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

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Abstract

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 CAR T-cell products have led to durable remissions of B-cell malignancies; four have been approved, each of which utilizes the murine derived CD19 scFv binding domain FMC63. Numerous studies have established the ability of these products to deeply deplete B-cells. An early clinical evaluation of an FMC63-41BB-CD3ζ CAR 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.

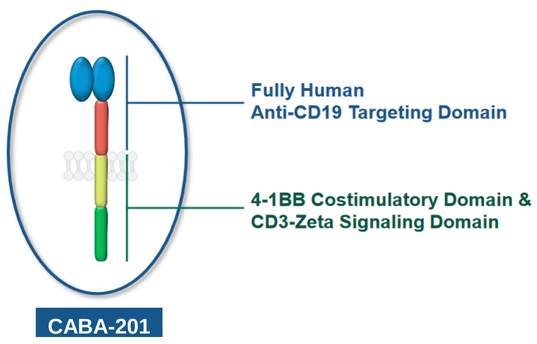
Methods: CABA-201, a fully human 41BB-CD3ζ containing CD19 CAR T-cell, was generated both from healthy donor apheresis and from myositis patients' (dermatomyositis subtype) peripheral blood mononuclear cells (PBMCs) via standard *ex vivo* expansion using antibody coated beads and lentiviral transduction. CABA-201 *in vitro* activity was evaluated in co-culture assays with either CD19+ NALM6 cells or with patient-matched myositis CD19+ B-cells. Activity was measured by Luminex assay for cytokine release, cytotoxicity via flow cytometry, or CAR T-cell activation via flow cytometry. *In vitro* 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 NSG-NALM6 model.

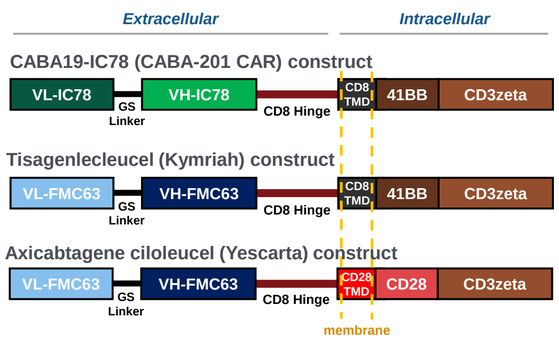
Results: CABA-201 generated from healthy donors showed specific *in vitro* activity against CD19+ NALM6 cells. Furthermore, CABA-201 generated from myositis patient PBMCs demonstrated specific *in vitro* activity against matched B-cells (Figure 1). The fully human CD19 binder CABA-201 had no off-target 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 NSG-NALM6 model.

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.

