



# Safety and clinical activity of autologous RNA chimeric antigen receptor T-cell therapy in myasthenia gravis (MG-001): a prospective, multicentre, open-label, non-randomised phase 1b/2a study

Volkan Granit\*, Michael Benatar\*, Metin Kurtoglu, Miloš D Miljković, Nizar Chahin, Gregory Sahagian, Marc H Feinberg, Adam Slansky, Tuan Vu, Christopher M Jewell, Michael S Singer, Murat V Kalayoglu, James F Howard Jr†, Tahseen Mozaffar† on behalf of the MG-001 Study Team‡

## Summary

**Background** Chimeric antigen receptor (CAR) T cells are highly effective in treating haematological malignancies, but associated toxicities and the need for lymphodepletion limit their use in people with autoimmune disease. To explore the use of CAR T cells for the treatment of people with autoimmune disease, and to improve their safety, we engineered them with RNA (rCAR-T)—rather than the conventional DNA approach—to target B-cell maturation antigen (BCMA) expressed on plasma cells. To test the suitability of our approach, we used rCAR-T to treat individuals with myasthenia gravis, a prototypical autoantibody disease mediated partly by pathogenic plasma cells.

**Methods** MG-001 was a prospective, multicentre, open-label, phase 1b/2a study of Descartes-08, an autologous anti-BCMA rCAR-T therapy, in adults (ie, aged  $\geq 18$  years) with generalised myasthenia gravis and a Myasthenia Gravis Activities of Daily Living (MG-ADL) score of 6 or higher. The study was done at eight sites (ie, academic medical centres or community neurology clinics) in the USA. Lymphodepletion chemotherapy was not used. In part 1 (phase 1b), participants with Myasthenia Gravis Foundation of America (MGFA) disease class III–IV generalised myasthenia gravis received three ascending doses of Descartes-08 to determine a maximum tolerated dose. In part 2 (phase 2a), participants with generalised myasthenia gravis with MGFA disease class II–IV received six doses at the maximum tolerated dose in an outpatient setting. The primary objective was to establish safety and tolerability of Descartes-08; secondary objectives were to assess myasthenia gravis disease severity and biomarkers in participants who received Descartes-08. This trial is registered with clinicaltrials.gov, NCT04146051.

**Findings** We recruited 16 individuals for screening between Jan 7, 2020 and Aug 3, 2022. 14 participants were enrolled (n=3 in part 1, n=11 in part 2). Ten participants were women and four were men. Two individuals did not qualify due to low baseline MG-ADL score (n=1) or lack of generalised disease (n=1). Median follow-up in part 2 was 5 months (range 3–9 months). There was no dose-limiting toxicity, cytokine release syndrome, or neurotoxicity. Common adverse events were headache (six of 14 participants), nausea (five of 14), vomiting (three of 14), and fever (four of 14), which resolved within 24 h of infusion. Fevers were not associated with increased markers of cytokine release syndrome (IL-6, IL-2, and TNF). Mean improvements from baseline to week 12 were –6 (95% CI –9 to –3) for MG-ADL score, –7 (–11 to –3) for Quantitative Myasthenia Gravis score, –14 (–19 to –9) for Myasthenia Gravis Composite score, and –9 (–15 to –3) for Myasthenia Gravis Quality of Life 15-revised score.

**Interpretation** In this first study of an rCAR-T therapy in individuals with an autoimmune disease, Descartes-08 appeared to be safe and was well tolerated. Descartes-08 infusions were followed by clinically meaningful decreases on myasthenia gravis severity scales at up to 9 months of follow-up. rCAR-T therapy warrants further investigation as a potential new treatment approach for individuals with myasthenia gravis and other autoimmune diseases.

**Funding** Cartesian Therapeutics and National Institute of Neurological Disorders and Stroke of the National Institutes of Health.

**Copyright** © 2023 Elsevier Ltd. All rights reserved.

## Introduction

Chimeric antigen receptor (CAR) T cells have been hailed as a versatile new class of effective, molecularly precise therapy. The CAR molecule combines the extracellular target binding domain of an antibody directed toward the desired target with the intracellular T-cell activation protein domains.<sup>1</sup> This combination enables T-cell

activation on contact with the target cell antigen, bypassing antigen presenting cells and many regulatory checkpoints.<sup>2</sup> However, due to their dependence on preconditioning lymphodepletion chemotherapy and association with severe toxicities, conventional CAR T cells have been reserved mainly for the treatment of individuals with advanced cancers.<sup>3</sup>

Lancet Neurol 2023; 22: 578–90

See [Comment](#) page 545

\*First authors contributed equally

†Senior authors contributed equally

‡Members are listed in the appendix

Department of Neurology, University of Miami, Miami, FL, USA (V Granit MD,

Prof M Benatar MD PhD); Cartesian Therapeutics, Gaithersburg, MD, USA

(M Kurtoglu MD PhD,

M D Miljković MD,

C M Jewell PhD,

M S Singer MD PhD,

M V Kalayoglu MD PhD);

Department of Neurology,

Oregon Health and Sciences

University, Portland, OR, USA

(N Chahin MD); Neurology

Center of Southern California,

San Diego, CA, USA

(G Sahagian MD); SFM Research,

Boca Raton, FL, USA

(M H Feinberg MD); Neurology

Associates, Orlando, FL, USA

(A Slansky MD); Department of

Neurology, University of

South Florida, Tampa, FL, USA

(Prof T Vu MD); Department of

Neurology, University of

North Carolina at Chapel Hill,

Chapel Hill, NC, USA

(Prof J F Howard Jr MD);

Department of Neurology,

University of California Irvine,

Irvine, CA, USA

(Prof T Mozaffar MD)

Correspondence to:

Prof James F Howard Jr,

Department of Neurology,

University of North Carolina at

Chapel Hill, Chapel Hill,

NC 27599–7025, USA

howardj@neurology.unc.edu

See Online for appendix

## Research in context

### Evidence before this study

We searched MEDLINE, Embase, and PubMed databases from inception to Feb 5, 2023, for relevant clinical studies in myasthenia gravis on the use of cell therapy, with no date or language restrictions. The search terms used were “cell therapy” (or “chimeric antigen receptor”), and “myasthenia gravis.” We did not identify any reports on the use of cell therapy, whether autologous or allogeneic, unmodified or engineered to express chimeric antigen receptors, in clinical studies of any phase. There was a single report on the use of engineered T cells in an experimental autoimmune myasthenia gravis mouse model.

### Added value of this study

MG-001 shows the feasibility of producing autologous RNA chimeric antigen receptor T cells (rCAR-T) from individuals with generalised myasthenia gravis receiving a background therapy of prednisone or steroid-sparing immunosuppressants, or both. The method of CAR expression through RNA engineering obviated the need for lymphodepletion chemotherapy, which is required for conventional, DNA-engineered CART cells.

Repeated rCAR-T infusions were not associated with cytokine release syndrome, neurotoxicity, or haematological adverse events typical of DNA CAR T cells. Treatment of participants with weekly or twice weekly infusions for six doses was associated with clinically meaningful decreases in all measures of myasthenia gravis severity, including induction of minimal symptom expression and elimination of dependence on intravenous immunoglobulin infusions in some participants. These effects were persistent at up to 9 months of follow-up.

### Implications of all the available evidence

This study shows the feasibility of rCAR-T as a novel treatment for generalised myasthenia gravis. rCAR-T might offer an improved safety profile compared with other forms of CAR-T therapy. Furthermore, this therapeutic approach could result in a numerical decrease on myasthenia gravis severity scales equal to or above what is considered clinically meaningful for months after treatment. Our findings require corroboration in an ongoing randomised, double-masked, placebo-controlled trial. More broadly, these results might support a new strategy that uses rCAR-T to combat autoimmunity beyond myasthenia gravis.

Conventional CAR T-cell engineering relies on DNA to express the CAR, and gene transfer underlies much of the observed toxicities of these cells.<sup>4</sup> The DNA is integrated permanently into the T-cell genome and replicates with each cell division.<sup>5</sup> Lymphodepletion, usually with fludarabine and cyclophosphamide, is necessary before administration to create the appropriate cytokine environment for the infused DNA CAR T cells to proliferate *in vivo* and reach their therapeutic concentration.<sup>4</sup> However, as the activated cells proliferate, the CAR signal is also amplified. This amplification leads to unpredictable pharmacokinetics and characteristic severe adverse events, such as cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome, that extend hospitalisation after treatment.<sup>6</sup> These aspects of DNA CAR T cells limit their suitability for use beyond advanced cancers. To date, within autoimmune disease indications, only patients with severe forms of systemic lupus erythematosus and neuromyelitis optica have received CAR T-cell therapies. These therapies have been restricted to DNA-based approaches and only in the context of expanded use and under extended hospital monitoring.<sup>7,8</sup>

To expand the range of disorders that are treatable with CAR T cells beyond cancer, we engineered these cells with RNA (rCAR-T), rather than DNA, on the premise that the temporary, non-replicable influence of mRNA would confer predictable pharmacokinetics and consequently a more favourable safety profile. rCAR-T uses the same advances in RNA engineering that enabled the widespread use of mRNA vaccines—ie, optimal 3' and 5' untranslated regions, poly(A) tail length, and 5' capping

to increase mRNA stability and enhance translational efficiency.<sup>9</sup> Since the CAR-encoding mRNA does not replicate together with the activated and proliferating rCAR T cells, the load of CAR<sup>+</sup> cells is determined and limited by the administered dose and declines over time, potentially enabling more precise pharmacokinetic control over the therapy than for CAR T cells engineered with DNA. Because our approach uses *ex vivo* T-cell proliferation, it does not require the specific cytokine environment that is induced by lymphodepletion. Although the therapeutic effects achieved with these approaches might be lasting, reaching the full therapeutic effect requires repeat dosing; thus, a robust manufacturing platform is required to generate enough autologous rCAR T cells.

