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Correlative Studies of CABA-201, a Fully Human, Autologous 4-1BB Anti-CD19 CAR T Cell Therapy in Patients with Immune-Mediated Necrotizing Myopathy and Systemic Lupus Erythematosus from the RESET-Myositis™ and RESET-SLE™ Clinical Trials

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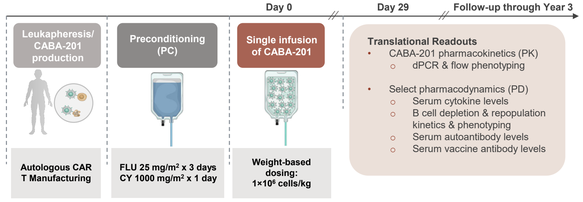
Background

CD19 targeting chimeric antigen receptor (CAR) T cells have demonstrated durable clinical responses in patients with idiopathic inflammatory myopathies (IIM), systemic sclerosis (SSc), and systemic lupus erythematosus (SLE) in an academic case series. The safety and tolerability of CABA-201, an autologous, fully human, 4-1BB anti-CD19-CAR T cell therapy is being evaluated in multiple phase I/II clinical trials for B-cell-mediated autoimmune diseases. The primary endpoint of these trials is safety and tolerability. Here we report on initial pharmacokinetic (PK), pharmacodynamic (PD), and other correlative data from the first 7 patients dosed across the RESET-Myositis (NCT06154252), RESET-SLE (NCT06121297), and RESET-SSc (NCT06328777) clinical trials. For IIM, we report initial exploratory data from two immune-mediated necrotizing myopathy (IMNM) patients and one dermatomyositis (DM) patient. For lupus, we report data from two SLE patients (one of which includes a Class V lupus nephritis (LN) patient ineligible for the LN cohort) and one LN patient. For SSc, we report data from one patient in the severe skin cohort (SSc-Skin).



Methods

CABA-201 cell PK profiles were assessed by dPCR for the vector in pre- and post-infusion PBMC samples. CABA-201 cells / µL blood reported values were determined with the patient's total PBMC count and the average CABA-201 vector copy number (VCN) for each patient's manufactured product. CABA-201 cells per µL blood was calculated using the following equation:

$$\frac{CAR\ T\ cells}{\mu L\ blood} = \frac{CAR\ copies}{\mu g\ DNA} * \frac{1 \mu g\ DNA}{1e5\ cells} * \frac{PBMC}{\mu L\ blood} * \frac{1}{VCN}$$

where an estimation of 1 µg DNA per 1x10^5 cells was used[1] and patient's PBMC count was determined using lymphocytes + monocytes counts[2]. Serum cytokines were measured via a multiplexed V-plex or U-plex mesoscale discovery (MSD) immunoassay. Flow cytometric analyses were performed on cell samples from apheresis, manufactured product (MP), and post-infusion time points to assess CAR expression in T cells and CD4/CD8 expression in CAR$^+$ T cells. Flow cytometry was also used to quantify B-cell numbers (via CD19 and CD20) and phenotypically determine maturity (via CD24 and CD38). All flow cytometry was performed using custom multi-color antibody panels; samples and controls were labelled and read on the Novocyte Quanteon flow cytometer (Agilent), and data were analyzed using FlowJo Software. Serum antibody panels were used to measure selected IIM-, SLE-, SSc-, and vaccine/infectious pathogen-associated antibodies in patient sera pre and post CABA-201 infusion utilizing the Luminex FlexMap. Serum antibody levels were reported as net median fluorescence intensity (MFI). T cell receptor (TCR) sequencing was performed on peripheral blood mononuclear cell (PBMC) samples isolated from LN-1 at various timepoints before and throughout the first month post-infusion. CAR$^+$ (CD8$^+$, CD8$^-$CD4$^-$ and CD4$^+$, where possible) and CAR$^-$ T cell populations were sorted via the MACSQuant Tyto prior to sequencing. Short-read sequencing (Illumina) of these populations was performed on libraries prepared using primer pools that amplify the beta chains of each TCR (Adaptive Biotech). A unique TCR clone was defined as a unique complementarity-determining region 3 nucleotide sequence. Data cut for this poster was 01-Nov-2024.

