AB821 is a CD8+ T cell selective IL-21 with enhanced bioavailability that reduces CD8+ T cell exhaustion to induce potent antitumor activity

#1048

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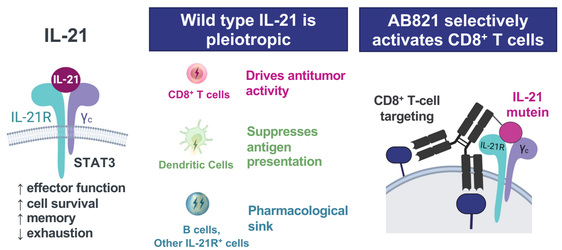
Introduction

Interleukin 21 (IL-21) is a pleiotropic cytokine that activates multiple lymphoid and myeloid immune cell subsets. IL-21 can have beneficial effects on antitumor immune responses due to its ability to activate Signal Transducer and Activator of Transcription 3 (STAT3) in CD8+ T cells and promote cytotoxicity, memory cell differentiation, and survival[1-5] and is therefore distinct from the predominantly proliferative effects of activating STAT5 signaling by IL-2. However, IL-21 can also induce immunosuppressive effects, such as suppression of antigen presentation in myeloid cells, which may directly oppose its antitumor effects on CD8+ T cells[6]. Although recombinant IL-21 did show monotherapy activity in the clinic[7-8] its clinical utility was limited, likely by its low bioavailability, dose-limiting toxicities and its potential immunosuppressive effects[8].

We hypothesized that maximizing the activity of IL-21 on CD8+ T cells while minimizing effects on non-CD8+ cells would enhance efficacy and improve tolerability. Here we describe AB821, a cis-targeted IL-21 fusion protein that selectively activates CD8+ T cells. We provide mechanistic insight into the function of AB821, demonstrating the ability of AB821 to restore cytotoxic function to exhausted CD8+ T cells, which results in potent anti-tumor activity, even in anti-PD-1 refractory mouse tumor models.

Overview of cis-targeting for IL-21

Figure 1: Overview of AB821, a CD8-targeted IL-21



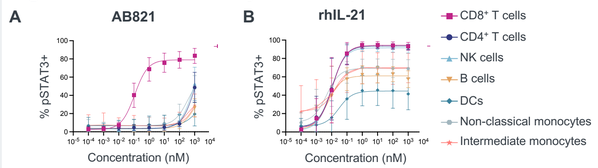
Within CD8+ T cells, IL-21R signaling via phosphorylated STAT3 (pSTAT3) can promote effector function, cell survival, and memory differentiation, as well as reduce exhaustion. However, other IL-21 responsive cell types may limit IL-21-based therapeutics from optimally activating CD8+ T cells by acting as a sink or even by mediating suppressive function, such as inhibiting dendritic cell function. AB821 is a fusion protein consisting of a CD8-targeting antibody fused to a charge and potency reduced IL-21. The molecular design of AB821 improves half-life and bioavailability while restricting IL-21 activity to the intended cellular target, CD8+ T cells.

Methods

- In vitro pSTAT3 assays were performed by incubating primary human blood leukocytes, human TIL, or mouse splenocytes with the indicated cytokine concentrations. Cells were fixed, washed, permeabilized with methanol, and stained for flow cytometry.
- Mouse tumor studies were performed by injecting tumor cells subcutaneously (SC) on the right flank. After the indicated number of days, mice were randomized to receive the indicated treatments via IV administration. Tumor volume and body weights were tracked over time. For flow cytometry assessment of mouse TIL, tumors were dissociated and single cell suspensions stained for flow cytometry.
- For repeated stimulation of human CD8+ T cells, cells were enriched by negative selection and cultured with 0.5 µg/mL plate-bound anti-CD3 (OKT3) with or without AB821. Supernatants were collected and assessed by MSD and cells were collected and stained for flow cytometry.
- Human TIL studies were performed on dissociated tumor samples. For IL-21R quantification, freshly dissociated samples were stained for IL-21R and receptor quantity was assessed using QuantBrite beads. For in vitro response assays, dissociated TIL were cultured for 3 days in vitro with indicated treatments. Supernatants were collected and assessed by MSD and cells were collected and stained for flow cytometry.