To test the suitability of our approach in autoimmunity, we engineered rCAR-T to treat individuals with generalised myasthenia gravis, a prototypical autoimmune disease in which autoantibodies target the neuromuscular junction, causing chronic, fluctuating, and potentially debilitating weakness and muscle fatigue. A substantial unmet medical need exists for people with myasthenia gravis whose disease does not respond to current (typically immunosuppressive) therapies or who have serious side-effects.<sup>10–14</sup> Autoantibody-producing plasma cells are a key cellular component of myasthenia gravis pathophysiology.<sup>15,16</sup> Existing myasthenia gravis therapies do not adequately or specifically target plasma cells.<sup>17</sup> The specific expression of B-cell maturation antigen (BCMA, also known as TNFRSF17) on the surface of mature plasma cells provides an opportunity to do so.

Here, we report the results of a prospective, multicentre,

open-label clinical trial to test the safety and preliminary clinical efficacy of Descartes-08, an anti-BCMA rCAR-T therapy,<sup>18</sup> in individuals with generalised myasthenia gravis.

## Methods

### Study design

MG-001 was a prospective, open-label, multicentre, non-randomised, phase 1b/2a trial evaluating the safety and clinical activity of Descartes-08, an anti-BCMA rCAR-T therapy, in adults with generalised myasthenia gravis who required immunosuppression. We conducted the trial across eight study sites in the US states of Florida, California, North Carolina, and Oregon; five study sites were academic medical centres and three were community neurology clinics.

The study was conducted in accordance with the principles of the Declaration of Helsinki, the Good Clinical Practice guidelines, and applicable US regulatory standards. Independent institutional review boards provided written approval of the protocol and amendments. All participants provided written informed consent. The protocol is included in the appendix (pp 10–81).

### Participants

Study participants were recruited from the existing patient population at each site and from outside referrals. Key inclusion criteria were: age 18 years or older; diagnosis of myasthenia gravis (Myasthenia Gravis Foundation of America disease class III–IV in part 1, class II–IV in part 2) with presence of a myasthenia gravis-associated autoantibody (anti-acetylcholine receptor [AChR], MuSK, or LRP4). If seronegative, unequivocal response to cholinesterase inhibitors and abnormal repetitive nerve stimulation or increased jitter on single-fibre electromyogram were required. Participants had to have a Myasthenia Gravis Activities of Daily Living (MG-ADL) score of at least 6 at both screening and baseline and require immunosuppression. Key exclusion criteria were the presence of a major chronic illness that was not well managed; intravenous immunoglobulin or plasma exchange within 4 weeks of baseline (ie, first infusion) visit; and the use of non-permitted immune modulators. The full eligibility criteria are shown in the appendix (pp 43–45). Sex was self-reported by participants from the options of male or female.

### Procedures

Eligible participants underwent leukapheresis to obtain peripheral blood mononuclear cells (PBMCs), from which Descartes-08 was prepared following Good Manufacturing Practices. Immunosuppression was not withheld before the collection of PBMCs. Descartes-08 is an autologous CD8<sup>+</sup> T-cell-only product that is transfected with RNA to express anti-BCMA targeting CAR protein over the course of a week. Only CD8<sup>+</sup> T cells are used for

manufacturing, since CD4<sup>+</sup> T cells are known for their memory function rather than direct killing function, which is not relevant to RNA-transfected CAR T cells that express the CAR molecule over days. Following leukapheresis, CD8 selection and manufacturing of autologous Descartes-08 cells, including their ex vivo proliferation and mRNA transfection, was performed. The autologous product was divided into several samples, frozen, and tested for sterility, cell quality, CAR expression, and potency. Samples of cells were thawed at each infusion visit and infused by a peripheral intravenous line.

For part 1, participants were admitted to the hospital for their first infusion and observed as inpatients for 3 days and thereafter daily as an outpatient until day 7. In part 1 of the study, each participant received three ascending once-weekly doses of Descartes-08 as a 15–30 min intravenous infusion of  $3.5 \times 10^6$  CAR<sup>+</sup> cells per kg (dose level 1),  $17.5 \times 10^6$  CAR<sup>+</sup> cells per kg (dose level 2), and  $52.5 \times 10^6$  CAR<sup>+</sup> cells per kg (dose level 3) to establish the maximum tolerated dose (MTD) at the safety interim analysis, before the study proceeded to part 2 (appendix p 42). The allowed dose margin was  $\pm 45\%$  for all dose levels. Participants in part 2 were assigned to a treatment group, as described later, at the investigator's discretion, taking into consideration each participant's preferences (predominantly decided by the time burden of each infusion schedule) and enrolment to date. Part 2 tested three dosing schedules of 15–30 min intravenous infusion at the MTD: twice weekly for 3 weeks (group 1), once weekly for 6 weeks (group 2), and once monthly for 6 months (group 3). Participants were to be evaluated at screening; infusion visits; weeks 8, 12, 16, and 20; and months 6, 9, and 12. Enrolment into part 2 would stop when sufficient data on safety, feasibility, and clinical activity had been gathered to permit the selection of the dosing schedule to be tested in later-stage trials. Participants who received Descartes-08 as outpatients were observed for an hour after infusion. Any participant with a fever of 38°C or higher (ie,  $\geq 100.4^\circ\text{F}$ ) within 7 days of infusion was admitted for 24 h of observation and evaluated for an infectious cause with blood and urine cultures. These admissions were not considered to be serious adverse events.

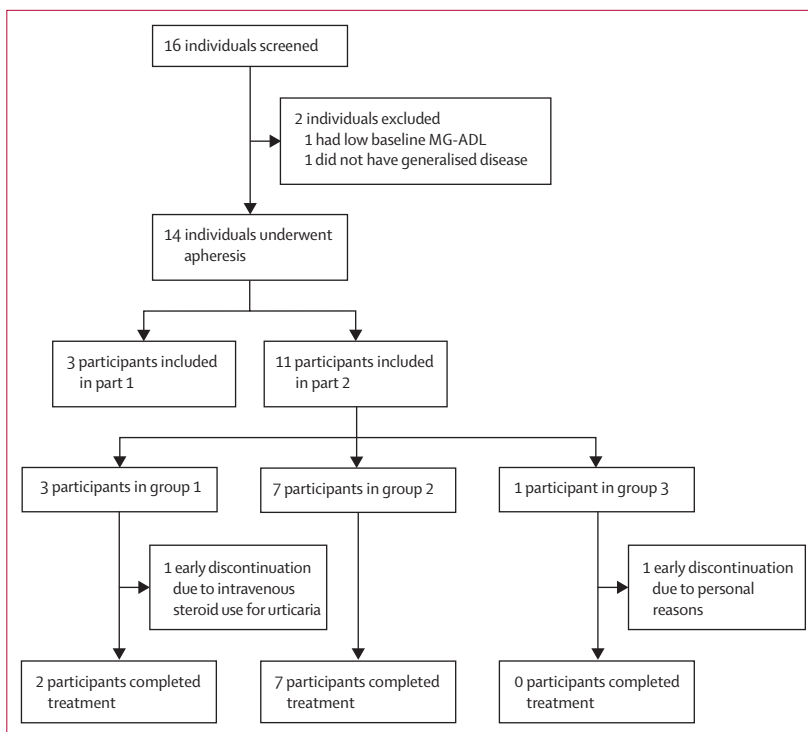
Permitted myasthenia gravis concomitant medications were corticosteroids (ie, equivalent to  $\leq 40$  mg prednisone per day), azathioprine, mycophenolate mofetil, pyridostigmine, and complement inhibitors, provided the dose was stable for at least 8 weeks before the first infusion. No dosing change was allowed for concomitant myasthenia gravis-specific medications during the study, other than corticosteroids. The dose of corticosteroids was not allowed to be increased, but it could be tapered at the site investigator's discretion after week 4. Intravenous immunoglobulin and plasma exchange were prohibited within 4 weeks of baseline and during the study. Other biological agents, including rituximab and efgartigimod, were prohibited within 8 weeks of baseline and during

the study.

