Preclinical Specificity and Activity of CABA-201, a Fully Human 4-1BB Containing CD19 CAR T Therapy for Treatment-Resistant Autoimmune Disease

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Abstract
Autoimmune diseases (AD) are common, with an incidence of 1 in 10 individuals. Most AD have standardized treatment regimens, but no curative therapy. Treatment consists of immunosuppressive therapy with associated toxicity. Targeted biologic therapies directed at cytokine pathways, costimulatory molecules, and B cells are also utilized but are limited by need for frequent infusions and high cost and incidence of adverse events. There is increasing evidence that B cells play a central role in AD pathogenesis, based upon responsiveness to B cell depletion with antibody-based therapeutics, but responses are typically transient, partly due to the incomplete depletion of B cells in secondary lymphoid tissues. Chimeric antigen receptor (CAR) T cells are a gene-engineered cellular immunotherapy which direct the T cell to a desired target. Multiple B cell targeted CD19 CAR T cell products have been demonstrated to induce durable remissions of refractory B cell malignancies. The commercially approved CD19 CAR T cell products utilize the murine derived CD19 αv binding domain (mNKD3). Studies have established the ability of these products to deeply deplete B cells in patients with hematologic malignancies. Proof of concept pilot data in patients evaluating the safety and efficacy of an FMC63-CD3ζ-CAR T cell product analogous to one of the commercially approved therapies, in treatment refractory AD patients, including with systemic lupus erythematosus, 3 with myasthenia, and 4 with systemic sclerosis suggest the potential to achieve deep and durable drug free remissions, with the first patient now beyond 24 months. We designed a new CABA-201 CAR T product, CABA-201, containing a fully human CD19 binder (IC78), that utilizes the 4-1BB costimulatory domain, which is reported to reduce the incidence and severity of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) relative to a CD28 costimulatory domain containing CD19 CAR T product. Preclinical studies were conducted to explore the specificity and activity of CABA-201, which were compared to the FMC63-41BB-CD3ζ CAR T cell product, analogous to one of the commercially approved therapies, in treatment refractory AD patients, including with systemic lupus erythematosus, 3 with myasthenia, and 4 with systemic sclerosis.

Results

A.
B.

Figure 1. Cytolysis of CABA-01 targeted CD19 positive target cells. Effectors cells (CABA-201, FMC63-CAR, or FMC63-41BB-CD3ζ) were co-incubated with target Nalm6 cells for 41 hours at a ratio of 1:1 (effector:target) to induce the target cell death. A) The cytotoxicity of target cells was measured using an IncuCyte cytotoxicity assay. B) Area under the curve (AUC) is shown as mean ± standard deviation (SD) from biologic triplicates. Staurosporine was used as toxicity controls. **p ≤ 0.01, ***p ≤ 0.001.

Figure 2. CABA-201 CAR T cells control Nalm6 tumor growth in NSG mice. A) Representative immunohistochemistry staining for GFP+ cells in blood, mesenteric lymph nodes, spleen, and liver of NSG mice injected with Nalm6 cells and co-cultured with 1x10^6 CABA-201 CAR T cells. B) Tumor growth was measured over 5 weeks. C) Representative images of GFP+ cells in the tumor tissue. The tumor size is shown as mean ± SD of biologic triplicates.

Figure 3. Off-target cytotoxicity of CABA-201 were not identified using human membrane proteome array against selected primary human cells. A) The anti-CD19 IC78 scFv was tested for reactivity against 5,344 human proteins, including 690 of all white blood cells, multiple myeloma and non-myeloma cancer cell lines, and 400 proteins selected to span the human genome. B) The fully human CD19 binder used in CABA-201 was clinically evaluated in ~20 oncology patients and had an acceptable safety profile leading to this study in autoimmune disease.

Figure 4. CABA-201 can be generated from autologous patient derived T cells. A) Representative IHC staining for GFP+ cells in blood, mesenteric lymph nodes, spleen, and liver of NSG mice injected with Nalm6 cells and co-cultured with 1x10^6 CABA-201 CAR T cells. B) Tumor growth was measured over 5 weeks. C) Representative images of GFP+ cells in the tumor tissue. The tumor size is shown as mean ± SD of biologic triplicates. Staurosporine was used as toxicity controls.

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 ~20 oncology patients and had an acceptable safety profile leading to this study in autoimmune disease
• CABA-201 demonstrated comparable cytotoxic activity to FMC63 CAR T cells against CD19+ target cells in vitro, and comparable in vivo potency was also seen in a NSG mouse model
• OFF-target efficacy of an FMC63-41BB-CD3ζ CAR T 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 screen.
• 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 demonstrates 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 II/II clinical trials in idiopathic inflammatory myopathy and myasthenia gravis.

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