nature medicine

Article

https://doi.org/10.1038/s41591-022-02182-7

Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: phase 1 UNIVERSAL trial interim results

Received: 1 July 2022

Accepted: 12 December 2022

Published online: 23 January 2023

Check for updates

Sham Mailankody[1,2] ✉, Jeffrey V. Matous[3], Saurabh Chhabra[4], Michaela Liedtke[5], Surbhi Sidana[6], Olalekan O. Oluwole[7], Shahbaz Malik[8], Rajneesh Nath[9], Faiz Anwer[10], Jose Carlos Cruz[11], Myo Htut[12], Erin E. Karski[13], Wade Lovelace[13], Myles Dillon[13], Eric Butz[13], Wendy Ying[13], Arun Balakumaran[13] & Shaji K. Kumar[14]

ALLO-715 is a first-in-class, allogeneic, anti-BCMA CAR T cell therapy engineered to abrogate graft-versus-host disease and minimize CAR T rejection. We evaluated escalating doses of ALLO-715 after lymphodepletion with an anti-CD52 antibody (ALLO-647)-containing regimen in 43 patients with relapsed/refractory multiple myeloma as part A of the ongoing first-in-human phase 1 UNIVERSAL trial. Primary objectives included determination of the safety and tolerability of ALLO-715 and the safety profile of the ALLO-647-containing lymphodepletion regimen. Key secondary endpoints were response rate and duration of response. Grade ≥3 adverse events were reported in 38 (88.0%) of patients. Cytokine release syndrome was observed in 24 patients (55.8%), with 1 grade ≥3 event (2.3%) and neurotoxicity in 6 patients (14%), with no grade ≥3 events. Infections occurred in 23 patients (53.5%), with 10 (23.3%) of grade ≥3. Overall, 24 patients (55.8%) had a response. Among patients treated with 320×10^6 CAR^+ T cells and a fludarabine-, cyclophosphamide- and ALLO-647-based lymphodepletion regimen ($n = 24$), 17 (70.8%) had a response including 11 (45.8%) with very good partial response or better and 6 (25%) with a complete response/stringent complete response. The median duration of response was 8.3 months. These initial results support the feasibility and safety of allogeneic CAR T cell therapy for myeloma.

Multiple myeloma (MM) remains an incurable cancer despite recent advances in treatment. The mainstay of MM treatment includes immunomodulatory drugs, proteasome inhibitors and anti-CD38 monoclonal antibodies. Although these have prolonged survival, patients eventually relapse, with each subsequent line of therapy rendering a patient more refractory to treatment[1]. Therapies targeting the B cell maturation antigen (BCMA) superfamily have emerged as a new class of therapy for treatment of myeloma. BCMA is a member of the tumor necrosis factor receptor (TNFR) superfamily (TNFRSF) that is expressed primarily on mature B lymphocytes and plasma cells[2]. BCMA maintains survival and proliferation of these cell types by binding to B cell-activating factor (BAFF) and a proliferation-inducing ligand (APRIL) and is specifically implicated in the survival and proliferation of MM cells[3,4]. Multiple modalities targeting BCMA have demonstrated activity including antibody–drug conjugates (ADCs)[5], bispecific antibodies[6,7] and autologous chimeric antigen receptor (CAR) T cell therapy[8,9].

A full list of affiliations appears at the end of the paper. ✉e-mail: mailanks@mskcc.org

https://doi.org/10.1038/s41591-022-02182-7

High overall response rates (ORRs) and duration of response (DOR) are seen, particularly with autologous BCMA-targeted CAR T cell therapies. As of December 2022, the US Food and Drug Administration (FDA) has approved four different BCMA-targeted therapies for treatment of patients with relapsed/refractory myeloma who have been treated with a proteasome inhibitor (PI), immunomodulator (IMiD) and anti-CD38 monoclonal antibody, including belantamab mafodotin-blmf, ide-cabtagene vicleucel (ide-cel), ciltacabtagene autoleucel (cilta-cel) and teclistamab (https://www.accessdata.fda.gov/drugsatfda_docs/label/2022/761291s000lbl.pdf)[5,8,9]. Belantamab mafodotin-blmf is a BCMA-directed antibody conjugated to a microtubule inhibitor (accelerated approval; approval rescinded in December 2022) and at approved doses achieved an ORR of 32% and the median DOR (mDOR) of 11 months[5,10]. Teclistamab, a bispecific antibody that targets both BCMA and CD3 (accelerated approval; full approval pending) and results from the phase 1/2 MajesTEC-1 study, showed an ORR of 63% and mDOR of 18.4 months[11,12]. Ide-cel and cilta-cel are autologous CAR T cell therapies targeting BCMA. Ide-cel achieved an ORR of 72% and an mDOR of 11 months[8,13], whereas cilta-cel has a reported ORR of 98% and the mDOR has not yet been reached[9,14].