CARTA
Chimeric **A**ntigen **R**eceptor **T** cells for **A**utoimmunity

*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

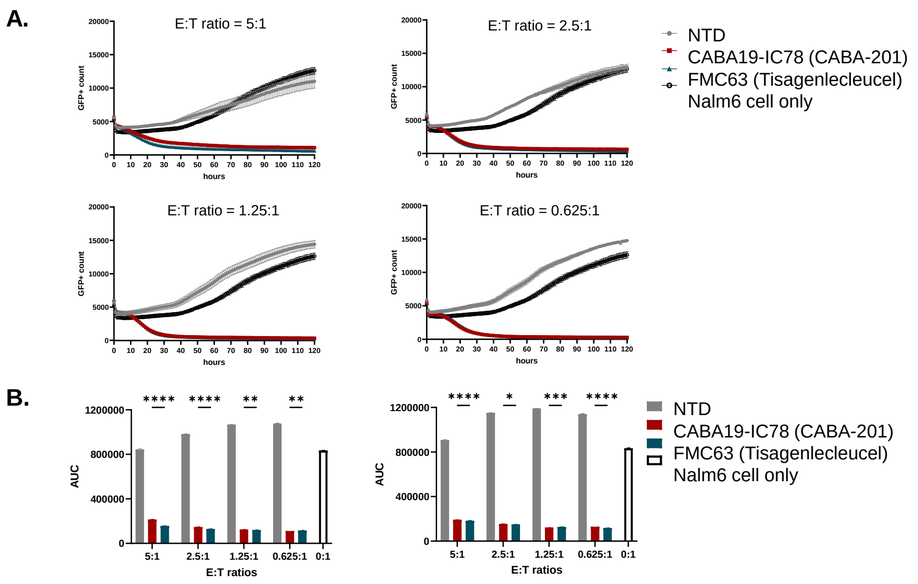


Figure 1. Cytotoxicity of CABA-201 toward CD19 positive target cells. Effector cells (CABA-201, FMC63 CAR, or NTD T cells) were co-incubated with target Nalm6 cells for 120 hours at indicated E:T ratios with 2 different donors. A) Cytolysis of wild-type CD19 positive Nalm6 cells was measured using an 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. **** $p \leq 0.0001$, *** $p \leq 0.001$, **$p \leq 0.01$, * $p \leq 0.05$.

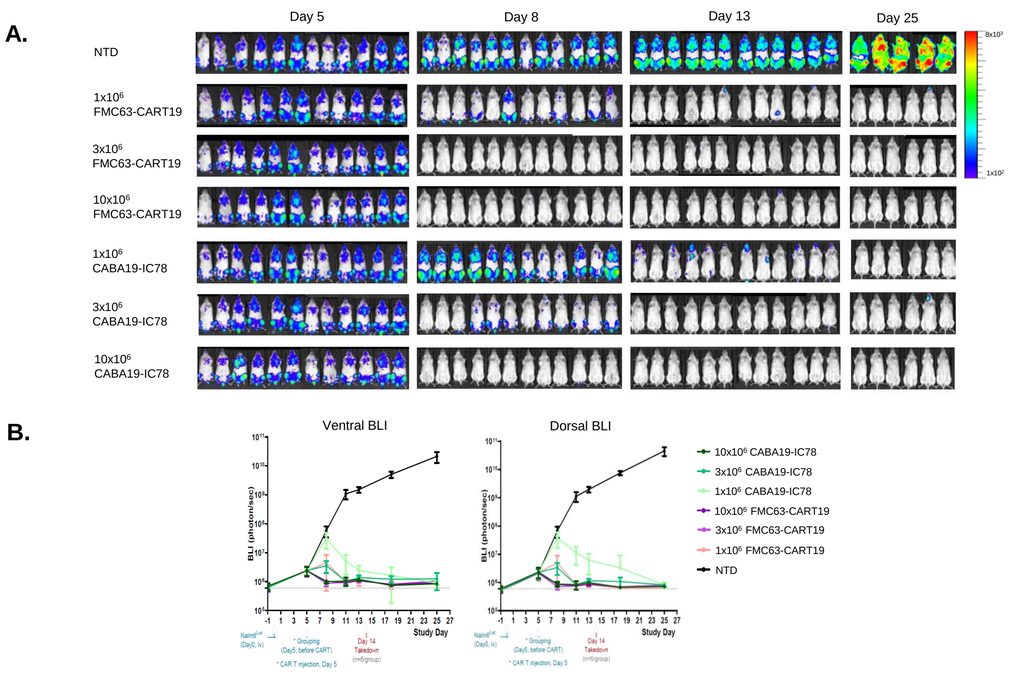


Figure 2. CABA-201 CAR T cells control Nalm6 tumor growth in NSG mice. A) Representative bioluminescence images (ventral) shown for each groups on Days 5, 8, 13 and Day 25. B) Both ventral and dorsal bioluminescence imaging kinetics (photons/sec) are plotted as mean +/- SD for each group.

