AB821 is a CD8+ T-cell selective IL-21 with enhanced bioavailability that mediates potent antitumor activity, cytotoxicity, and expansion of memory CD8+ T cells

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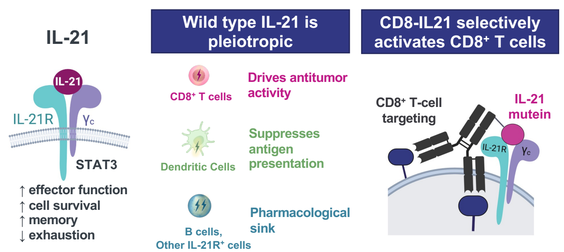
Introduction

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 the development of a cis-targeted IL-21 (AB821) fusion protein that exhibits improved bioavailability, selectively activates CD8+ T cells, increases CD8+ T-cell cytotoxicity and memory, and demonstrates potent antitumor activity and synergy with anti-programmed death protein 1 (PD-1).

Overview of cis-targeting for IL-21

Figure 1: Overview of CD8-targeted IL-21



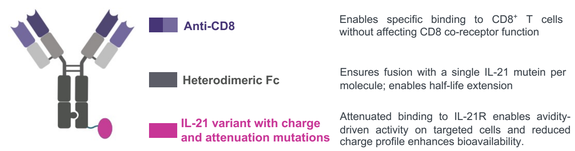
Within CD8+ T cells, IL-21R signaling via phosphorylated STAT3 (pSTAT3) can promote effector function, cell survival, and memory differentiation, as well as reduce exhaustion. However, other IL-21 responsive cell types may limit IL-21-based therapeutics from optimally activating CD8+ T cells by acting as a sink or even by mediating suppressive function, such as inhibiting dendritic cell function. To design molecules with CD8-restricted IL-21 signaling, we employed a cis-targeting approach by first attenuating the affinity of IL-21, and then restoring functionality on CD8+ T cells by fusing the IL-21 mutein to an antibody targeting CD8+ T cells.

Methods

- In vitro pSTAT3 assays were performed by incubating primary human blood leukocytes with the indicated cytokine concentrations. Cells were fixed, washed, permeabilized with methanol, and stained for flow cytometry.
- For activity assays with CD8+ T cells, cells were enriched by negative selection and cultured with indicated molecules with or without 0.5 µg/mL plate-bound anti-CD3 (OKT3). Supernatants were collected and assessed by MSD and cells were collected and stained for flow cytometry.
- Humanized mouse studies were performed by dosing CD34+ stem-cell humanized NSG mice (Jackson Labs) with a single dose of the indicated molecule. Blood was sampled and cells stained for flow cytometry at indicated time points.
- Mouse tumor studies were performed by injecting tumor cells subcutaneously (SC) on the right flank. After 8 days, mice were randomized to receive the indicated treatments via IV administration. Tumor volume or diameter and body weights were tracked over time.
- For non-human primates (NHP) studies, a single dose of the indicated molecule was administered IV. Peripheral blood was taken at the indicated time points for hematology, clinical chemistry, and flow cytometry assessments.

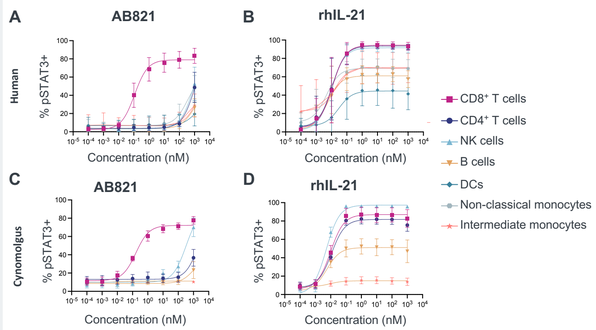
Results: AB821 design and selectivity

Figure 2: AB821: a human CD8-IL21 with improved bioavailability



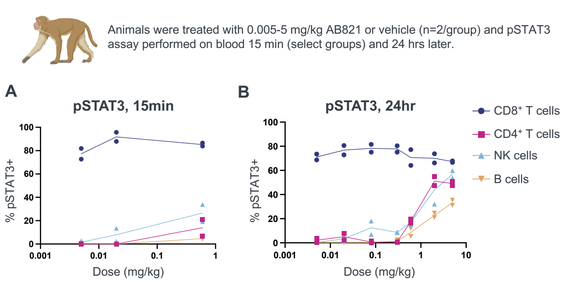
Human IL-21 is highly charged (high pI) which may limit its bioavailability. AB821 is a fusion between an affinity-attenuated and charge-reduced variant of human IL-21 and a CD8-targeting antibody. Charge reduction of IL-21 results in improved biophysical properties and exposure in vivo.

Figure 3: AB821 selectively activates pSTAT3 in CD8+ T cells



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

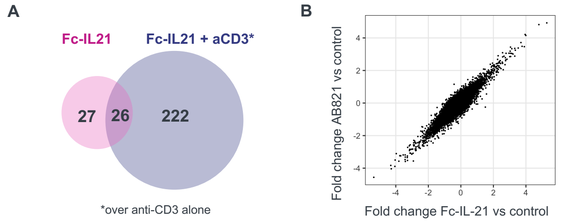
Figure 4: AB821 selectively activates STAT3 in CD8+ T cells in cynomolgus monkeys in vivo



AB821 activity was assessed using in vivo pSTAT3 assay. (A) 15 mins after dosing, 80% of CD8+ T cells are positive for pSTAT3, even at the lowest dose tested, with minimal detection of pSTAT3 in non-CD8+ T cells. (B) 24 hrs later, pSTAT3 signal is retained at all dose levels. At doses >0.6mg/kg, pSTAT3 is detected in non-CD8+ T cells at 24 hours.