Although the recent FDA approvals of autologous BCMA-targeted CAR T cell therapies mark an important treatment advance for patients with MM, there are several logistical challenges to autologous CAR T that may prevent widespread access to CAR T therapy. These include the number of cells available and suitable for collection possibly being scarce because patients are often lymphopenic[15,16], manufacturing constraints and, perhaps most importantly, because of the lengthy vein-to-vein time, which makes bridging therapy necessary for most patients. These factors have resulted in wait lists for treatment and some patients die before they can receive treatment[17]. Allogeneic CAR T cell therapy aims to bridge these logistical hurdles by being an off-the-shelf CAR T product that can be accessed without the need for leukapheresis and the subsequent lengthy manufacturing times[18]. Previous studies of donor-derived, allogeneic anti-CD19 CAR T therapy have reported complete responses in patients with heavily pretreated B cell acute lymphoblastic leukemia and this efficacy has been associated with a manageable safety profile[19,20].

ALLO-715 contains an integrated, self-inactivating, third-generation, recombinant lentiviral vector that expresses a second-generation anti-BCMA CAR containing a single-chain variable fragment (scFv) derived from a human anti-BCMA antibody and the intracellular domains of 4-1BB and CD3ζ (Extended Data Fig. 1). The extracellular region of the BCMA CAR also contains two mimotopes that confer susceptibility to the anti-CD20 monoclonal antibody rituximab and functions as an intracellular off-switch in the presence of rituximab[21,22]. There are two additional changes using the transcription activator-like effector nuclease (TALEN) technology: (1) knockout of the T cell receptor alpha constant (TRAC) and (2) knockout of cluster of differentiation (CD) 52. Knocking out TRAC reduces the risk of graft-versus-host disease (GvHD) by reducing the expression of the T cell receptor (TCR)-αβ complex at the cell surface, to prevent TCR-αβ-mediated recognition of histocompatibility antigens. CD52 is a cell-surface glycoprotein found on a variety of host immune cell types, including lymphocytes, monocytes, macrophages, eosinophils and dendritic cells; host immune cells can mediate a host-versus-graft reaction, leading to the elimination of ALLO-715. $CD52^+$ cells can be effectively depleted by anti-CD52 antibodies such as ALLO-647, a therapeutic immunoglobulin G1 monoclonal antibody. Inactivation of CD52 in the CAR T cells enables cell expansion and persistence of ALLO-715 in patients who are lymphodepleted with ALLO-647.

UNIVERSAL is a first-in-human phase 1 trial of ALLO-715 (NCT04093596) in patients who have relapsed and refractory myeloma. UNIVERSAL consists of three parts: parts A, B and C. Part A evaluates the safety, efficacy, cellular kinetics, immunogenicity and pharmacodynamics of a single dose of ALLO-715 after lymphodepletion (LD) with an ALLO-647-containing regimen. Part B evaluates ALLO-715 in combination with nirogacestat and part C evaluates a consolidation regimen of ALLO-715 in which two doses are given approximately 2 weeks apart. In the present study, we report a nonprespecified interim analysis of cohort A. Cohort A is continuing to enroll patients.