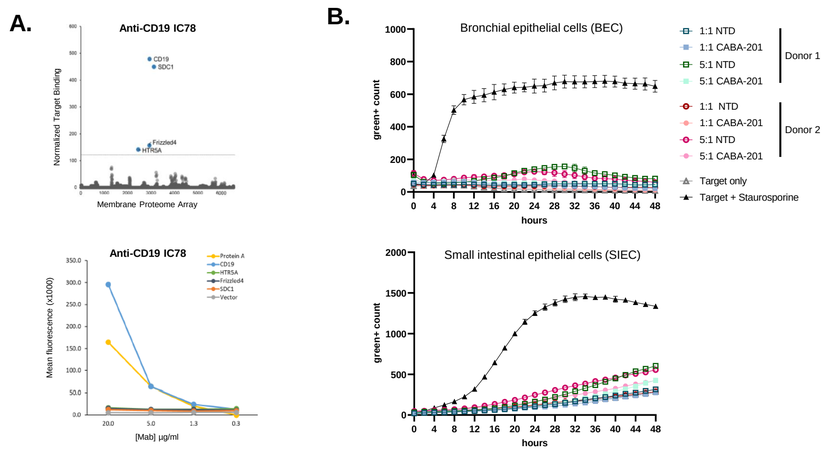
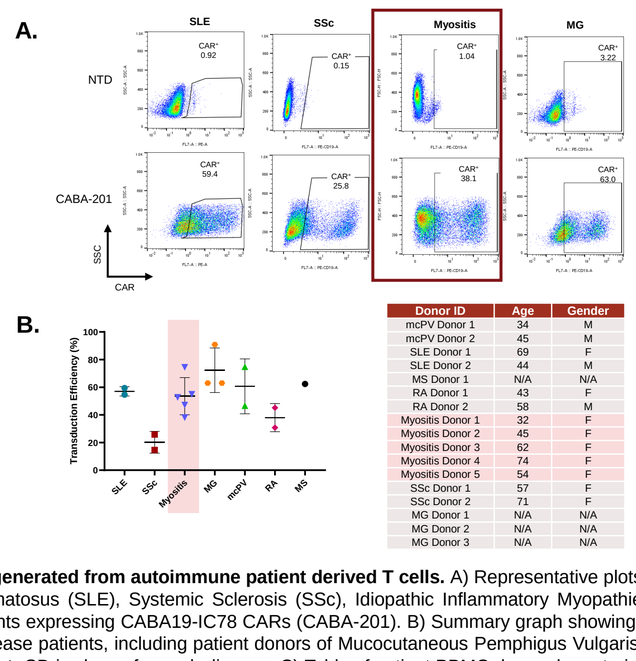


Figure 3. Off-target cytotoxic interactions of CABA-201 were not identified using human membrane proteome arrays or against selected primary human cells. A) The anti-CD19 IC78 scFv was tested for reactivity against 5,344 human membrane proteins, including 94% of all single-pass, multi-pass and GPI-anchored 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.



Donor ID	Age	Gender
mcPV Donor 1	34	M
mcPV Donor 2	45	M
SLE Donor 1	69	F
SLE Donor 2	44	M
MS Donor 1	N/A	NA
RA Donor 1	43	F
RA Donor 2	58	M
Myositis Donor 1	32	F
Myositis Donor 2	45	F
Myositis Donor 3	62	F
Myositis Donor 4	74	F
Myositis Donor 5	54	F
SSc Donor 1	57	F
SSc Donor 2	71	F
MG Donor 1	N/A	N/A
MG Donor 2	N/A	N/A
MG Donor 3	N/A	N/A

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 Erythematosus (SLE), Systemic Sclerosis (SSc), Idiopathic Inflammatory Myopathies (Myositis) and Acetylcholine receptor Myasthenia Gravis (MG) patients expressing CABA19-IC78 CARs (CABA-201). B) Summary graph showing transduction efficiency of primary T cells from multiple autoimmune disease patients, including patient donors of Mucocutaneous Pemphigus Vulgaris (mcPV), Rheumatoid Arthritis (RA), and Multiple Sclerosis (MS). Mean ± SD is shown for each disease. C) Table of patient PBMC donor characteristics by indication. M = Male; F = Female; N/A = Not Available.

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 PBMC of different autoimmune patients 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. Additionally, representative histogram of CD25 and CD69 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. B) Additional CABA-201 cytotoxicity and activation data generated from SLE, SSc, and myositis donors 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 ~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 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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