[1] Baumer et al. 2018 Scientific Reports
[2] Boris et al. 2020 Molecular Therapy Methods & Clinical Development

Results

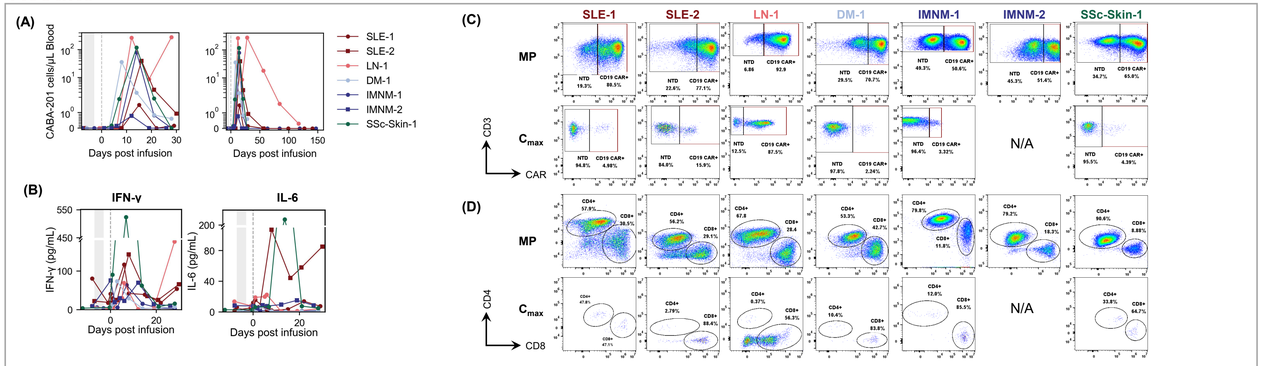


Figure 1. CABA-201 characterization. (A) CABA-201 PK profile in patients over time in days elapsed from CABA-201 infusion (left: 30-day follow-up, right: all time). **(B)** Levels of selected serum cytokines over time in days elapsed from CABA-201 infusion. In **(A-B)** vertical gray shading indicates window in time for PC across all subjects and vertical dotted line indicates infusion at day 0. **(C)** Flow cytometry plots of the manufactured product (MP) and PBMCs at the time of maximum CABA-201 exposure post-infusion (C_{max}; Day 15 for SLE-1, SLE-2, IMNM-1 and SSc-Skin-1, Day 29 for LN-1, Day 8 for DM-1, and no CAR T cell detected post-infusion for IMNM-2). Percentage of T cells that are CAR$^+$ in the MP and at post-infusion C_{max} **(D)** Flow cytometry plots showing the percentage of CAR$^+$ T cells expressing CD4 and CD8 in the MP and post-infusion C_{max}.