### Outcomes

The primary objective of part 1 was to establish tolerability, with the primary endpoints being the MTD and the type and frequency of adverse events in all eligible participants. The primary endpoint of part 2 was safety (ie, frequency and severity of adverse events) to final follow-up in all eligible participants. Secondary endpoints, which were evaluated in all participants who had at least one disease evaluation after day 1 (baseline) visit, were mean changes from baseline at each follow-up visit for up to 12 months in four validated scales of myasthenia gravis severity: MG-ADL, Quantitative Myasthenia Gravis (QMG), Myasthenia Gravis Composite (MGC), and Myasthenia Gravis Quality of Life 15-revised (MG-QoL-15r), as well as the Myasthenia Gravis Post-Intervention Status. MG-ADL is an eight-item, 24-point, patient-reported scale that assesses the effects of myasthenic symptoms on daily functioning. By convention, a 2-point change is considered clinically meaningful.<sup>19</sup> Minimal symptom expression is defined as MG-ADL of 0 or 1.<sup>20</sup> QMG is a standardised, quantitative, 39-point scoring system consisting of 13 provider-assessed items, which include hand grip strength and forced vital capacity. MGC is a ten-item, 60-point weighted instrument composed of selected components of the MG-ADL and QMG scores. A 3-point change in QMG and MGC is considered clinically meaningful.<sup>21,22</sup> MG-QoL-15r is a 15-item, 30-point quality-of-life, patient-reported instrument.<sup>23</sup> Currently, there is no consensus as to what is considered a clinically meaningful change in MG-QoL-15r. Anti-AChR antibody titres were measured by radioimmunoassay in a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Quest Diagnostics, Secaucus, NJ, USA). Anti-MuSK and anti-LRP4 antibodies were measured by semiquantitative cell-based cluster assay (University of Oxford, Oxford, UK).<sup>24</sup>

In a prespecified exploratory analysis, we evaluated pharmacokinetics using quantitative RT-PCR for CAR mRNA in all participants who received at least one dose of Descartes-08. ELISA was used to measure the concentration of soluble BCMA (R&D Systems, Minneapolis, MN, USA) in a prespecified analysis, and amounts of BAFF (TNFSF13B; R&D Systems, Minneapolis, MN, USA) and APRIL (TNFSF13; Invitrogen, Washington, DC, USA) were measured in a post-hoc analysis. Soluble BCMA is a surrogate measure of total plasma cells; BAFF and APRIL are markers of B-cell survival. Tetanus, diphtheria, pertussis, meningococcus, and SARS-CoV-2 antibody titres and immunoglobulin concentrations were measured in a CLIA-certified laboratory (Quest Diagnostics) to evaluate plasma cell function and humoral immunity after Descartes-08 in a prespecified analysis. Immunophenotyping of B cells, T cells, and dendritic cells was performed by flow cytometry. Serum cytokine concentrations were measured using a multiplex



**Figure 1: Trial profile**

MG-ADL=Myasthenia Gravis Activities of Daily Living. All 14 eligible participants were included in the primary analysis of the endpoint.

bead-based assay (BioLegend, San Diego, CA, USA) in a prespecified analysis. Post-hoc, high-throughput, next-generation sequencing of complementarity-determining region 3 from cDNA was conducted to follow distinct T-cell receptor clonotypes (Adaptive Biotechnologies, Seattle, WA, USA).

### Statistical analysis

As this was an early-phase, open-label study with a dose-escalation regimen, no formal power analysis was performed. Baseline demographics and primary endpoints (ie, type and frequency of adverse events) were analysed in all enrolled participants using descriptive statistics. Categorical variables were expressed as percentages, and continuous variables were expressed as mean and SD, or median and range for variables with skewed distribution (including those with mean:SD ratio <2). Secondary and exploratory endpoints in all participants who received at least one dose of Descartes-08 were expressed as mean change with a 95% CI, or proportion with SE. SE of proportion was calculated as  $\sqrt{\hat{p}(1-\hat{p})/n}$ , where  $\hat{p}$  is the sample proportion and  $n$  is the sample size. We calculated critical values for the 95% CI on the basis of the  $t$  distribution using a two-tailed test with significance level 0.05 and  $n-1$  degrees of freedom since the sample size was less than 30 in all analyses. To assess normality, we first used a quantile-quantile plot to compare quantiles of our data to quantiles

	Part 1 participants (n=3)	Part 2 participants (n=11)			All participants (n=14)
		Group 1 (n=3)	Group 2 (n=7)	Group 3 (n=1)	
Age, years	57 (16)	43 (26)	52 (17)	70	52 (18)
Sex					
Female	2 (67%)	2 (67%)	5 (71%)	1 (100%)	10 (71%)
Male	1 (33%)	1 (33%)	2 (29%)	0	4 (29%)
Weight, kg	83 (26)	80 (23)	88 (20)	71	84 (21)
BMI, kg/m <sup>2</sup>	31 (5.8)	26 (0.9)	34.5 (8.4)	28.5	31.6 (8.1)
Race and ethnicity					
White, non-Hispanic	1 (33%)	2 (67%)	7 (100%)	1 (100%)	11 (79%)
White, Hispanic	1 (33%)	0	0	0	1 (7%)
Asian	1 (33%)	1 (33%)	0	0	2 (14%)
MGFA class at screening					
II	0	0	3 (43%)	0	3 (21%)
III	3 (100%)	2 (67%)	4 (57%)	1 (100%)	10 (71%)
IV	0	1 (33%)	0	0	1 (7%)
Age at disease onset, years	44 (25–58)	25 (15–54)	26 (14–79)	44	40 (14–79)
Duration of disease, years	13 (5–25)	14 (4–15)	16 (3–27)	26	14 (3–27)
Myasthenia gravis antibody status					
Anti-AChR antibody	3 (100%)	3 (100%)	4 (57%)	1 (100%)	11 (79%)
Anti-MuSK antibody	0	0	2 (29%)	0	2 (14%)
Seronegative (for AChR, MuSK, and LRP4 antibodies)	0	0	1 (14%)	0	1 (7%)
Baseline score					
QMG	14.3 (3.5)	17.3 (1.5)	15.9 (4.5)	8.0	15.3 (4.1)
MG-ADL	8.7 (3.8)	10.7 (3.5)	10.7 (3)	6.0	10.0 (3.2)
MGC	19.3 (6.7)	24.7 (4.7)	23.0 (5.2)	13.0	21.9 (5.7)
MG-QoL-15r	23.3 (3.1)	19.1 (9)	19.7 (5.1)	12.0	19.9 (5.8)
Previous myasthenia gravis therapies (standard of care)					
Pyridostigmine	3 (100%)	3 (100%)	7 (100%)	1 (100%)	14 (100%)
Prednisone	3 (100%)	3 (100%)	7 (100%)	1 (100%)	14 (100%)
Other immunosuppressants	3 (100%)	3 (100%)	7 (100%)	1 (100%)	14 (100%)
Eculizumab	0	0	2 (29%)	0	2 (14%)
Rituximab	0	0	2 (29%)	0	2 (14%)
Previous intravenous immunoglobulin	1 (33%)	3 (100%)	7 (100%)	1 (100%)	12 (86%)
Previous plasma exchange	0	3 (100%)	5 (71%)	0	8 (57%)
Diagnosis of thymoma	0	3 (100%)	0	0	0
Previous thymectomy	1 (33%)	2 (67%)	3 (43%)	0	6 (43%)
Previous myasthenia gravis crisis requiring intubation	1 (33%)	1 (33%)	2 (19%)	0	4 (29%)
Myasthenia gravis ongoing therapy					
Pyridostigmine	2 (67%)	3 (100%)	6 (86%)	0	11 (79%)
Prednisone	3 (100%)	2 (67%)	5 (71%)	0	10 (71%)
Azathioprine	0	0	1 (14%)	0	1 (7%)
Mycophenolate mofetil	1 (33%)	0	0	0	1 (7%)

Data are mean (SD), n (%), or median (range). Part 1 involved inpatient dose escalation. For part 2, group 1 received twice weekly dosing, group 2 received weekly dosing, and group 3 received monthly dosing. Baseline characteristics of individual participants are shown in the appendix (p 5). AChR=acetylcholine receptor. MG-ADL=Myasthenia Gravis Activities of Daily Living. MGC=Myasthenia Gravis Composite. MGFA=Myasthenia Gravis Foundation of America. MG-QoL-15r=Myasthenia Gravis Quality of Life 15-revised. QMG=Quantitative Myasthenia Gravis.

**Table 1: Demographics and baseline characteristics**

of normal distribution. When n was 4 or more, we used a Shapiro-Wilk test, with  $p > 0.05$  considered to be normal distribution,<sup>25,26</sup> and presented individual data points when n was 3 or fewer. When absolute change in exploratory biomarkers from baseline showed skewed

distribution, relative change was used instead. All analyses were performed using Mathematica version 13.1.0.0 (Wolfram Research, Champaign, IL, USA).

Safety monitoring was performed by the site investigator,

the sponsor (Cartesian Therapeutics) medical monitor, and an external monitoring committee. Data were entered by research staff at each site into a 21 Code of Federal Regulations Part 11 compliant electronic database, which was analysed by the study sponsor. In part 1, safety was evaluated after each infusion by the site investigator and sponsor medical monitor. Throughout the study, the clinical data were reviewed periodically by a sponsor-funded safety monitoring committee comprising experts with no other relationship with the sponsor or trial.