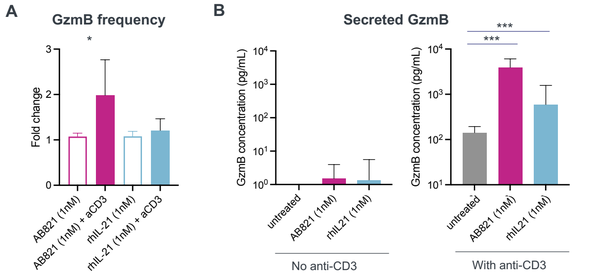
Results: AB821 mechanism of action

Figure 5: IL-21 synergizes with TCR signaling to induce transcriptional changes that are fully reproduced by AB821



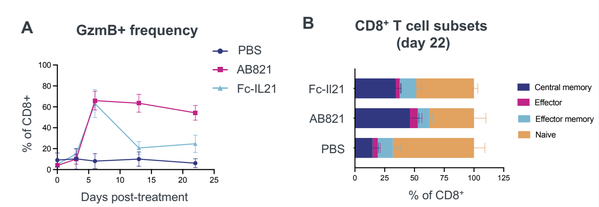
Gene expression was assessed by RNAseq in human CD8+ T cells cultured for 48 hrs (A) or 72 hrs (B) with AB821 or a non-targeted, charge-reduced Fc-IL21 fusion with WT potency (Fc-IL21) (A) Fc-IL21 gene signature is significantly enhanced in combination with anti-CD3 treatment compared to Fc-IL21 alone. (B) Transcriptional profiles of AB821 and Fc-IL21 are highly similar at 72 hours of treatment in combination with anti-CD3

Figure 6: AB821 increases frequency of GzmB+ CD8+ T cells and GzmB secretion in combination with TCR stimulation



CD8+ T cells were stimulated for 72hrs with AB821 or rhIL-21 on standard plates or plates coated with 0.5 µg/mL anti-CD3. (A) Frequency of GzmB+ CD8+ T cells was assessed by flow cytometry and (B) GzmB secretion was assessed by MSD in the supernatant, were both increased by AB821 treatment. *p<0.05, ***p<0.001 by non-parametric t-test.

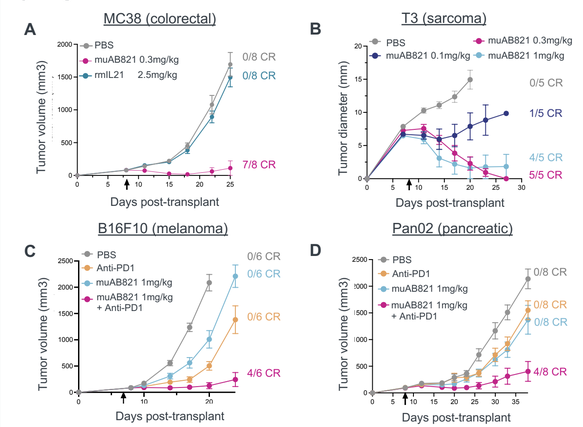
Figure 7: AB821 increases GzmB and T-cell central memory phenotype in a humanized mouse model



AB821 or a non-targeted, charge-reduced Fc-IL21 fusion was administered at 0.3 mg/kg to CD34+ hematopoietic stem cell humanized mice. (A) AB821 induced a sustained increase in the frequency of GzmB+ cells within CD8+ T cells. (B) At Day 22, a shift to increased frequency of central memory was observed in AB821-treated animals.

Results: AB821 antitumor activity

Figure 8: muAB821 demonstrates potent antitumor activity and synergizes with anti-PD1



A murine surrogate of AB821 (muAB821) with comparable charge adjustment and in vitro potency was generated. Antitumor activity was assessed in the syngeneic tumor models (A) MC38 colorectal carcinoma, (B) T3 sarcoma, (C) B16F10 melanoma, and (D) Pan02 pancreatic adenocarcinoma. In generally immunotherapy-responsive models (A-B), muAB821 demonstrated strong single agent activity, with complete responses at 0.3-1 mg/kg. Wild type mouse IL-21 (2.5 mg/kg 2x weekly) showed no single agent activity in the MC38 model. In the anti-PD1-resistant models (C,D), muAB821 demonstrated clear synergy with anti-PD1, whereas minimal activity of both individual agents was observed. No body weight loss was observed in any study.

Conclusions

- AB821 is a cis-targeted, charge adjusted IL-21 that potently and selectively activates CD8+ T cells in vitro and in vivo.
- In human CD8+ T cells, AB821 activity synergizes with TCR stimulation to increase CD8+ T-cell cytotoxicity and support T cell memory.
- A murine surrogate of AB821 demonstrates potent single-dose monotherapy activity down to 0.1 mg/kg in MC38 and T3 tumor models synergizes with anti-PD1 in difficult-to-treat antiPD-1 resistant tumor models

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