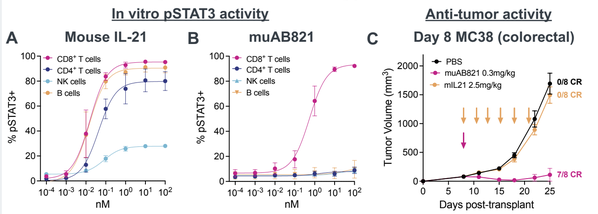
Results: AB821 molecular design and antitumor activity

Figure 2: AB821 selectively activates pSTAT3 in CD8+ T cells



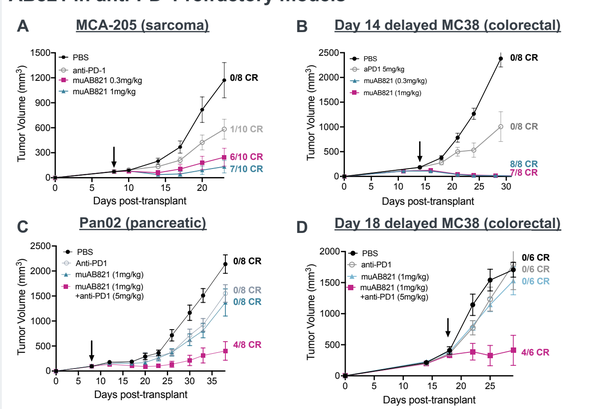
pSTAT3 was measured for (A) recombinant human IL-21 (rhIL-21) and (B) AB821 in human PBMCs. AB821 potently induces pSTAT3 selectively in CD8+ T cells, with minimal effect in other IL-21 responding cell types at <100 nM.

Figure 3: muAB821, the murine surrogate of AB821, has superior antitumor activity compared to wild type mouse IL-21



pSTAT3 was measured for (A) mouse IL-21 (mIL-21) and (B) muAB821 in mouse splenocytes. muAB821 has a comparable activity profile to AB821. (C) muAB821 (single dose, 0.3mg/kg) was superior in clearing MC38 tumors compared to mIL-21 (2.5mg/kg every 2-3 days)

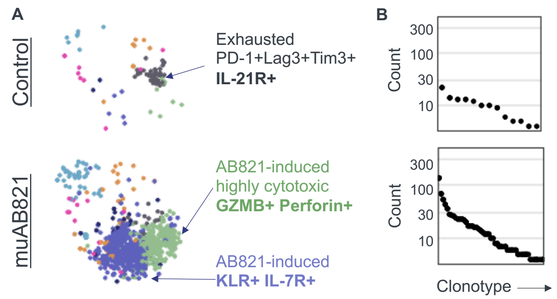
Figure 4: Potent antitumor activity of a mouse surrogate of AB821 in anti-PD-1 refractory models



A murine surrogate of AB821 (muAB821) with comparable charge adjustment and in vitro potency was generated. Antitumor activity was assessed in anti-PD-1 refractory syngeneic tumor models (A) MCA-205, (B) delayed MC38 dosed on day 14 of tumor growth, (C) Pan02, and (D) delayed MC38 dosed on day 18 of tumor growth. muAB821 demonstrated strong single agent activity (A, B) and strong synergy with anti-PD-1 (C,D) in models resistant to anti-PD-1 therapy

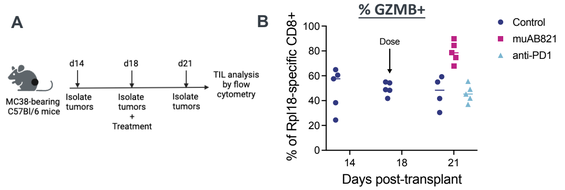
Results: AB821 mechanism of action

Figure 5: The exhausted CD8+ T cell population is replaced by distinct functional CD8+ T cell populations by AB821 treatment