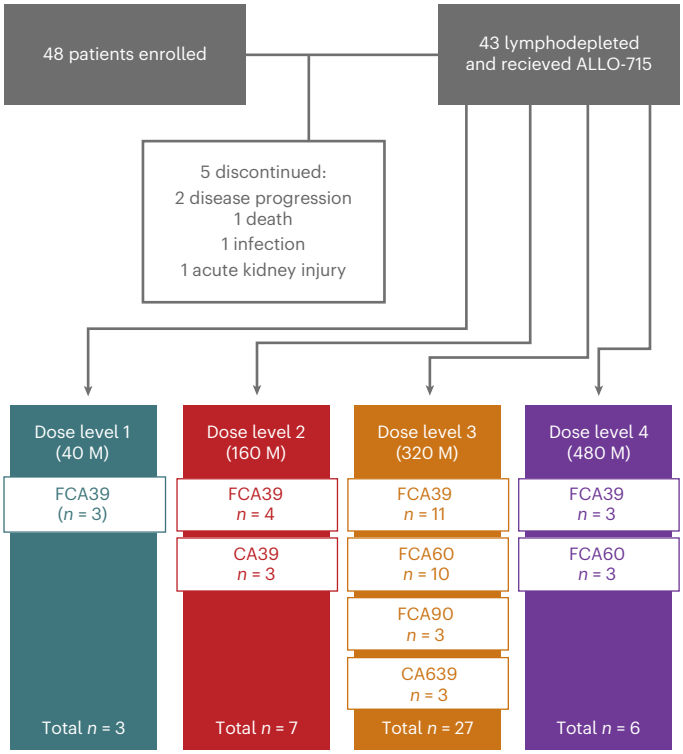


Fig. 1 | Consort diagram. M, 10^6 CAR^+ T cells. LD nomenclature: C, cyclophosphamide 300 mg m^{-2} on days −5, −4 and −3; F, fludarabine 30 mg m^{-2} on days −5, −4 and −3; A39, ALLO-647 13 mg m^{-2} on days −5, −4 and 0; A60, ALLO-647 20 mg per day on days −5, −4 and −3; A90, ALLO-647 30 mg per day on days −5, −4 and −3.

Results

Patients

The primary objectives of part A of the phase 1 UNIVERSAL trial were safety and tolerability of ALLO-715 at increasing dose levels, and also assessing the safety profile of ALLO-647 used as LD in combination with fludarabine and/or cyclophosphamide before ALLO-715 infusion. Between 10 September 2019 and 14 October 2021, 48 patients with relapsed/refractory MM were enrolled into part A of UNIVERSAL. Eligible patients were aged ≥18 years and must have received at least 3 previous lines of therapy including a PI, IMiD or anti-CD38 monoclonal antibody. Patients were also required to be refractory to their last line of treatment (progression during or within 60 days of their last dose), and have measurable disease, an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 and adequate organ function. Patients who had received previous non-CAR T BCMA-targeted therapy (bispecific, ADC or other) were permitted. Previous exposure to a BCMA-directed CAR T was excluded. All patients were screened for the presence of donor (product)-specific anti-human leukocyte antigen (HLA) antibodies (DSAs) and those with positive DSA tests ($n = 6$) were excluded. Five patients did not proceed to LD due to progressive disease, death or acute events (Fig. 1). The median time from enrollment to start of LD was 5 days (range 0–20 days) and 43 patients received both LD and ALLO-715, which was derived from 3 healthy donors in the present study. The trial followed a standard 3 + 3 dose escalation

https://doi.org/10.1038/s41591-022-02182-7

Table 1 | Baseline characteristics of patients who received ALLO-715

Characteristic		Total (*n*=43)
Age, median (range) (years)		64 (46, 77)
Gender, *n* (%)	Male	27 (62.8)
	Female	16 (37.2)
Race, *n* (%)	White	35 (81.4)
	Black or African–American	5 (11.6)
	Asian	2 (4.7)
	Native Hawaiian or Pacific Islander	1 (2.3)
Ethnicity, *n* (%)	Hispanic or Latino	2 (4.7)
	Non-Hispanic or Latino	39 (90.7)
	Not reported	2 (4.7)
ECOG PS, *n* (%)[a]	0	21 (48.8)
	1	22 (51.2)
ISS stage, *n* (%)	I	12 (27.9)
	II	22 (51.2)
	III	8 (18.6)
	Unknown	1 (2.3)
High-risk cytogenetics[b], *n* (%)		16 (37.2)
Extramedullary disease, *n* (%)		9 (20.9)
High tumor burden at screening[c], *n* (%)		14 (32.6)
Bone marrow plasma cell infiltration, median (range) (%)		20 (0, 97)
Time since initial diagnosis, median (range) (years)		4.9 (0.9, 26.4)
Number of previous anti-myeloma regimens, median (range)		5 (3, 11)
Previous autologous SCT, *n* (%)		39 (90.7)
Triple refractory, *n* (%)[d,e]		39 (90.7)
Penta-refractory, *n* (%)[e,f]		18 (41.9)

[a]ECOG performance status (PS) scores range from 0 to 5, with higher scores indicating greater disability. [b]High-risk cytogenetics defined as the presence of any of the following: del17p, t(14;16) or t(4;14). [c]High tumor burden defined as ≥50% of plasma cell in bone marrow. [d]Triple refractory disease defined as refractory to an IMiD, a PI and an anti-CD38 monoclonal antibody. [e]Refractory defined as disease progression on or within 60 d of the last dose of the most recent drug(s) given. [f]Penta-refractory disease defined as refractory to two IMiDs, two PIs and an anti-CD38 monoclonal antibody. ISS, International Staging System; SCT, stem-cell transplantation.

