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# Clinical and translational findings following MuSK-CAART infusion without preconditioning in patients with Myasthenia Gravis (MuSCAARTes™ trial)

Cabaletta Bio®

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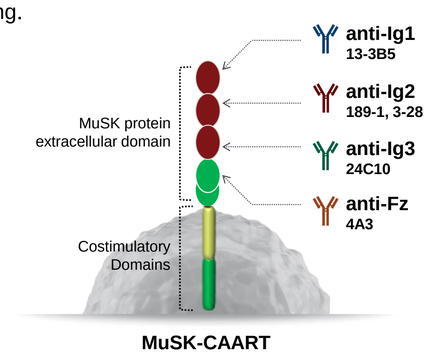
## Background

Muscle-specific tyrosine kinase (MuSK) myasthenia gravis (MG) is a rare but potentially severe disease, in which patients develop pathogenic autoantibodies that specifically target the MuSK protein within the neuromuscular junction. The current standard of care for MG includes broadly immunosuppressive therapies (rituximab and FcRn inhibitors) that are not curative. Ideally, therapy would selectively eliminate pathogenic memory B cells that are MuSK specific while sparing non-autoreactive immune cells. As chimeric antigen receptor engineered T cells (CAR-T) have demonstrated long lasting remission of B cell-mediated cancers, we developed engineered chimeric autoantibody receptor T (CAART) cells to assess if remission of B cell mediated autoimmune disease is possible through selective memory B cell ablation. By binding B cells expressing the MuSK specific B cell receptor (BCR) through the MuSK autoantigen expressed on its targeting domain, MuSK-CAART is designed to specifically eliminate only autoreactive B cells. Here, we report on the interim clinical and translational data from a Phase 1, open-label, study of autologous MuSK-CAART to evaluate the safety and early translational profile of MuSK-CAART in patients with anti-MuSK antibody positive MG (NCT05451212). As of this poster, the MusCAARTes trial is not currently dosing patients as we continue to evaluate clinical and translational data from the A1 (5 x $10^8$ cell dose) and A2 (2.5 x $10^9$ cells dose) cohorts, where patients were treated with MuSK-CAART in absence of lymphodepleting preconditioning.




MuSK-CAART

## Methods

Flow cytometric analyses were performed on the infusion product & post-infusion peripheral blood mononuclear cell (PBMC) samples to assess transduction efficiency, CD4/CD8 ratio, HLA-DR expression. CAART cell flow cytometry was performed using a custom multi-color panel; samples and controls were labelled and read on the MACSQuant16 flow cytometer (Miltenyi Biotec). Data was analyzed using the FloJo Software. Cells were gated utilizing the following gating hierarchy: Time → Singlets → Cells → Live → CD3+ → CAAR-T+ followed by CD4/CD8, and HLA-DR of the CAAR+ T cells. MuSK-CAART cell cytotoxicity assays were performed *in vitro* using the IncuCyte® platform. MuSK-CAART cell pharmacokinetic profiles were assessed by qPCR for the vector in pre- and post-infusion PBMC samples. MuSK-CAART cells / uL blood reported values were determined with patient total PBMC count and the average vector copy number (VCN) for each patient manufactured product. CAART cells per µL blood was calculated using the following equation:

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

where an estimation of 1 µg DNA per 1e5 cells was used [1] and patient PBMC count was determined using lymphocytes + monocytes counts [2]. Serum IFN-γ levels were measured via a multiplexed mesoscale discovery (MSD) immunoassay. T cell receptor (TCR) sequencing was performed on PBMC samples isolated from patient A2-1 at various timepoints before and throughout the first two months post-infusion. CAAR+ and CAAR- 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. Anti-MuSK autoantibody levels were evaluated on pre- and post- infusion serum samples via anti-MuSK radioimmune assay (RIA) at a CLIA laboratory (Mayo Clinic). MG activities of daily living (MG-ADL) scores were assessed by clinical investigator.

