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Presented at the
ASGCT Annual Meeting 2023
May 16-20, 2023
Los Angeles, CA

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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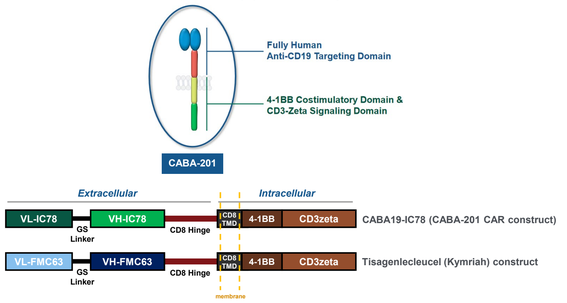
Abstract

Over 4% of the world population is estimated to live with autoimmune disease. Treatment typically requires systemic immunosuppressive therapy that have associated toxicities and are not curative. 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 typically transient due to the incomplete depletion of B cells in secondary 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 CAR T cell products have led to durable remissions of B cell leukemias and lymphomas; three have been approved by regulators globally, each of which utilizes the murine derived CD19 scFv binding domain FMC63. Data from numerous studies have established the ability of these products to deeply deplete B cells. An early proof of concept pilot study evaluating the safety and efficacy of an FMC63-41BB-CD3ζ CAR T cell product, analogous to one of the approved therapies, in 5 patients with treatment refractory systemic lupus erythematosus suggests the potential to achieve rapid, deep and durable drug-free remissions. We designed a new CD19 CAR T product candidate (CABA-201) containing a clinically evaluated (NCT05091541) fully human CD19 binder (IC78), designed to minimize immune mediated interference with activity. In addition, the construct utilizes the same 4-1BB costimulatory domain used in the pilot study above, which is reported to have a reduced incidence and severity of cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) in oncology patients. Preclinical studies were conducted to explore the specificity and activity of CABA-201 compared to the specificity and activity of the FMC63-41BB-CD3ζ construct using the same cell production method. CABA-201 demonstrated comparable cytotoxic activity to FMC63 CAR T cells against CD19⁺ Nalm6 cells in vitro, and comparable in vivo potency was observed in a dose ranging study in the NSG-Nalm6 tumor model. No evidence of off-target cytotoxic activity of CABA-201 was identified against a panel of selected primary human cells, and no off-target binding against IC78 was detected in a membrane proteome array, or in clinical studies evaluating IC78 in a tandem CAR formation. CABA-201 generated from primary T cells from multiple autoimmune disease patients showed robust CAR surface expression and effective elimination of target autologous CD19⁺ B cells in vitro. Together, these data support the tolerability and activity of CABA-201, and provide a clinically relevant benchmark for dose related potency in clinical studies planned for initiation later this year.

CARTA
Chimeric Antigen Receptor T cells for Autoimmunity

*Potential to **'reset the immune system'** in patients with **autoimmune diseases driven by B cells**, through generalized transient B cell depletion and **repopulation of healthy B cells***



Results

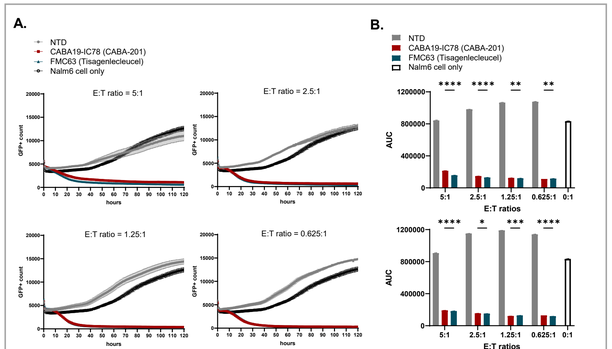


Figure 1. Cytotoxicity of CABA-201 toward CD19 positive target cells. Effector cells (CABA-201, FMC63 CAR, or NTD T cells) were co-incubated with target Nalm6 cells for 120 hours at indicated E:T ratios with 2 different donors. A) Cytolysis of wild-type CD19 positive Nalm6 cells was measured using an imaging-based IncuCyte cytotoxicity assay, and GFP+ count is shown as mean ± SD in triplicates. Representative results from 1 donor is shown. Effector:Target (E:T) ratio is based on total T-cell number. B) Area under the curve (AUC) is shown as mean ± SD from graphs shown in a) and from 1 another donor. Two-way ANOVA was used to compare differences in the average AUC at each E:T ratio between FMC63 CART19 cells and CABA19-IC78 CART cells. **** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$.

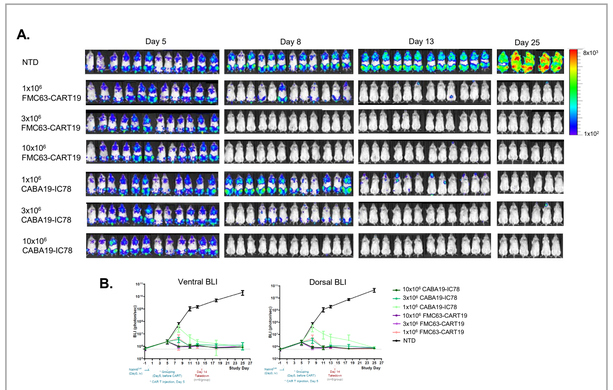


Figure 2. CABA-201 CAR T cells control Nalm6 tumor growth in NSG mice. A) Representative bioluminescence images (ventral) shown for each group on Days 5, 8, 13 and Day 25. B) Both ventral and dorsal bioluminescence imaging kinetics (photons/sec) are plotted as mean +/- SD for each group.