Table 2 | All grade AEs occurring in ≥20% of patients, grade ≥3 events occurring in two or more patients with CRS and neurotoxic effects in patients who received ALLO-715

n (%)	Total (*N*=43)	
	Any grade	Grade ≥3
Any AE[a]	43 (100)	38 (88)
Hematological		
Neutropenia	30 (69.8)	30 (69.8)
Anemia	24 (55.8)	20 (46.5)
Thrombocytopenia	22 (51.2)	18 (41.9)
Lymphopenia	14 (32.6)	14 (32.6)
Leukopenia	5 (11.6)	5 (11.6)
Gastrointestinal		
Nausea	27 (62.8)	0
Diarrhea	19 (44.2)	1 (2.3)
Constipation	12 (27.9)	0
Infectious		
CMV viremia	14 (32.6)	2 (4.7)
Pneumonia	4 (9.3)	3 (7)
Sepsis	2 (4.7)	2 (4.7)
Metabolic and nutritional		
Hypokalemia	14 (32.6)	1 (2.3)
Decreased appetite	13 (30.2)	2 (4.7)
Hypophosphatemia	11 (25.6)	2 (4.7)
Other		
Fatigue	26 (60.5)	2 (4.7)
Headache	16 (37.2)	0
Cough	12 (27.9)	0
Pyrexia	12 (27.9)	1 (2.3)
Peripheral edema	11 (25.6)	0
Hypertension	10 (23.3)	6 (14)
Insomnia	9 (20.9)	0
Hypoxia	3 (7)	2 (4.7)
Failure to thrive	2 (4.7)	2 (4.7)
Aspartate aminotransferase increased	5 (11.6)	2 (4.7)
Cytokine release syndrome[b]	24 (55.8)	1 (2.3)
Neurological toxic effect[c]	6 (14)	0

[a]Shown are AEs occurring in ≥20% of patients who received ALLO-71. [b]CRS grading according to the American Society for Transplant and Cellular Therapy (2019)[3]. [c]Neurological toxic effects identified using a broad search: noninfectious encephalopathy/delirium standardized MedDRA query (SMQ) reviews with adjudication were then completed to identify cases of potential neurological toxicity[35]

design with 4 dose levels of ALLO-715. Escalating dose levels were tested sequentially and all patients who started treatment received all doses as per their dose escalation assignment. Doses of ALLO-647 and ALLO-715 were not escalated simultaneously. In addition, the experimental design allowed for expansion cohorts of up to 12 patients to further characterize the safety and efficacy of specific LD and ALLO-715 dose levels; 43 patients received ALLO-715 and were treated at 4 target dose levels (DLs) of: DL1, 40×10^6 ($n = 3$); DL2, 160×10^6 ($n = 7$); DL3, 320×106 ($n = 27$); and DL4, 480×10^6 ($n = 6$) CAR^+ T cells, respectively. Several LD regimens were also evaluated including fludarabine (90 mg m^{-2}) and cyclophosphamide (900 mg m^{-2}) combined with ALLO-647 at a 3-day dose of 39 mg (FCA39 ($n = 21$)), 60 mg (FCA60 ($n = 13$)) or 90 mg (FCA90 ($n = 3$)). Cyclophosphamide and ALLO-647 were also tested without fludarabine (CA39 ($n = 6$)). A total of 33 patients discontinued treatment: 24 due to progressive disease, 7 due to death and 2 due to withdrawal of consent. The cutoff date for clinical analysis was 14 October 2021.

Patient characteristics are shown in Table 1. The median age was 64 years (range 46–77 years) and 63% were male. The median time from the diagnosis of myeloma was 4.9 years (range 0.9–26.4 years) and 37% had a high-risk cytogenetics profile defined as the presence of del(17p), t(14;16) or t(4;14). In addition, 21% of patients had extramedullary disease. Patients had received a median of 5 (range 3–11) previous treatment regimens and 91% had triple refractory disease, that is, refractory to a PI, an IMiD and an anti-CD38 monoclonal antibody, whereas 42% had penta-refractory disease, that is, refractory to 2 PIs, 2 IMiDs and an anti-CD38 monoclonal antibody. Three patients had received previous BCMA-targeted treatment. All patients were refractory to their last line of treatment. No patients received bridging therapy and all patients observed a 2-week wash-out between their last treatment regimen and the start of LD.