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

## Overview of Clinical Metrics

Two cohorts are included in this report as part of a dose escalation clinical protocol (NCT05451212). Patients did not receive lymphodepleting preconditioning prior to the single infusion of MuSK-CAART cells.

| Cohort | Total MuSK-CAART Cell Dose | Fold Increase in Dose | Subjects per Cohort |
|---|---|---|---|
| A1 | 5x$10^8$ | 1x | 2 |
| A2 | 2.5x$10^9$ | 5x | 4 |

## Patient demographics

| Subject ID | Age, years | Sex | Disease Duration, years | MGFA Clinical Classification | Baseline Anti-MuSK Ab Level, nmol/L | Baseline MG-ADL | Prior MG Medications |
|---|---|---|---|---|---|---|---|
| A1-1 | 47 | F | 8.0 | Class IIIa | 0.38 | 9 | Prednisone Azathioprine IVIg |
| A1-2 | 81 | F | 2.6 | Class IIb | 0.23 | 0 | Prednisone Methotrexate |
| A2-1 | 70 | F | 1.2 | Class IIb | 0.02 | 5 | Firdapse |
| A2-2 | 64 | F | 5.1 | Class IIb | 12.2 | 4 | Methotrexate |
| A2-3 | 61 | F | 8.7 | Class IIIb | 2.97 | 3 | Pyridostigmine IVIg |
| A2-4 | 39 | F | 15.2 | Class IIIa | 14.7* | 6 | None |

## Serious Adverse Events

| Subject ID | Serious Adverse Event | Grade | Duration | Related to Therapy |
|---|---|---|---|---|
| A2-1 | Severe Hypokalemia | 4 | 3 days | No |
| A2-2 | CRS | 1 | 3 days | Yes |
| A2-3 | CRS | 2 | 3 days | Yes |
| A2-3 | Hemophagocytic Lymphohistiocytosis (HLH) | 4 | 11 days | Yes |

*Only available from screening. The values for the other 5 subjects are from pre-infusion

## Results

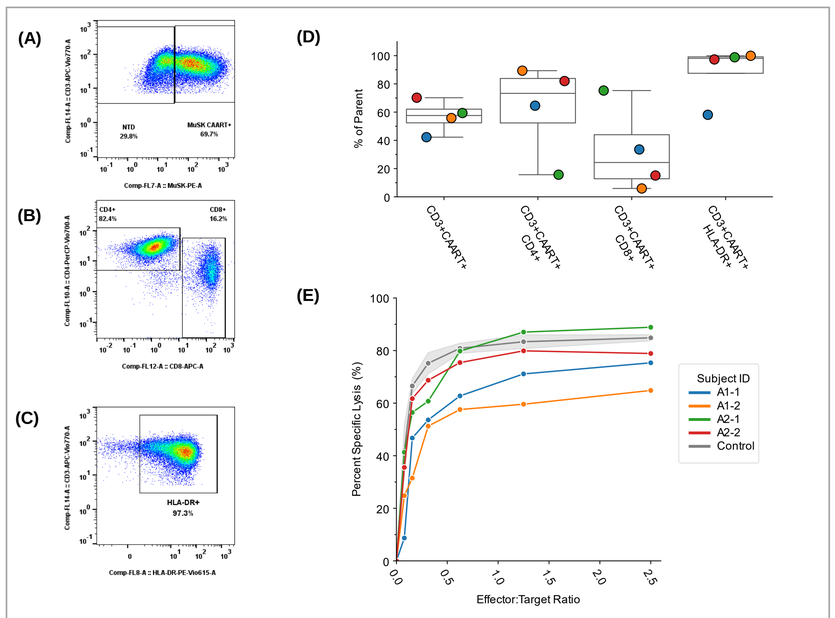



**Figure 1. Infusion Product Characterization.** **(A)** Representative flow plot depicting transduction efficiency of the manufactured product (MP) measured by flow cytometry and defined as the percentage of patients' T cells in the MP that are MuSK-CAAR+. **(B)** Representative flow cytometry of MuSK-CAAR+ T cells expressing CD4 and CD8 from the MP from one patient. **(C)** Representative flow cytometric analysis of the MP and the MuSK-CAAR+ T cells expressing HLA-DR, used as a marker of cellular activation. **(D)** Cumulative flow data from the MP from all four patients represented as the percentage of MuSK-CAART cells that are CD4+, CD8+, and HLA-DR+. **(E)** In vitro antigen-specific lysis of GFP+ anti-MuSK surface immunoglobulin-expressing NALM6 target cells by MuSK-CAAR+ effector cells from patients' MP. Cell lysis curves show the number of GFP+ target cells present (±SD) at effector to target ratios ranging from 0:1 to 2.5:1 over 120 hours.