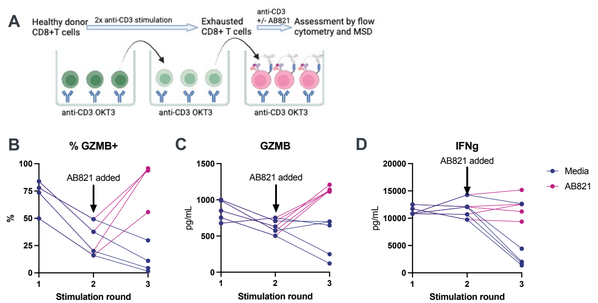
MC38-bearing mice were treated with 0.5mg/kg muAB821 on day 14 of tumor growth. After 3 days, tumors were harvested and analyzed by single cell RNAseq. (A) In PBS treated animals, most CD8+ T cells are exhausted. However, in muAB821 treated animals, two distinct clusters of highly cytotoxic effector cells are observed with a high degree of clonal expansion (C).

Figure 6: AB821 increases expression of GZMB in exhausted CD8+ T cells in a delayed MC38 tumor model



MC38 tumor-antigen-specific (Rpl18) CD8+ T cells were identified by staining with tetramers at timepoints described in (A). (B) muAB821 treatment increased the fraction of GZMB-expressing antigen-specific CD8+ T cells.

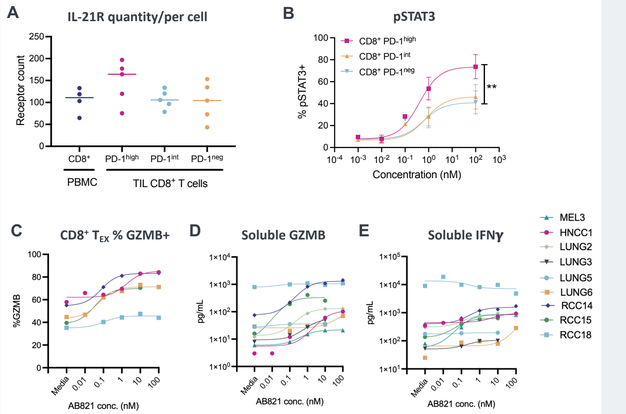
Figure 7: AB821 restores functionality to exhausted human CD8+ T cells



CD8+ T cells were exhausted by repeated anti-CD3 stimulation followed by treatment with AB821 as indicated in (A). AB821 restored the frequency of expression (A) and secretion (B) of GZMB and prevented loss of IFNγ secretion (C) following repeated stimulation. Each line represents an individual donor.

Results: AB821 activity in human TIL

Figure 8: Human TIL CD8+ T_{EX} cells respond to AB821 by increasing expression and secretion of key effector molecules



Human tumor samples were dissociated and assessed by flow cytometry immediately following dissociation (A, B) or after 3 days of culture with indicated treatment conditions (C-E). (A) PD-1^{high} CD8+ T cells express higher quantity of IL-21R and (B) respond more strongly to AB821 based on pSTAT3 levels. (C) Frequency of GZMB expression in exhausted CD8+ T cells, identified as PD-1+CD39+Tim3+, was increased by AB821 treatment. (D, E) AB821 treatment also increased secretion of both GZMB and IFNγ from dissociated human TIL in culture.

Conclusions

- AB821 is a cis-targeted, charge and potency reduced IL-21 that potently and selectively activates CD8+ T cells in vitro and in vivo.
- A murine surrogate of AB821 demonstrates potent single-dose monotherapy activity and synergy with anti-PD-1 in difficult to treat, anti-PD-1 refractory mouse syngeneic tumor models
- Exhausted CD8+ T cells are a primary target cell for AB821 therapy, where AB821 serves to improve functionality by promoting expression and secretion of key effector molecules such as GZMB and IFNγ.

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