This trial is registered with [clinicaltrials.gov](https://clinicaltrials.gov), NCT04146051.

### Role of the funding source

Cartesian Therapeutics had a role in the design of the study, data collection, interpretation, analysis, writing of the manuscript, and the decision to submit.

### Results

Site investigators recruited 16 individuals for screening between Jan 7, 2020 and Aug 3, 2022. Two individuals did not qualify due to low baseline MG-ADL score (n=1) or lack of generalised disease (n=1; figure 1). Of the 14 participants included (ten women and four men), three participants were assigned to part 1 of the study, and 11 participants were assigned to part 2 (n=3 to receive MTD twice weekly for 3 weeks [group 1], n=7 to receive MTD once weekly for 6 weeks [group 2], and n=1 to receive MTD once monthly for 6 months [group 3]). 14 patients, aged 18–83 years, meeting all eligibility criteria, received at least one dose of Descartes-08, and were included in the safety analysis. Most participants had Myasthenia Gravis Foundation of America class III disease (table 1). Most participants continued use of pyridostigmine, and most continued receiving corticosteroids throughout the study (mean dose 19.5 mg/day [SD 12.1]). All participants had previously received at least one of intravenous immunoglobulin, corticosteroids, non-steroidal immune suppressants, or plasma exchange.

We successfully produced Descartes-08 from all participants despite their use of immunosuppressants, with a potency similar to that from a healthy volunteer (appendix p 3). Overall, final cell products were median 99.1% CD8<sup>+</sup> (range 99.1–99.7), 77.0% CD3<sup>+</sup>CD56(NCAM1)<sup>-</sup> (95% CI 69.2–76.7), and 92.0% P1<sup>+</sup> (95% CI 89.1–95), with low exhaustion markers CD57 (B3GAT1) and PD1, and downregulation of IKZF2 compared with original PBMCs (appendix p 6). Three participants in part 1 received a median of  $6.4 \times 10^9$  CAR<sup>+</sup> cells (range 6.3–7.2) over three infusions. In part 2, 11 participants received a median of  $17.3 \times 10^9$  CAR<sup>+</sup> cells (range 9.65–33.12) divided across a median of six infusions (range three to six). Two (18%) of 11 participants in part 2 withdrew and did not complete all planned infusions: one due to urticaria (group 1) and another for personal reasons unrelated to safety (group 3).

	Grade*	Part 1 (n=3)	Part 2: all groups (n=11)	Part 2: group 1 (n=3)	Part 2: group 2 (n=7)	Part 2: group 3 (n=1)
Hand numbness	2	1 (33%)	0	0	0	0
Headache	1	1 (33%)	5 (45%)	1 (33%)	3 (43%)	1 (100%)
Muscle soreness	1	1 (33%)	1 (9%)	0	1 (14%)	0
Nausea	1	1 (33%)	4 (36%)	2 (67%)	2 (29%)	0
Rash	3	0	1 (9%)	1 (33%)	0	0
Itchy throat	1	0	2 (18%)	0	1 (14%)	1 (100%)
Vomiting	1	0	3 (27%)	2 (67%)	1 (14%)	0
Weakness	1	0	2 (18%)	2 (67%)	0	0
Line infiltration	1	0	1 (9%)	1 (33%)	0	0
Fever	1	0	4 (36%)	1 (33%)	3 (43%)	0
Shortness of breath†	1	0	2 (18%)	1 (33%)	1 (14%)	0
Chills	1	0	2 (18%)	1 (33%)	1 (14%)	0
Diarrhoea	1	0	1 (9%)	1 (33%)	1 (14%)	0
Gum inflammation	1	0	1 (9%)	0	1 (14%)	0
Teeth sensitivity	1	0	1 (9%)	0	1 (14%)	0
Night sweats	1	0	1 (9%)	0	1 (14%)	0
Restless leg	1	0	1 (9%)	0	1 (14%)	0
Light-headedness	1	0	1 (9%)	0	1 (14%)	0

Data are n (%). Each adverse event was counted once per patient, at the highest grade reported. Relatedness includes possibly, probably, or likely. \*There were no adverse events of grade 3 or higher reported in part 1, and no grade 2 or grade 4 events reported in part 2, where grade 1 is mild, grade 2 is moderate, grade 3 is severe, and grade 4 is life-threatening. †Not associated with hypoxia.

**Table 2: Adverse events related to Descartes-08 per investigators' assessment**

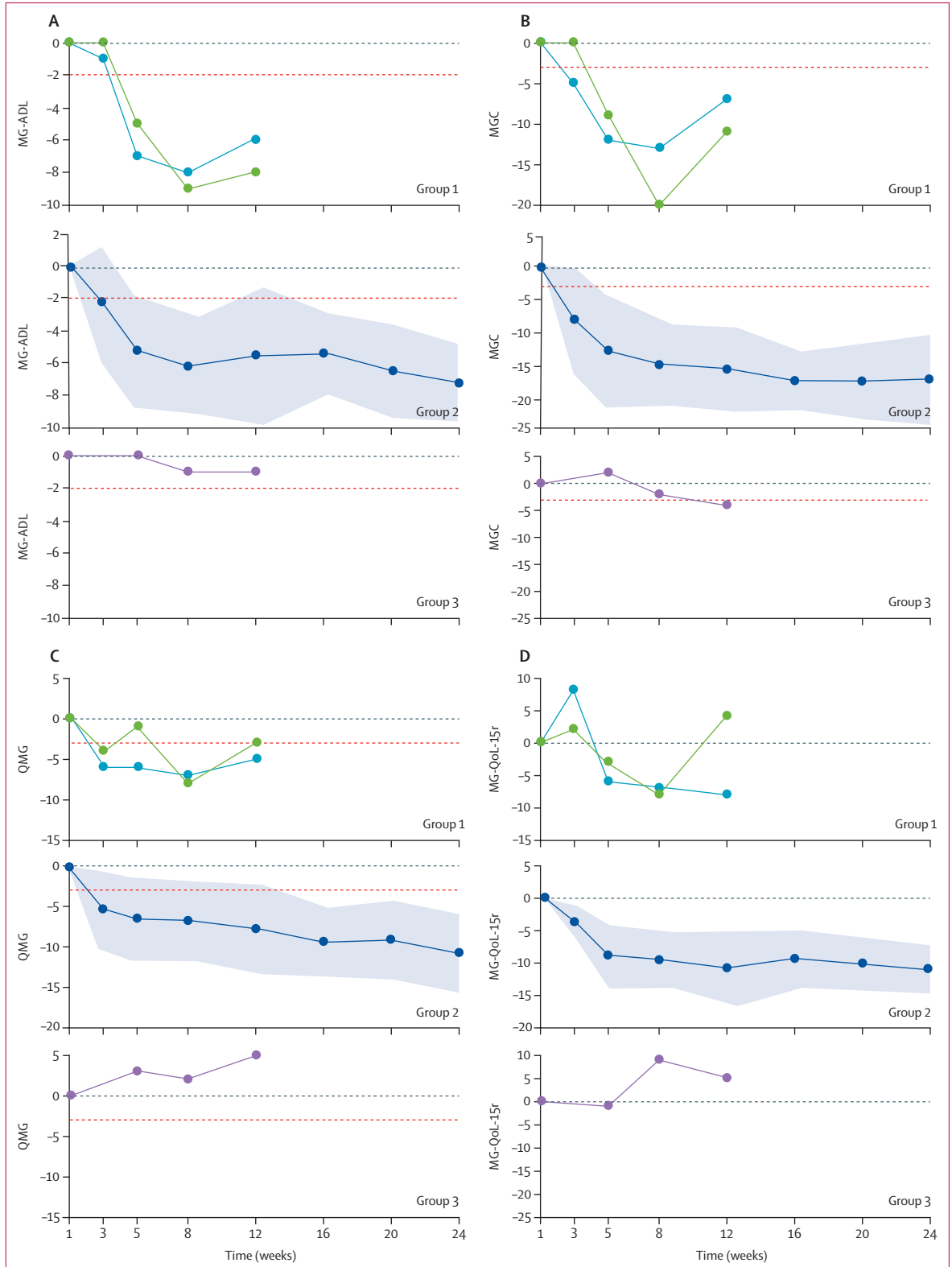
There were no dose-limiting toxicities, treatment-related serious adverse events, or adverse events of grade 3 or higher in part 1 (table 2), making dose level 3 (ie,  $52.5 \times 10^6$  CAR<sup>+</sup> cells per kg) the MTD. One serious adverse event unrelated to Descartes-08 (ie, grade 2 influenza requiring admission to hospital, which occurred after apheresis but before treatment initiation) was reported in part 1. Two serious adverse events were reported in part 2. The first was grade 3 urticaria 24 h after the third infusion in a participant with a previous history of drug-induced urticaria; skin biopsy was consistent with a typical drug reaction, and serum tryptase and cytokine levels were normal (appendix p 7). The urticaria was deemed possibly related to Descartes-08 and resolved completely after the administration of intravenous steroids. Per protocol, the participant was taken off-study due to steroid administration, and no myasthenia gravis severity assessments were performed after treatment. The second serious adverse event was a non-ST segment elevation myocardial infarction occurring 72 h after the sixth infusion in an 83-year-old man with a history of hypertension and hyperlipidaemia. Coronary angiography showed multivessel disease requiring revascularisation, and the patient fully recovered. The investigator deemed the event to be unrelated to Descartes-08.