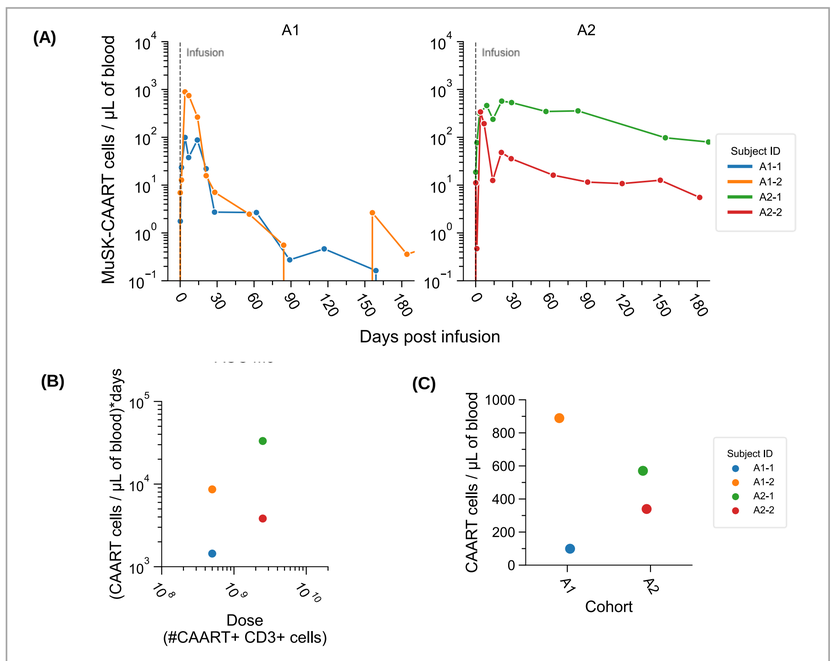



**Figure 2. MuSK-CAART post-infusion persistence.** Post-infusion MuSK-CAART cell persistence was measured by qPCR on genomic DNA extracted from peripheral whole blood samples. **(A)** Persistence plotted as MuSK-CAART cells/µL of blood, which is calculated using leukocyte counts at each visit, the vector copy number and transduction efficiency of the manufactures product, and the copies of CAART transgene/µg of DNA for each visit. X-axis corresponds to days elapsed since last infusion. **(B)** Area under the curve representation of persistence over time plots through the first 3 months represented as the number of CAART cells by time. **(C)** Peak expansion of MuSK-CAART cells in circulation ($C_{max}$) in cells/µL of blood captured for each patient.

