁺ T cell expansion in non-human primates
- In mouse models, restricting the activity of IL-2 to CD8⁺ T cells improves anti-tumor efficacy and tolerability as compared to an untargeted “not α ” IL-2.
- Cytokine release assays on human PBMCs show low propensity for inflammatory cytokine production with AB248 compared to IL-2 and “not α ” IL-2
 - NK cells drive IFN γ production following IL-2 and “not α ” IL-2 treatment
 - AB248 avoids NK-cell driven, non-specific cytokine secretion by human PBMCs
- Unlike NK cells, CD8⁺ T cells do not produce IFN γ following an IL-2 signal alone; T cell receptor activation is also required
 - AB248 augments IFN γ in CD8⁺ T cells also receiving a TCR signal