No patient had evidence of functional immunosuppression (ie, new hypogammaglobulinaemia or disappearance of previously therapeutic concentrations of

**Figure 2: Change from baseline in disease severity scores for participants enrolled in part 2**

Individual changes from baseline are shown for participants in groups 1 and 3, and mean changes from baseline are shown for participants in group 2. Change from baseline in MG-ADL score (A), MGC score (B), QMG score (C), and MG-QoL-15r score (D). Group 1 received twice weekly infusions (n=2, first post-treatment follow-up at week 5). Group 2 received once weekly infusions (n=7 up to week 16, n=6 up to week 20, and n=5 up to week 24; first post-treatment follow-up at week 8). Group 3 received monthly infusions (n=1; first follow-up after treatment was at week 36 but was not reached due to withdrawal from the study). One of three participants in group 1 withdrew from the study before the first assessment after treatment and is not presented here. The single group 3 participant withdrew from the study after receiving three of six planned monthly infusions. The shaded bands represent 95% CI. Dashed red lines indicate clinically meaningful decrease. Currently, there is no consensus as to what is considered a clinically meaningful change in MG-QoL-15r.

MG-ADL=Myasthenia Gravis Activities of Daily Living. QMG=Quantitative Myasthenia Gravis. MGC=Myasthenia Gravis Composite. MG-QoL-15r=Myasthenia Gravis Quality-of-Life 15-revised.



	All participants who completed treatment in part 2 (n=9)	By treatment group		By myasthenia gravis type		
		Group 1 (n=2)	Group 2 (n=7)	AChR antibody-positive (n=6)	MuSK antibody-positive (n=2)	Seronegative (n=1)
<b>Mean score change (95% CI)*</b>						
MG-ADL	-5.9 (-9 to -2.8)	-6, -8	-6 (-15 to 3)	-6 (-11 to -1)	-3, -4	-8
QMG	-7 (-11 to -3)	-5, -3	-8 (-20 to 4)	-5 (-10 to 0)	-9, -5	-17
MGC	-14 (-19 to -9)	-7, -11	-15 (-29 to -1)	-14 (-21 to -7)	-14, -7	-22
MG-QoL-15r	-9 (-15 to -3)	-8, 4	-11 (-23 to 1)	-8 (-17 to 1)	-10, -6	-14
<b>Number of participants with improvement (%)</b>						
MG-ADL decrease $\geq 2$ points	8 (89%)	2 (100%)	6 (86%)	5 (83%)	2 (100%)	1 (100%)
MGC decrease $\geq 3$ points	9 (100%)	2 (100%)	7 (100%)	6 (100%)	2 (100%)	1 (100%)
QMG decrease $\geq 3$ points†	8 (89%)	2 (100%)	6 (86%)	5 (83%)	2 (100%)	1 (100%)
MG-ADL decrease $\geq 6$ points‡	5 (56%)	2 (100%)	3 (43%)	4 (67%)	0	1 (100%)

Data are for participants in groups 1 and 2 of part 2 who completed all six infusions and 12-week follow-up. One group 1 participant withdrew from the study before the first assessment after treatment. Clinical efficacy outcomes for the single group 3 participant are shown in figure 1. AChR=acetylcholine receptor. \*Individual values are presented for groups of  $\leq 2$  participants. †All participants who had the prespecified  $\geq 2$ -point improvement in QMG also had a  $\geq 3$ -point improvement. ‡Post-hoc analysis of depth of response.

**Table 3: Measures of disease severity at week 12**

vaccine titres) or opportunistic infection. Headache, fever, and nausea were the most frequently reported treatment-related adverse events in part 2. All fevers occurred 4–6 h after infusion and resolved within 24 h. None of these participants had positive urine or blood cultures or received empirical antibiotics. Serum cytokine concentrations from febrile participants showed increases in IFN $\gamma$  and its downstream chemokines, CXCL10 and CCL2, but not in IL-2, IL-6, or TNF (appendix p 7). There were no episodes of hypotension, hypoxia, or requirement for the use of tocilizumab or steroids for treatment or prevention of cytokine release syndrome.

Participants in part 1 showed variable reductions in mean MG-ADL, QMG, MGC, and QoL-15r scores at weeks 3–24 (appendix p 3). One participant tapered their dose of prednisone from 40 mg daily before treatment to 25 mg daily at final (ie, 12-month) follow-up.

Participants in part 2 who were enrolled in groups 1 and 2 and had at least one disease evaluation after day 1 (baseline) had decreases compared with baseline in all disease severity scores by week 5; in group 2, these reductions increased further by week 8 and subsequently plateaued (figure 2). Persistent numerical improvement in myasthenia gravis scales pointing towards clinical symptom improvement were observed in all nine participants who reached week 8, and most participants at week 12 (table 3). Myasthenia Gravis Post-Intervention Status results are shown in the appendix (p 5). By week 16, all group 1 participants had withdrawn from the study due to adverse events (n=1) or worsening symptoms requiring additional treatment (n=2); all seven participants in group 2 continued to have myasthenia gravis scale scores below those at baseline during a median of 6 months' follow-up (range 4–9 months). Three (43%) of seven participants in group 2

had minimal symptom expression during at least one follow-up visit; two of three maintained minimal symptom expression at the most recent (ie, 6-month) follow-up. In group 2, one participant who required weekly intravenous immunoglobulin infusions before enrolment did not require further intravenous immunoglobulin during follow-up at 4 months, and another participant who had required biweekly infusions did not require intravenous immunoglobulin at 6 months. There were no changes in concomitant myasthenia gravis-specific medications reported in any participants included in part 2 during the study period. Planned final follow-up was at month 12; however, not all participants had reached final follow-up at the time of data collection for the primary endpoint.

Eight (73%) of 11 participants in part 2 had anti-AChR antibody titres noted in their medical records. In five (63%) of eight participants, these autoantibodies were present at baseline (median 50.90 nmol/L [range 0.86–571.57]) and decreased by 22% [95% CI 1–43] at week 8 (figure 3E). One of two participants with documented anti-MuSK disease had antibodies at screening; no change was observed during treatment (appendix p 7). Results for the semi-quantitative cell-based cluster assay for anti-MuSK and anti-LRP4 antibodies are shown in the appendix (p 8).

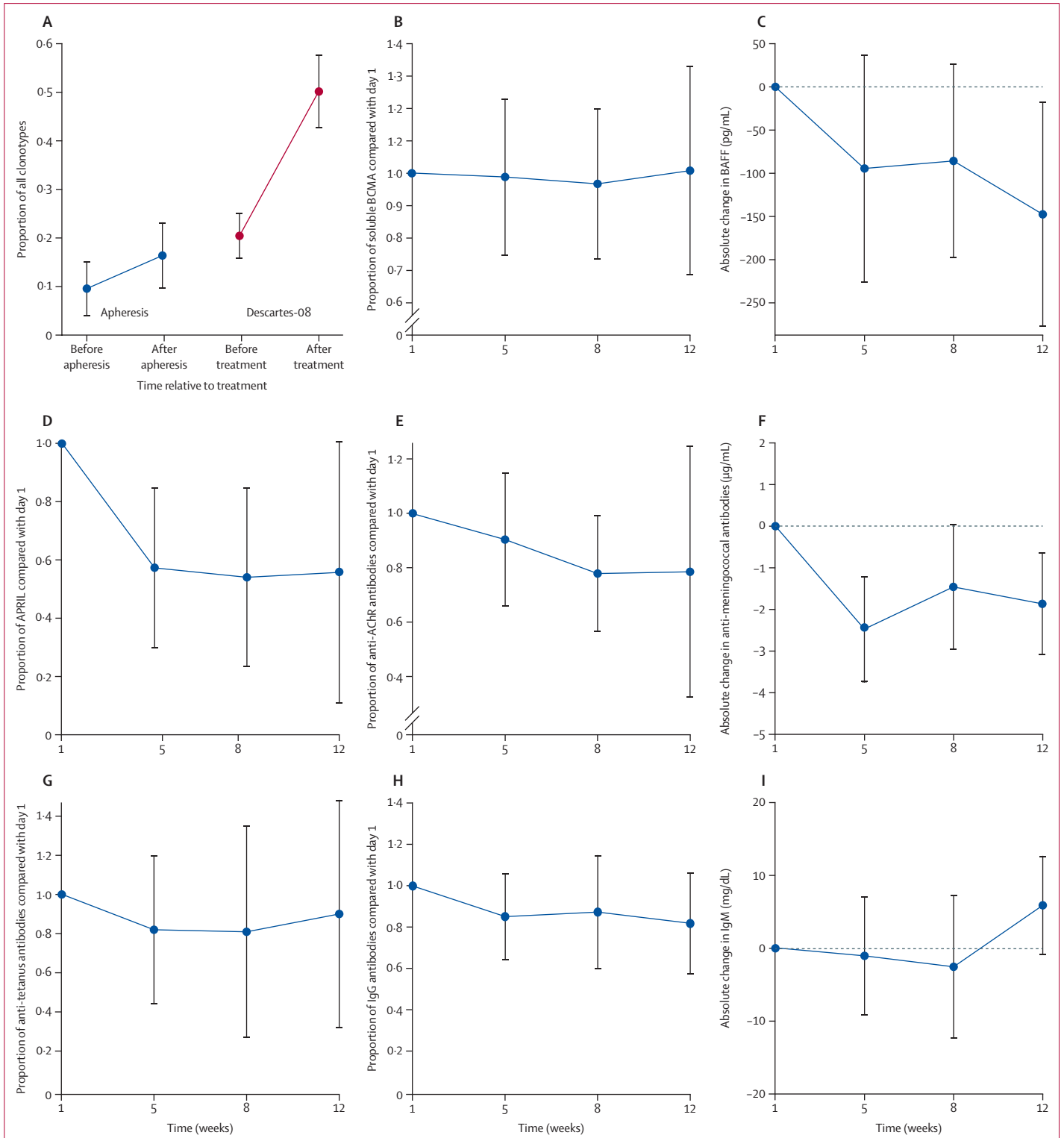
CAR RNA was detected in peripheral blood 1–2 h after infusion and at no other timepoint (appendix p 4). Bone marrow biopsies are not usually done on people with myasthenia gravis, so bone marrow was not assessed for the presence of CAR T cells or CAR RNA.