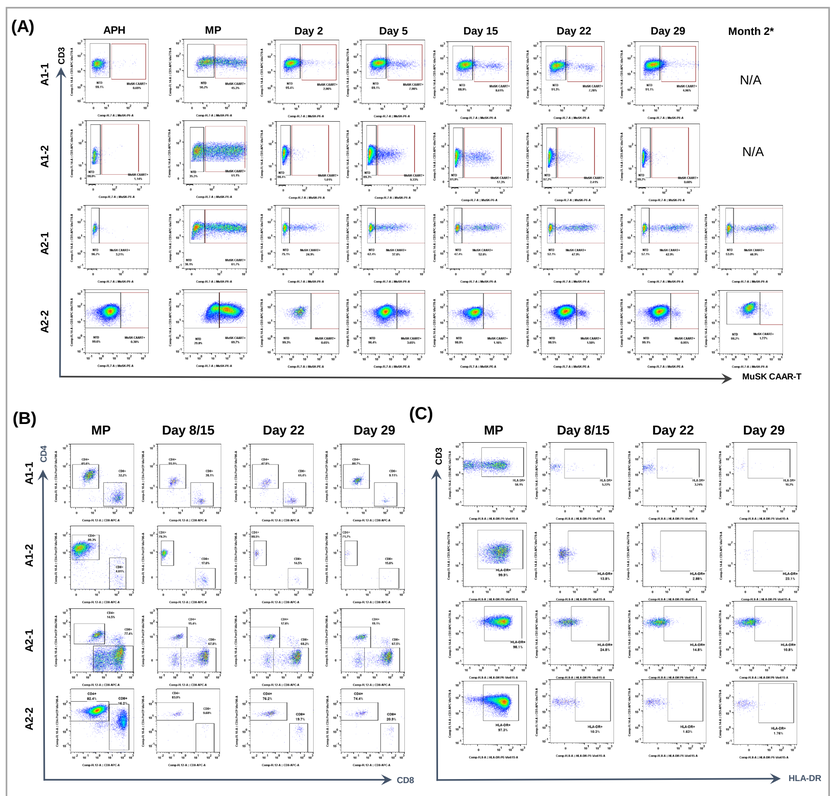



**Figure 3. MuSK-CAART post-infusion flow cytometric profiling.** Flow plots for the first four patients dosed with MuSK-CAART cells. **(A)** Percent of CD3+ T cells expressing the MuSK-CAAR at baseline (Apheresis sample), in the manufactured product (MP) and at various time points after infusion for all four subjects. Each row includes flow cytometric data from a given patient over time. *The two patients assessed in cohort A2 had additional longitudinal follow-up through Month 2 due to higher levels of persisting MuSK-CAART cells. **(B)** Flow cytometry of MuSK-CAAR+ T cells expressing CD4 and CD8 from the MP and at selected timepoints within the first month following infusion. **(C)** Flow cytometry plots depicting HLA-DR expression in MuSK-CAAR+ T cells across all four patient in the MP and throughout the first month after infusion for the four patients analyzed.

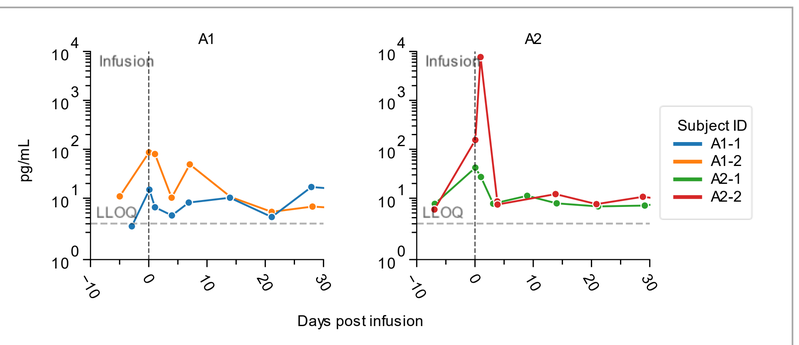



**Figure 4. Post-infusion serum IFNγ levels.** Baseline (pre-infusion) and various post-infusion serum samples were analyzed from four patients in Cohorts A1 and A2 for IFNγ (pg/mL) via MSD multiplex immunoassay. X-axis corresponds to days elapsed since last infusion. Dashed line depicts lower limit of quantification (LLOQ) of assay.

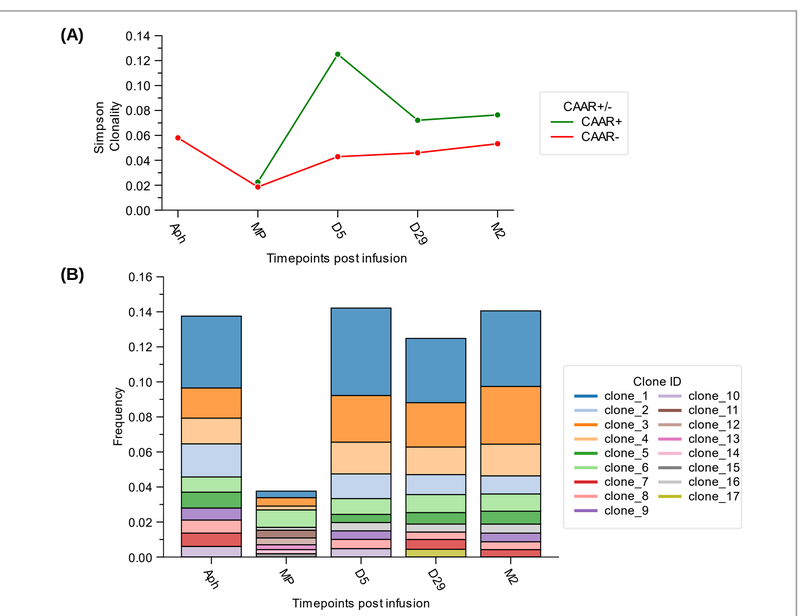



**Figure 5. TCR Sequencing Results.** Apheresis (Aph), Manufactured Product (MP), Day 5 (D5), Day 29 (D29) and Month 2 (M2) post-infusion serum samples from patient A2-1 were analyzed for unique TCR sequences. **(A)** Simpson clonality, a measure of diversity ranging from 0 to 1 where 1 indicates a monoclonal sample, was compared between CAART+ and CAART- samples at each timepoint. **(B)** Only 17 unique clones across all timepoints make up the top 10 clones by frequency for each time point.