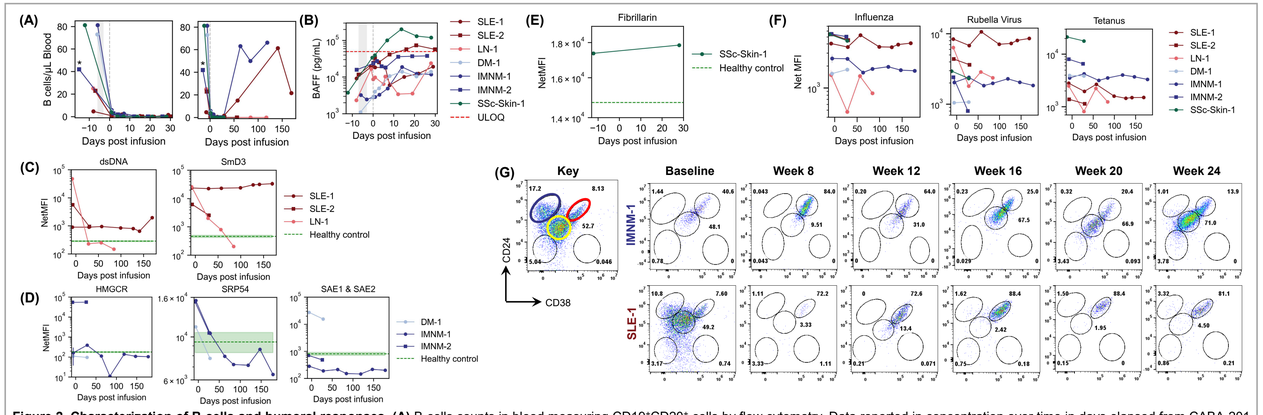


Figure 2. Characterization of B-cells and humoral responses. (A) B-cells counts in blood measuring CD19$^+$CD20$^+$ cells by flow cytometry. Data reported in concentration over time in days elapsed from CABA-201 infusion through the first month (left panel) and over all time (right panel). Pre-infusion B-cell levels were measured in PBMCs from the pre-preconditioning (pre-PC) visit for all subjects other than IMNM-2 where apheresis was used (*). **(B)** Serum BAFF levels from pre-PC through the first month post-CABA-201 infusion. **(C-F)** Serum antibody levels at various time points at pre-PC and after CABA-201 infusion. Y-axis is net MFI and dashed green horizonal lines with shading depict healthy donor sera levels represented as mean ±1 sd. Research assay has a higher sensitivity to autoantibodies and vaccine antibodies as compared to clinical assay. **(C)** Serum levels of select SLE autoantibodies in SLE/LN patients. **(D)** Serum levels of select IIM autoantibodies in IIM patients. **(E)** Serum levels of Fibrillarin in SSc-Skin patient. **(F)** Serum levels of select vaccine/infectious pathogen antibodies before and after CABA-201 infusion. **(G)** Phenotype of CD19$^+$CD20$^+$ B-cells in two patients pre-infusion and through reconstitution post-infusion were characterized by flow cytometry with CD24 and CD38. Dot plot on the left indicates key gates of interest: transitional naïve B-cells (Red), naïve B-cells (yellow), memory B-cells (blue).

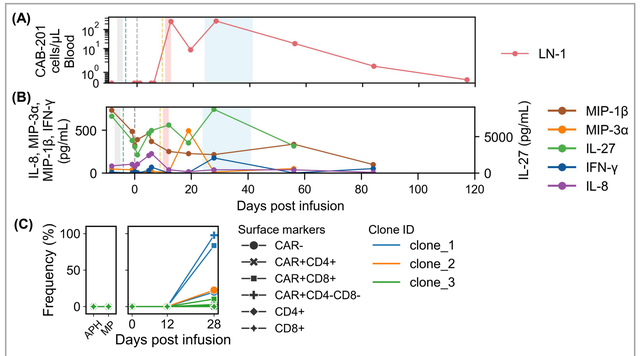


Figure 3. Detailed Translational findings from LN-1. (A) CABA-201 PK overlaid on timeline of clinical events reported in LN-1. Vertical dashed lines represent hospitalization with a fever of indeterminate etiology (blue), CABA-201 infusion (grey), and CRS (orange). Shaded areas represent timing of lymphodepletion (grey), grade 4 ICANS (day 10-12; red) and fever with pancytopenia (day 24-41; blue). **(B)** Serum cytokine levels over the first 8-12 weeks post-CABA-201 infusion. MIP-1β, MIP-3α, IL-27, and IL-8 were either not elevated or detected in other subjects. **(C)** TCR sequencing depicting the top 3 clones in the apheresis, MP, and PBMCs collected at days 12 and 28 post-infusion in LN-1. Apheresis samples were sorted into CD4+ and CD8+ populations. Other samples were sorted into CAR$^-$, CAR$^+$CD4$^+$, CAR$^+$CD8$^+$, and CAR$^+$CD4$^-$CD8$^-$ where possible. Day 28 T cells comprised of three dominant clones (clones 1-3), regardless of the CAR, CD4, or CD8 expression.

Conclusions

- We report on early translational data on 7 patients with various autoimmune diseases (1 DM, 2 IMNM, 2 SLE, 1 LN, and 1 SSc) treated with CABA-201 across three clinical trials.
- Peak expansion (C_{max}) was observed between Day 8 and Day 15, with the exception of LN-1, which had a biomodal expansion profile (C_{max} observed at Day 29).
- CABA-201 manufactured product CAR T cells were CD4 dominant and exhibited an inversion to CD8 dominance at C_{max} for most patients.
- Peripheral B-cells were rapidly depleted following CABA-201 infusion and began to reconstitute as early as 8 weeks post-infusion.
  - Reconstituted B-cells primarily exhibited a transitional naïve phenotype.
  - Despite clinical improvement in all three subjects, as of the most recent follow up, disease-associated antibodies were reduced in 2 of the 3 subjects with extended follow up after CABA-201 infusion (IMNM-1, SLE-1, LN-1).
  - Vaccine and infectious pathogen antibodies remained stable for most patients.
- LN-1 had multiple inflammatory events that started before preconditioning (PC) and had highly elevated pro-inflammatory cytokines (MIP-1β and IL-27) which continued after infusion, and some findings suggest occult infection (supported by TCR clonal sequencing).
  - Prior to PC, an inflammatory process possibly consistent with infection or disease flare was present (elevated IL-27 and MIP-1β).
  - Shortly after CABA-201 infusion, patient had elevated IL-8 consistent with ICANS.
  - Starting at day 18, an inflammatory event occurred (elevated MIP-3α, IFN-γ, and IL-27) correlated with endogenous TCR (non-CAR-T) expansion.
- These data further support the potential for CABA-201 to provide an immune system reset that could lead to durable disease response without the need for chronic immunosuppression.