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 IL2R in the pathogenesis of VLS sparked the development of second-generation IL-2 molecules referred to as "not" IL-2s, which signal through the IL2Rβγ receptor. Early clinical data from several "not" IL-2R agonists in melanoma and renal cell carcinoma suggest lower objective response rates compared to IL2. However, clinical data from several "not" IL-2 agonists in melanoma and renal cell carcinoma suggest lower objective response rates compared to IL2.

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

Methods

- Mouse phospho-STAT5 (pSTAT5) assays were performed by incubating the indicated concentrations of cytokine molecules for 20 minutes with single cell suspensions derived from mouse spleenocytes, fixed using 4% PFA, permeabilized with MOICh, and stained for pSTAT5.
- Cis-acting efficacy studies were performed by injecting 5 × 10⁶ B16F10 or 1 x 10⁶ MC-38 cells subcutaneously on the right flank of C57BL6J mice. After 5 days, mice were randomized to treatment groups and dosed subcutaneously with cis-acting cytokine. 200 μg of mAB248 (1 mg/kg) was injected intraperitoneally (i.p.) twice a day to treatment and placebo groups on days 4 and 5 of tumor growth prior to treatment, and weekly thereafter.
- Intravenous pharmacokinetic studies: tumors and blood were harvested 5 days after treatment and processed for flow cytometry.
- For cytokine-driven studies, CD8-targeted IL-2 was dosed intravenously and peripheral blood was taken at the indicated time points for humoral and cytokine biomarker analysis.
- Human PBMCs assays were used to measure the concentration of cytokine molecules in 24 hours. Cells were plated on six, surface stained, washed, and then fixed, permeabilized with BD Cytofix, stained intracellularly, and run on the flow cytometer.

Figure 2: Generating a cis-targeted cytokine

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

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

Results

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

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

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

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 untargeted "not" IL-2-α IL-2 comparator.
- Cis-acting 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" IL-2-α IL-2 comparator.
- muAB248 shows marked synergy with anti-PD1 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 cell toxicity induced body weight loss in mice following treatment with a "not" IL-2, but are dispensable for efficacy. In contrast, CD8+ T cells are essential for efficacy and do not contribute to body weight loss.
- Cis-acting IL-2 safely expands CD8+ T cells in cynomolgous monkeys without significant pharmacodynamic effects on other IL2R-targeting cell types.
- AB248 is a cis-targeted IL-2 molecule in development for the treatment of solid tumors.

References