Preclinical specificity and activity of CABA-201, a fully human 4-1BB containing CD19 CAR T therapy for treatment-resistant myositis

Background: Idiopathic inflammatory myopathies are a group of rare immune mediated diseases that affect primarily skeletal muscle and can also impact other organs including the skin, lungs, and joints. There is increasing evidence that B cells play a central role in disease pathogenesis, based upon responsiveness to B-cell depletion by antibody-based therapeutics; however, responses are transient due to the incomplete depletion of B-cells in lymphoid tissue. Chimeric antigen receptor (CAR) T-cells are a novel gene-engineered cellular immunotherapy where a synthetic T-cell receptor is expressed to redirect the T-cell to a desired target. Several B-cell targeted CD19/CD20 T-Cell products have led to durable remissions of B-cell malignant diseases; four have been approved, each of which utilizes the murine derived CD19 scFv binding domain FM63. Numerous studies have established the ability of these products to deeply deplete B-cells. An early clinical evaluation of an FM63-4-1BB-CDC2 T-cell product, analogous to one of the approved therapies, in patients with treatment refractory myositis suggest the potential to safely achieve durable drug-free remissions in patients with treatment refractory disease.

Methods: Methods: CABA-201, a fully human 4-1BB-CDC2 containing CD19 CAR T-cell, was generated both from healthy donor apheresis and from myositis patients (dermatomyositis subtype) peripheral blood mononuclear cells (PBMCs) via standard ex vivo expansion using antibody coated beads and terminal transduction. CABA-201 in vitro activity was evaluated in co-culture assays with either CD19+ NALM cells or with patient-matched myositis CD19+ B-cells. Activity was measured by Luminescence assay for cytokine release, cytotoxicity via flow cytometry, or CAR T-cell activation via flow cytometry. In vivo safety was assessed via CAR T-cell co-culture against selected primary human cells and via membrane proteome assay. In vivo studies assessed the function of CABA-201 in an NIS NALM model.

Results: Results: CABA-201 generated from healthy donors showed specific in vitro activity against CD19+: NALM cells. Furthermore, CABA-201 generated from myositis patient PBMCs demonstrated specific in vitro activity against matched B-cells (Figure 1). The fully human CD19 binder CABA-201 had no off-target binding, and CABA-201 did not show any activity against selected non-B-cell primary human cells. Finally, in vivo studies confirmed the safety and activity of CABA-201 in the NIS-NALM model.

Conclusions: Conclusions: Together, these data support the safety and activity of CABA-201 and provide a clinically relevant benchmark for dose related potency in clinical studies.

Abstract

Methods: that

Abstract

AUC

GFP+ count

GFP+ count

Figure 1. Cytotoxicity of CABA-201 towards NALM-19 positive target cells. Effective cells (CABA-201, FM63 CAR, or NTD T cells) were co-incubated with target NALM cells for 120 hours at indicated E:T ratios with 2 different donors. A) Cytotoxicity of ex vivo generated CABA-201 positive NALM cells was measured using an imaging based assay for T cell recognition. B) Representative results from one donor in shown. Effective E:T ratio is based on total T cell number. B) Area under the curve (AUC) is shown as mean ± SD from three graphs shown in A. In vivo study showed similar pattern. C) Immunohistochemical analysis of NALM cells and CABA-201 CAR T cells. 

Figure 2. CABA-201 CAR T cells control Nalm6 tumor growth in NIS mice. A) Representative bisulphite sequencing images (zoomed) shown for each group on Days 5, 8, 13 and Day 20. B) Both ventral and dorsal bisulphite sequencing imaging (photo in x) are plotted as mean ± SD for each group.

Figure 3. Off-target cytotoxic interactions of CABA-201 were not identified using human membrane proteome arrays or by selected primary human cells. A) The anti-CD19 IC78 CAR was selected for specificity against 201 human immune proteome, excluding all B cells, monocytes and T cells. IC78 anti-CD19 CARs did not bind to most proteins represented in the MPA (top), total IgG, FcγR, CD19, and FMC7 were control/forward in the validation assessment (bottom), and only binding to the positive control Protein A, and CD19 was observed and concentration dependent. No binding to Ig1E, FcγR1, CD19, SDC1 compared to the negative control (vector alone) was detected. There were no cross-reactive target specificities. B) Representative results of CABA-201 cytotoxicity assays using two donor T cell batches co-incubated for 48 hours with BEC (top) SDC (bottom) at indicated E:T ratio. Lysis was measured by incorporation of green dye over time via time-cycled setup. Green count is shown as mean ± SD in triplicate. Staurosporine was used as toxicity controls.

Figure 4. CABA-201 can be generated from autoimmune patient derived T cells. A) Representative plots showing primary human T cells isolated from Systemic Lupus Erythematous (SLE), Scleroderma (SSC), idiopathic inflammatory Myositis (Myositis) and Ankylosing spondylitis (AS) patients expressing CABA-201 CARs (CABA-201). B) Summary graph showing transcriptional efficacy of primary T cells from multiple autoimmune disease patients, including patient donors of Musculoskeletal Pathogenesis (mPGV) (SLE, Rheumatoid Arthritis (RA), and Musculoskeletal Disease (MSD). Mean ± SD is shown for each disease. C) Table of patient PBMC donor characteristics by indication: M = Male, F = Female, NA = Not Available.

Figure 5. Autoimmune patient derived CABA-201 T cells effectively kill their intended autologous patient target B cells. A) Effective T cells (CABA-201 or NT T cells) generated from PMBCs of different autoimmune patients were co-cultured with B cells isolated from the same donor as the indicated E:T ratio for 24 hours. Percentage of CD19 positive cells is shown for each representative matched donor pair. Don’t be represented means no detectable matched donor pair following co-culture. 1:1 E:T ratio of 1.1, 5:1 E:T ratio of 5.1. B) Additional CABA-201 cytotoxicity and activation data generated from SLE, SSC, and Myositis murine are shown.

Conclusions

• CABA-201 has been designed and specifically engineered for patients with autoimmune diseases
• The fully human CD19 binder used in CABA-201 was clinically evaluated in ~26 oncology patients and had an acceptable safety profile leading to this study in autoimmune disease
• CABA-201 demonstrated comparable cytotoxic activity to FM63 CAR T cells against CD19+ target cells in vitro, and comparable in vivo potency was also seen in a NIS mouse model
• Off-target cytotoxic activity of CABA-201 was not identified against a panel of selected primary human cells, and no off-target interactions or binding against IC78 were detected in a membrane proteome array and a tissue cross-reactivity panel
• CABA-201 generated from patients with multiple autoimmune diseases showed robust CAR surface expression and effective elimination of target autologous CD19+ B cells

This pre-clinical data demonstrating the potential of CABA-201 to provide improvement in a broad range of autoimmune diseases where B cells have a pathogenic role has led to the initiation of Phase 1/2 clinical trials in Scleroderma, SLE, Myositis, and Myasthenia Gravis.

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