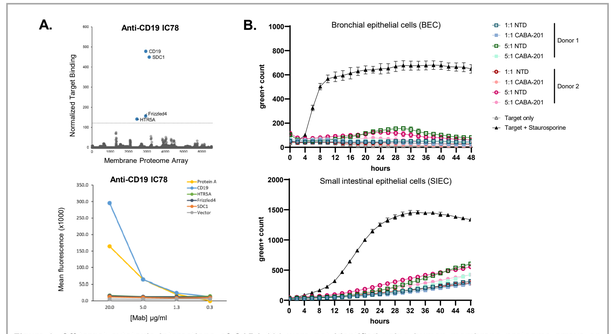


Figure 3. Off-target cytotoxic interactions of CABA-201 were not identified using human membrane proteome arrays or against selected primary human cells. A) The anti-CD19 IC78 scFv was tested for reactivity against 5,344 human membrane proteins, including 94% of all single-pass, multi-pass and GPI-anchored proteins. Anti-CD19 IC78 scFv did not bind to most proteins represented in the MPA (top). Identified "hits" (SDC1, Frizzled4, and HTR5A) were carried 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 HTR5A, Frizzled4, SDC1 as compared to the negative control (vector alone) was detected. There were no cross-reactive targets identified. B) Representative results of CABA-201 cytotoxicity assays using two donor T-cell batches co-incubated for 48 hours with BEC (top) SIEC (bottom) at indicated E:T ratios. Lysis was measured by incorporation of green dye over time via IncuCyte assay. Green+ count is shown as mean ± SD in triplicates. Staurosporine was used as toxicity controls.

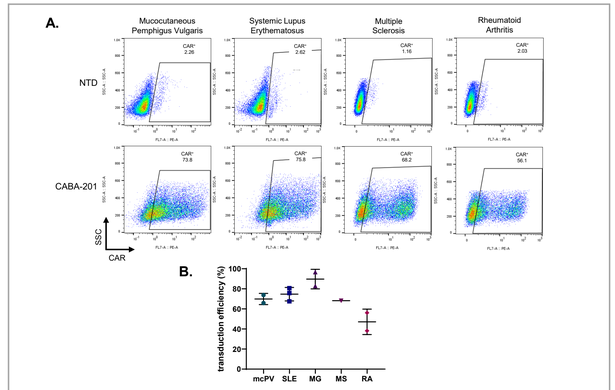


Figure 4. CABA-201 can be generated from autoimmune patient derived T cells. A) Representative plots showing primary human T cells isolated from mucocutaneous Pemphigus Vulgaris (mcPV), Systemic Lupus Erythematosus (SLE), Multiple Sclerosis (MS), and Rheumatoid Arthritis (RA) patients expressing CABA19-IC78 CARs (CABA-201). B) Summary graph showing transduction efficiency of primary T cells from multiple autoimmune disease patients. MG, Myasthenia Gravis. Mean ± SD is shown for each disease.

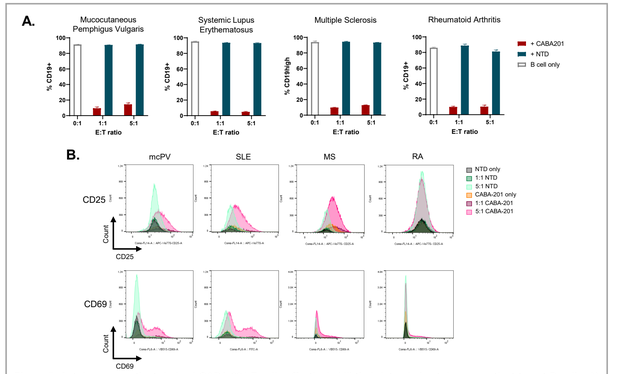


Figure 5. Autoimmune patient derived CABA-201 T cells effectively kill their intended autologous patient target B cells. A) Effector T cells (CABA-201 or NTD T cells) generated from mcPV, SLE, MS, and RA donors were co-cultured with B cells isolated from the same donor at the indicated E:T ratios for 24 hours. Percentage of CD19 positive cells is shown for each representative matched donor pair. Each bar represents mean ± SD of triplicates. B) Representative histogram of CD25 (upper panel) and CD69 (lower panel) surface expression on effector T cells is shown for each representative matched donor pair following co-culture. 1:1 = E:T ratio of 1:1, and 5:1 = E:T ratio of 5:1.

Conclusions

- CABA-201 has been specifically engineered for patients with autoimmune diseases
- Fully human CD19 binder in CABA-201 was clinically evaluated in ~20 oncology patients with safety profile appropriate for study in autoimmunity
- CABA-201 demonstrated comparable cytotoxic activity to FMC63 CAR T cells against CD19⁺ target cells in vitro, and comparable in vivo potency was observed
- No off-target cytotoxic activity of CABA-201 was identified against a panel of selected primary human cells, and no off-target interactions against IC78 were detected in a membrane proteome array
- No off-target binding of IC78 was observed in tissue cross-reactivity panel
- CABA-201 generated from primary T cells of multiple autoimmune disease patients showed robust CAR surface expression and effective elimination of target autologous CD19⁺ B cells from the same patients
- CABA-201 has a potential to provide improvement in a broad range of autoimmune diseases where B cells have a role initiating or maintaining disease

Acknowledgement *IASO Biotherapeutics*