Soluble BCMA, a surrogate marker of total plasma cells, was measured in all participants in group 1 and group 2 and showed a highly skewed between-participant distribution at all assessed timepoints (median 42.08 ng/mL [range 11.90–154.59] at day 1, 27.92 ng/mL [17.69–110.72]



at week 5, 26·27 ng/mL [16·06–104·40] at week 8, and 30·49 ng/mL [20·97–112·88] at week 12) with no appreciable change over time following Descartes-08

administration (figure 3B). Circulating concentrations of BAFF and APRIL were also assessed (figure 3C, D). The mean concentration of BAFF was 1050·3 pg/mL (95% CI



770.8–1329.8) at baseline and decreased after treatment by up to 148.0 pg/mL (18.0–278.0) at week 12. APRIL, which had a highly skewed distribution, (median at 50.90 pg/mL [range 0.86–571.57] at day 1) also decreased by up to 46% [95% CI 23–85] at week 8 and 40% [0–90] at week 12.

To evaluate plasma cell function and humoral immunity after Descartes-08, immunoglobulin concentrations and titres of anti-meningococcal and anti-tetanus antibodies were measured in all participants included in part 2 at screening and follow-up. Anti-meningococcal IgG antibody titres were measurable in six participants (mean 4.4 µg/mL [95% CI 2.1–6.7]) and decreased by 2.5 µg/mL [1.2–3.8] at week 5, 1.5 µg/mL [0–3] at week 8, and 1.9 µg/mL [0.7–3.1] at week 12 (figure 3F). Anti-tetanus IgG antibodies were detected in all nine participants (median 93.7 units per mL [range 8.5–850.9] at day 1) and did not change (figure 3G). The median total IgG among the nine participants was 1325 mg/dL [range 383–2862] and decreased by up to 18% [95% CI –6 to 42] by week 12, whereas IgA and IgM concentrations were unchanged during treatment (figures 3H–I). IgE was undetectable in all participants.

Comparison of T-cell receptor clonotypes by use of T-cell receptor sequencing on PBMCs before and 57–85 days after infusion showed newly expanded clones dominating the overall T-cell repertoire (figure 3A). Clonotypes that expanded from screening to day 1 did not show similar dominance, suggesting that the expansion was an effect of Descartes-08 (appendix p 4). Other than transient increases of IFN $\gamma$ , there were no consistent changes during or after treatment in any of the 13 cytokines measured (appendix p 6).

## Discussion

In our prospective, open-label, multicentre trial in 14 patients with generalised myasthenia gravis, Descartes-08 appeared to be safe and was associated with changes on a range of myasthenia gravis outcome measures that directionally suggest potential clinical improvement; most notably, there was resolution of dependence on intravenous immunoglobulin infusion in two participants and induction of minimal symptom expression in three other participants. These improve-

ments were maintained in all participants who received weekly infusions for 6 weeks at 6–12 months of follow-up.

This study showed the feasibility of preparing autologous rCAR-T for individuals on immunosuppressive therapy, of using the cells without lymphodepletion chemotherapy, and of administering rCAR-T in the outpatient setting with minimal monitoring needed after infusion due to the notable safety profile of the product. Although DNA-based CAR T-cell therapies are also moving to the outpatient setting, they still require close monitoring after infusion, with daily clinic visits and reservation of hospital beds in case severe toxicities develop. In accordance with time-restricted expression of RNA-based CAR molecules *in vitro*,<sup>18</sup> mRNA detection in our study was transient.

Consistent with the hypothesised mechanism of targeting plasma cells, we observed decreases in BAFF and APRIL, B-cell survival factors, and ligands of BCMA that have previously been shown to correlate with myasthenia gravis severity.<sup>27</sup> There were only small numerical decreases in vaccine antibody titres and IgG concentrations, with a negligible decrease in soluble BCMA and no evidence of immunosuppression (ie, increased occurrence of infections or complete depletion of protective vaccine titres). These observations suggest that, as expected, few plasma cells were affected by rCAR-T.

In theory, Descartes-08 could have inhibited all humoral immunity, and fear of immune suppression has prompted preclinical work on targeting specific plasma cell subsets.<sup>28</sup> In practice, however, we did not observe hypogammaglobulinaemia, susceptibility to infection, or other evidence of broader plasma cell destruction. The measurable effect of Descartes-08 on the plasma cell niche was, therefore, modest compared with the magnitude of numerical myasthenia gravis scale improvement observed. A possible explanation for this discordance is the propensity for pathogenic plasma cell clones to reside in primary and secondary lymphoid organs, such as the thymus and bone marrow,<sup>15,29</sup> a compartment that is more accessible to CAR T cells than the loose connective tissue of the gastrointestinal tract, in which most non-pathogenic plasma cells reside.<sup>30,31</sup> BCMA is also expressed on plasmacytoid dendritic cells when they are activated through Toll-like receptors<sup>32</sup> and might be an additional target for Descartes-08 cells. Chronic innate activation of plasmacytoid dendritic cells drives their secretion of type I interferons, promoting autoimmunity.<sup>33</sup> Other mechanisms, such as suppression of autoreactive T-helper cell clones by KIR $\cdot$ CD8 $^+$  T cells, cannot be excluded.<sup>34</sup> Notably, we observed large and persistent changes in the T-cell receptor clonotype repertoire, the mechanism of which we have yet to elucidate.

Two studies of conventional CAR T-cell treatments in people with autoimmune disorders emphasise how toxicity might limit the broader use of DNA-engineered cells despite their therapeutic potential. In one study, five individuals with refractory systemic lupus erythematosus

**Figure 3: Exploratory biomarkers of participants enrolled to part 2**  
(A) Relative frequency of T-cell receptor clonotypes that expanded from screening to day 1 (apheresis) and from day 1 to day 57–85 (Descartes-08 treatment), shown as a proportion (0–1) of all clonotypes detected at that timepoint. Data were available for three participants, one each with AChR antibody-positive, MuSK antibody-positive, and seronegative myasthenia gravis. Individual clonotypes are shown in the appendix (p 4). Mean change from baseline in serum soluble BCMA (B), BAFF (C), APRIL (D), anti-AChR antibody (E), anti-meningococcal antibodies (all serogroups; F), anti-tetanus antibodies (G), total IgG (H), and total IgM levels (I) in group 1 (n=2) and group 2 (n=7) participants in part 2. Only participants with detectable anti-AChR antibodies at baseline were included in (E), n=5. All error bars represent 95% CI. AChR=acetylcholine receptor.