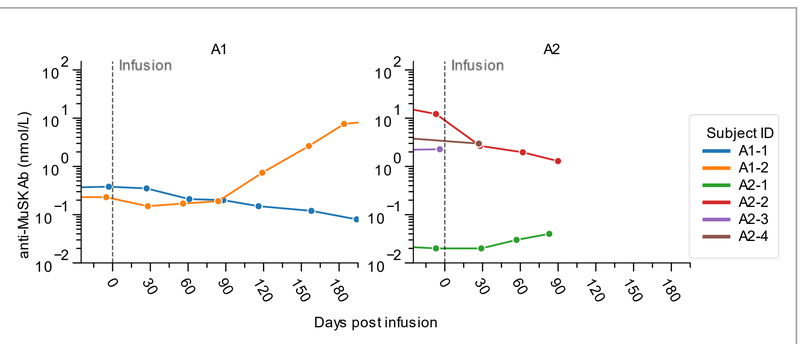



**Figure 6. Serum anti-MuSK autoantibody levels before and after MuSK-CAART cell infusion. (A)** Anti-MuSK auto-Ab levels were determined by RIA (Mayo Clinic) in serum samples from six patients in Cohort A1 and A2 at baseline and for up to six months post-infusion.

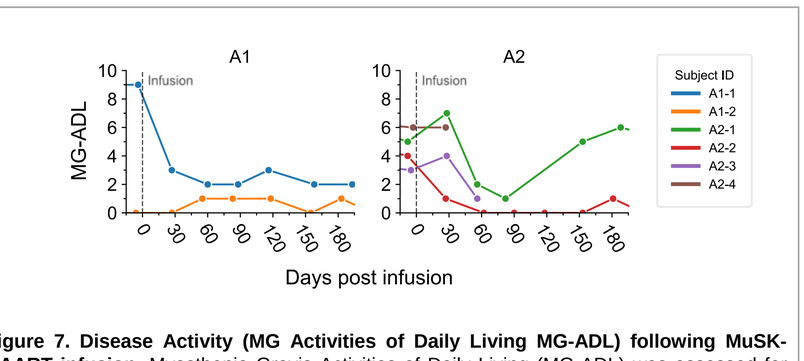



**Figure 7. Disease Activity (MG Activities of Daily Living MG-ADL) following MuSK-CAART infusion.** Myasthenia Gravis Activities of Daily Living (MG-ADL) was assessed for each of the six patients at screening, pre- and post-infusion timepoints. Depicted here are MG-ADL values at pre-infusion and post-infusion up to six months. A decrease in 2 points from baseline (pre-infusion) represents a clinically significant improvement.

## Conclusions

- MuSK-CAART cells were detected in the circulation of all patients by qPCR and flow cytometry at both dose levels examined, 5x$10^8$ and 2.5x$10^9$ transduced cells (data unavailable for A2-3 and A2-4).
  - Peak expansion ($C_{max}$) was similar for the two dose levels investigated whereas total MuSK-CAART cell exposure over the first 3 months (AUC) was higher for patients dosed with 2.5x$10^9$ compared to 5x$10^8$ cells. Patient A2-1 exhibited prolonged high exposure through the first six months post-infusion.
  - Flow cytometric analysis of post-infusion MuSK-CAART cell levels paralleled qPCR results. Persisting CAAR+ T cells generally maintained the CD4/CD8 ratio established at baseline in the manufactured product. MuSK-CAART cell activation, measured by HLA-DR, was reduced compared to the MP within the first 2 weeks post-infusion.
  - Serum IFNγ levels peaked immediately after MuSK-CAART cell infusion at both dose levels and returned to baseline within the first month after infusion.
- T cell clonality in patient A2-1 is highly diverse at all time points and there is no major change in the distribution of top clones except for the infusion product which displays the most diversity.
- Two out of six patients (A1-1 and A2-2) exhibited a >20% auto-antibody drop within the first month after treatment with MuSK-CAART.
- Two out of six patients (A1-1 and A2-2) exhibited a clinically meaningful improvement in MG activities of daily living (MG-ADL) within the first month that was sustained (insufficient follow up time for A2-3 and A2-4).
- MuSK-CAART treatment of anti-MuSK positive MG patients showed severe adverse events only at a 2.5 x $10^9$ cell dose. No severe adverse events were observed at a 5 x $10^8$ cell dose.
- In summary, there is evidence of biological activity and clinical effect with MuSK-CAART cells alone, without preconditioning, suggesting that it may be possible to achieve clinical activity with CART cells in autoimmune disease without preconditioning.