received a single infusion of  $1 \times 10^6$  DNA-modified anti-CD19 CAR T cells per kg under an expanded access protocol.<sup>7</sup> Although drug-free remission was maintained for a median of 8 months of follow-up, all participants required inpatient admission and preconditioning chemotherapy, and all developed haematological toxicity and cytokine release syndrome. In the other study, 12 patients with relapsed–refractory neuromyelitis optica received  $0.5–1 \times 10^6$  DNA-modified anti-BCMA CAR T cells per kg in a phase 1 trial.<sup>8</sup> Most participants (11 [92%] of 12) had remission; however, all had grade 1–2 cytokine release syndrome and grade 3 or higher adverse events, including neutropenia (12 [100%]), anaemia (six [50%]), and thrombocytopenia (three [25%]). More than half (seven [58%]) developed infections, including three (25%) of 12 with serious cytomegalovirus infections and one (8%) with serious pneumonia. Other plasma cell-targeting therapies are also associated with severe toxicities. Administration of daratumumab, an anti-CD38 monoclonal antibody, in seven patients with autoantibody-associated neurological disorders resulted in five grade 3 or higher toxicities, including one treatment-related death.<sup>35</sup> Consistent with our initial hypothesis that rCAR-T has less potential for toxicity than does conventional CAR T-cell therapy, because it obviates the need for lymphodepletion chemotherapy and has predictable pharmacokinetics, we observed no cytokine release syndrome, neurotoxicity, or haematological toxicities in any of the participants treated with Descartes-08. Although some cytokine release syndrome grading systems do classify any occurrence of fever as grade 1 cytokine release syndrome, unchanged IL-2, IL-6, and TNF concentrations during the fever episodes (appendix p 7), the timing of the fevers, and the absence of associated symptoms that are typical for cytokine release syndrome<sup>36,37</sup> contravene this classification. The safety profile of Descartes-08 allowed for repeat dosing and outpatient infusions for all 11 participants who were included in part 2. Only one participant required admission to hospital due to urticarial rash, which resolved within 24 h of steroid treatment. Notably, allergic reactions have been reported with repeated infusions of rCAR-T.<sup>38</sup>

Currently, myasthenia gravis is treated with broad immunosuppression, intravenous immunoglobulin, and plasma exchange, or with approved disease-modifying drugs that target specific elements of the pathogenic pathway. With the exception of the non-selective B-cell inhibitor rituximab, all of these treatments target mechanisms that are downstream of antibody production, and even rituximab was not associated with a meaningful decrease in anti-AChR antibody titres.<sup>39</sup> Complement inhibitors work at the level of the neuromuscular junction, require chronic administration (eg, eculizumab every 2 weeks or ravulizumab every 8 weeks), and carry a black box warning for meningococcal infections and sepsis.<sup>12,13</sup> Efgartigimod, a ligand of the neonatal Fc

receptor, is not associated with meningococcal infections and is administered as four once-weekly infusions; however, this treatment is intended for cyclic administration with median duration of the first cycle of 10 weeks.<sup>11</sup> By contrast, the numerical decreases in myasthenia gravis severity scales associated with Descartes-08 appear to persist for many months after all infusions were completed at week 6. Therefore, Descartes-08 might be useful as an infrequent, as-needed treatment, avoiding side-effects from continuous exposure to immunomodulators or immunody has the inherent limitations of a small, open-label trial, including the potential for placebo effect, and little ability to draw inferences from secondary, exploratory, and subgroup analyses. First, the soluble BCMA assay used in the study was developed for evaluation of participants with multiple myeloma, who have significantly higher serum soluble BCMA concentrations than the ones we observed in myasthenia gravis.<sup>40</sup> Therefore, the assay might not have been suitable for detecting small changes from already low baseline values. Second, three participants had Myasthenia Gravis Foundation of America class II myasthenia gravis, which can improve to minimal symptom expression even with conventional treatments. However, our inclusion criteria required that participants had substantial disease symptoms (MG-ADL  $\geq 6$ ), despite being on longstanding conventional regimens that were stable for at least 8 weeks before Descartes-08 treatment. Although it is unlikely that the observed magnitude of symptom improvement could be attributed solely to this unchanged maintenance dose, or to the placebo effect, in the absence of a control group, such a possibility cannot be excluded. The consistent temporal relationship between Descartes-08 initiation and measured myasthenia gravis scale improvements, with a latency of about 5–8 weeks and persistence for up to 12 months, also supports the notion that those improvements were not spontaneous or due solely to concomitant medications. Third, only two participants had MuSK antibody-positive myasthenia gravis, and only one participant had seronegative myasthenia gravis. Analyses of PBMCs obtained from patients with MuSK antibody-positive myasthenia gravis suggest that plasmablasts contribute to the production of MuSK-specific autoantibodies in individuals with relapse, and that BCMA density on plasmablasts might be lower than on plasma cells.<sup>41,42</sup> Although it appears that the two participants with MuSK antibody-positive myasthenia gravis had a different magnitude of response than others (table 3), a larger sample size is required to confirm this hypothesis.

In addition to Descartes-08 appearing to be safe and well tolerated in our study, the magnitude of measured responses is promising, as the proportion of participants in our study who had a decrease in myasthenia gravis scales equal to or greater than what is considered to be clinically meaningful appeared to be greater than the

reported placebo effects in other myasthenia gravis trials.<sup>11–14</sup> Comparison to historical controls is not conclusive, and therefore a more complete assessment of efficacy is underway in a randomised, placebo-controlled study, using six once-weekly doses of Descartes-08 for myasthenia gravis (NCT04146051).

#### Contributors

All authors had full access to the study design information and reviewed, edited, and provided final approval of the manuscript content. VG, MB, MK, MSS, MVK, and TM designed the study. VG, NC, GS, MHF, AS, TV, JFH, and TM were responsible for investigation and data collection. MDM was responsible for data analysis. MK and JFH accessed and verified the data. VG, MB, CMJ, MSS, JFH, and TM were responsible for data interpretation. VG and MDM wrote the original draft. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Declaration of interests

VG has received honoraria as a consultant or advisory board member from Alexion Pharmaceuticals, argenx, Immunovant, and Amylyx Pharmaceuticals and is employed by Biohaven Pharmaceuticals. MB received trial support and consulting fees from Alexion, Cartesian, Horizon, Immunovant, Sanofi, Takeda, UCB, and Ra Pharma. MK, MSS, and MVK are employees of and have ownership interest in Cartesian Therapeutics. MDM and CMJ are employees of Cartesian Therapeutics. CMJ is appointed as an employee of the University of Maryland and VA Maryland Health Care System. The views in this paper do not reflect the views of the state of Maryland or the US Government. GS has received research support from Cartesian Therapeutic, Immunovant, and argenx, paid to his institution; received consulting fees from UCB Pharma and Immunovant; received honoraria from argenx and Alexion; received travel support from argenx and Immunovant; and holds unpaid positions at the Myasthenia Gravis Foundation of California and the American Association of Neuromuscular and Electrodiagnostic Medicine. MHF has received honoraria as a consultant or advisory board member from argenx. TV is the University of South Florida site principal investigator for myasthenia gravis clinical trials sponsored by Alexion (now part of AstraZeneca), argenx, (now part of UCB), Horizon (now part of Viela Bio), Janssen (now part of Momenta), Regeneron, and Cartesian Therapeutics, and receives speaking or consulting honoraria relating to myasthenia gravis from Alexion, argenx, and UCB. JFH has received research support (paid to his institution) from Alexion Pharmaceuticals, argenx, Cartesian Therapeutics, the US Centers for Disease Control and Prevention, the Myasthenia Gravis Foundation of America, the Muscular Dystrophy Association, the National Institutes of Health (including the National Institute of Neurological Disorders and Stroke and the National Institute of Arthritis and Musculoskeletal and Skin Diseases), the Patient-Centered Outcomes Research Institute, Ra Pharmaceuticals (now UCB), and Takeda Pharmaceuticals; honoraria from Alexion Pharmaceuticals, argenx, Immunovant, NMD Pharma, Novartis Pharmaceuticals, Ra Pharmaceuticals (now UCB), Regeneron Pharmaceuticals, Sanofi US, and Viela Bio (now Horizon Therapeutics); he has also received non-financial support from Alexion Pharmaceuticals, argenx, Ra Pharmaceuticals (now UCB), and Toleranzia. TM has served in an advisory capacity for Alexion, Amicus, Annji, argenx, Arvinas, Ask Bio, Audentes, AvroBio, Horizon Therapeutics, Immunovant, Maze Therapeutics, Momenta (now Janssen), Sanofi-Genzyme, Spark Therapeutics, UCB, and Zogenix; serves on the speaker's bureau for argenx and Sanofi-Genzyme; serves on the medical advisory board for the Myositis Association, Neuromuscular Disease Foundation, Myasthenia Gravis Foundation of California, and Myasthenia Gravis Foundation of America; receives research funding from the Myositis Association, the Muscular Dystrophy Association, the National Institutes for Health, Alexion, Amicus, Annji, argenx, Audentes (now part of Astellas) Gene Therapy, Bristol-Myers-Squib, Cartesian Therapeutics, Grifols, ML-Bio, Momenta, Ra Pharmaceuticals, Sanofi-Genzyme, Spark Therapeutics, and Valerion; and serves on the data safety monitoring board for Acceleron, Avexis, Sarepta, and the National Institutes for Health. All other authors declare no competing interests.

#### Data sharing

Access to anonymised, individual, and trial-level data (analysis datasets) will be provided by request from qualified researchers performing independent, rigorous research, after review and approval of a research proposal and statistical analysis plan and execution of a data sharing agreement. Data requests can be submitted at any time and the data will be accessible for 12 months. Requests can be submitted to [trials@cartesianrx.com](mailto:trials@cartesianrx.com).

#### Acknowledgments

We thank all the study participants, trial teams, and members of the Study Monitoring Committee: Gil Wolfe, Syed Abbas Ali, and Mihriye Mete. We also thank our patients and their families. Research reported in this publication was supported by the National Institute of Neurological Disorders and Stroke of the National Institutes of Health under awards number R25NS088248 and NS115426–01A1. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health. MG-001 study was sponsored by Cartesian Therapeutics.

#### References

- June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med* 2018; **379**: 64–73.
- Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer* 2021; **21**: 145–61.
- Singh AK, McGuirk JP. CAR T cells: continuation in a revolution of immunotherapy. *Lancet Oncol* 2020; **21**: e168–78.
- Amini L, Silbert SK, Maude SL, et al. Preparing for CAR T cell therapy: patient selection, bridging therapies and lymphodepletion. *Nat Rev Clin Oncol* 2022; **19**: 342–55.
- Shao L, Shi R, Zhao Y, et al. Genome-wide profiling of retroviral DNA integration and its effect on clinical pre-infusion CAR T-cell products. *J Transl Med* 2022; **20**: 514.
- Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: mechanisms, manifestations and management. *Blood Rev* 2019; **34**: 45–55.
- Mackensen A, Müller F, Mougiakakos D, et al. Anti-CD19 CAR T cell therapy for refractory systemic lupus erythematosus. *Nat Med* 2022; **28**: 2124–32.
- Qin C, Tian D-S, Zhou L-Q, et al. Anti-BCMA CAR T-cell therapy CT103A in relapsed or refractory AQP4-IgG seropositive neuromyelitis optica spectrum disorders: phase 1 trial interim results. *Signal Transduct Target Ther* 2023; **8**: 5.
- Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines—a new era in vaccinology. *Nat Rev Drug Discov* 2018; **17**: 261–79.
- Gilhus NE. Myasthenia Gravis. *N Engl J Med* 2016; **375**: 2570–81.
- Howard JF Jr, Bril V, Vu T, et al. Safety, efficacy, and tolerability of efgartigimod in patients with generalised myasthenia gravis (ADAPT): a multicentre, randomised, placebo-controlled, phase 3 trial. *Lancet Neurol* 2021; **20**: 526–36.
- Howard JF Jr, Utsugisawa K, Benatar M, et al. Safety and efficacy of eculizumab in anti-acetylcholine receptor antibody-positive refractory generalised myasthenia gravis (REGAIN): a phase 3, randomised, double-blind, placebo-controlled, multicentre study. *Lancet Neurol* 2017; **16**: 976–86.
- Vu T, Meisel A, Mantegazza R, et al. Terminal complement inhibitor ravulizumab in generalized myasthenia gravis. *NEJM Evid* 2022; published online April 26. <https://doi.org/10.1056/EVIDoa2100066>.
- Howard JF Jr, Nowak RJ, Wolfe GI, et al. Clinical effects of the self-administered subcutaneous complement inhibitor zilucoplan in patients with moderate to severe generalized myasthenia gravis: results of a phase 2 randomized, double-blind, placebo-controlled, multicenter clinical trial. *JAMA Neurol* 2020; **77**: 582–92.
- Zografou C, Vakrakou AG, Stathopoulos P. Short- and long-lived autoantibody-secreting cells in autoimmune neurological disorders. *Front Immunol* 2021; **12**: 686466.
- Jin W, Yang Q, Peng Y, et al. Single-cell RNA-Seq reveals transcriptional heterogeneity and immune subtypes associated with disease activity in human myasthenia gravis. *Cell Discov* 2021; **7**: 85.
- Beecher G, Putko BN, Wagner AN, Siddiqi ZA. Therapies directed

- against B-cells and downstream effectors in generalized autoimmune myasthenia gravis: current status. *Drugs* 2019; **79**: 353–64.
- 18 Lin L, Cho S-F, Xing L, et al. Preclinical evaluation of CD8+ anti-BCMA mRNA CAR T cells for treatment of multiple myeloma. *Leukemia* 2021; **35**: 752–63.
- 19 Muppidi S, Silvestri NJ, Tan R, Riggs K, Leighton T, Phillips GA. Utilization of MG-ADL in myasthenia gravis clinical research and care. *Muscle Nerve* 2022; **65**: 630–39.
- 20 Vissing J, Jacob S, Fujita KP, O'Brien F, Howard JF. 'Minimal symptom expression' in patients with acetylcholine receptor antibody-positive refractory generalized myasthenia gravis treated with eculizumab. *J Neurol* 2020; **267**: 1991–2001.
- 21 Barnett C, Katzberg H, Nabavi M, Bril V. The quantitative myasthenia gravis score: comparison with clinical, electrophysiological, and laboratory markers. *J Clin Neuromuscul Dis* 2012; **13**: 201–05.
- 22 Burns TM, Conway M, Sanders DB. The MG Composite: a valid and reliable outcome measure for myasthenia gravis. *Neurology* 2010; **74**: 1434–40.
- 23 Burns TM, Sadjadi R, Utsugisawa K, et al. International clinimetric evaluation of the MG-QOL15, resulting in slight revision and subsequent validation of the MG-QOL15r. *Muscle Nerve* 2016; **54**: 1015–22.
- 24 Rodriguez Cruz PM, Huda S, López-Ruiz P, Vincent A. Use of cell-based assays in myasthenia gravis and other antibody-mediated diseases. *Exp Neurol* 2015; **270**: 66–71.
- 25 Anderson TW, Darling DA. A test of goodness of fit. *J Am Stat Assoc* 1954; **49**: 765–69.
- 26 Shapiro SS, Wilk MB. An analysis of variance test for normality (complete samples). *Biometrika* 1965; **52**: 591–611.
- 27 Uzawa A, Kuwabara S, Suzuki S, et al. Roles of cytokines and T cells in the pathogenesis of myasthenia gravis. *Clin Exp Immunol* 2021; **203**: 366–74.
- 28 Oh S, Mao X, Manfredo-Vieira S, et al. Precision targeting of autoantigen-specific B cells in muscle-specific tyrosine kinase myasthenia gravis with chimeric autoantibody receptor T cells. *Nat Biotechnol* 2023; published online Jan 19. <https://doi.org/10.1038/s41587-022-01637-z>.
- 29 Fujii Y, Monden Y, Hashimoto J, Nakahara K, Kawashima Y. Acetylcholine receptor antibody production by bone marrow cells in a patient with myasthenia gravis. *Neurology* 1985; **35**: 577–79.
- 30 Simonetta F, Alam IS, Lohmeyer JK, et al. Molecular imaging of chimeric antigen receptor T Cells by ICOS-ImmunoPET. *Clin Cancer Res* 2021; **27**: 1058–68.
- 31 Carpenter RO, Evbuomwan MO, Pittaluga S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin Cancer Res* 2013; **19**: 2048–60.
- 32 Schuh E, Musumeci A, Thaler FS, et al. Human plasmacytoid dendritic cells display and shed B cell maturation antigen upon TLR engagement. *J Immunol* 2017; **198**: 3081–88.
- 33 Lande R, Ganguly D, Facchinetti V, et al. Neutrophils activate plasmacytoid dendritic cells by releasing self-DNA-peptide complexes in systemic lupus erythematosus. *Sci Transl Med* 2011; **3**: 73ra19.
- 34 Li J, Zaslavsky M, Su Y, et al. KIR<sup>+</sup>CD8<sup>+</sup> T cells suppress pathogenic T cells and are active in autoimmune diseases and COVID-19. *Science* 2022; **376**: eabi9591.
- 35 Scheibe F, Ostendorf L, Prüss H, et al. Daratumumab for treatment-refractory antibody-mediated diseases in neurology. *Eur J Neurol* 2022; **29**: 1847–54.
- 36 Hu Y, Li J, Ni F, et al. CAR-T cell therapy-related cytokine release syndrome and therapeutic response is modulated by the gut microbiome in hematologic malignancies. *Nat Commun* 2022; **13**: 5313.
- 37 Hay KA, Hanafi L-A, Li D, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood* 2017; **130**: 2295–306.
- 38 Maus MV, Haas AR, Beatty GL, et al. T cells expressing chimeric antigen receptors can cause anaphylaxis in humans. *Cancer Immunol Res* 2013; **1**: 26–31.
- 39 Nowak RJ, Coffey CS, Goldstein JM, et al. Phase 2 trial of rituximab in acetylcholine receptor antibody-positive generalized myasthenia gravis: the BeatMG Study. *Neurology* 2021; **98**: e376–89.
- 40 Alomari M, Kunacheewa C, Manasanch EE. The role of soluble B cell maturation antigen as a biomarker in multiple myeloma. *Leuk Lymphoma* 2023; **64**: 261–72.
- 41 Stathopoulos P, Kumar A, Heiden JAV, Pascual-Goñi E, Nowak RJ, O'Connor KC. Mechanisms underlying B cell immune dysregulation and autoantibody production in MuSK myasthenia gravis. *Ann N Y Acad Sci* 2018; **1412**: 154–65.
- 42 Stathopoulos P, Kumar A, Nowak RJ, O'Connor KC. Autoantibody-producing plasmablasts after B cell depletion identified in muscle-specific kinase myasthenia gravis. *JCI Insight* 2017; **2**: e94263.