https://doi.org/10.1038/s41591-022-02182-7

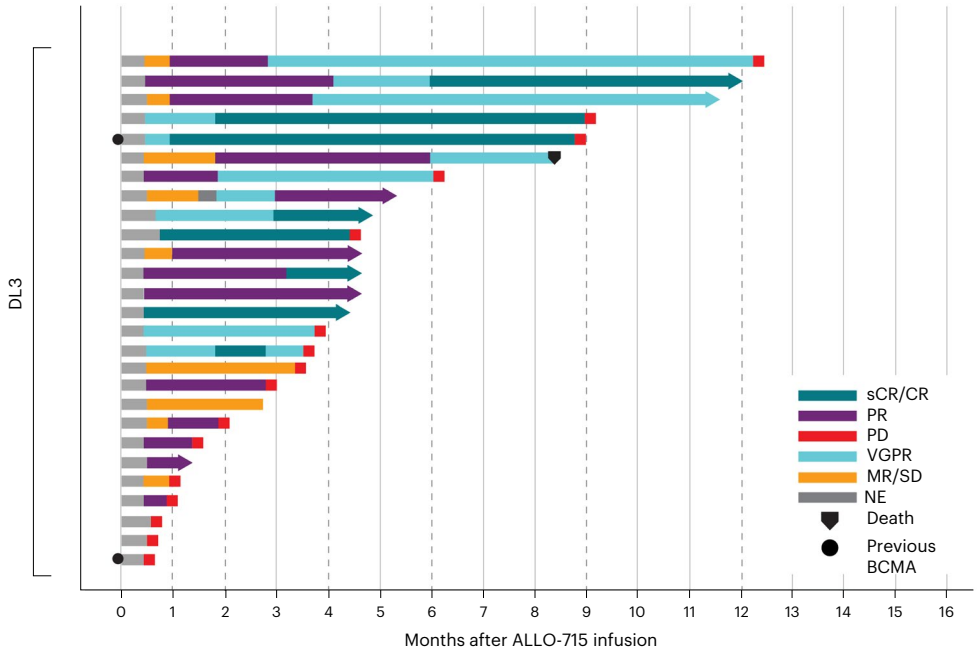


Fig. 2 | Duration of response in patients who received 320 × 10^6 CAR$^+$ T cells with an FCA[a] LD regimen. [a]FCA indicates conditioning with fludarabine, cyclophosphamide and varying doses of ALLO-647. MR/SD, minimal response/stable disease; NE, not estimatable; PD, progressive disease; PR, partial response.

Safety and tolerability of ALLO-647 and ALLO-715

During dose escalation, one dose-limiting toxicity (DLT) of grade 5 fungal pneumonia (related to LD) on day 8 was observed at a dose of 160 × 10^6 CAR$^+$ T cells with an LD regimen of cyclophosphamide (total dose: 900 mg m^{-2}) and ALLO-647 total dose of 39 mg (CA39). All 43 patients reported at least one adverse event (AE) (Table 2), with 88% of patients having AEs that were grade ≥3. Hematological events were expected given the use of lymphodepleting chemotherapy. The most frequent AEs were neutropenia in 69.8%, anemia in 55.8% and thrombocytopenia in 51.2%. Of ten deaths that occurred during the study after ALLO-715 administration, seven were related to disease progression and three were instances of grade 5 infections: fungal pneumonia, adenoviral hepatitis and sepsis.

Infections occurred in 53.5% of patients with 23.3% of patients experiencing a grade ≥3 infection (Extended Data Table 1). The only infection reported in >10% of patients was cytomegalovirus (CMV) (n = 14; 32.6%). Grade 1 or 2 CMV reactivation occurred in 12 patients, consistent with CMV viremia and 12 of the 14 patients received treatment including 10 with oral valganciclovir and 2 with letermovir (1 as treatment and 1 for viral control). Two patients had grade 3 CMV reactivation and were treated with intravenous antiviral therapy. Of these two, one patient had no sign of endorgan damage. The second patient experienced grade 3 CMV disease in the setting of adenovirus hepatitis and human herpesvirus 6 infection with subsequent death from the adenoviral hepatitis. The only other grade ≥3 infection reported in more than one person was pneumonia (n = 3, 7%). Prolonged cytopenias (defined as cytopenias existing at study day 56 and present for the preceding ≥21 days) occurred in 8 (19%) subjects, of whom 2 had events of prolonged neutropenia and thrombocytopenia (bicytopenia) and 2 each had prolonged neutropenia or anemia. One subject had prolonged thrombocytopenia and one prolonged pancytopenia. The cytopenias were resolved in seven patients at a median time of 2.6 months (range 1.9–8.4 months), whereas the patient with prolonged pancytopenia remained cytopenic at the time of death from adenoviral hepatitis.

Cytokine release syndrome (CRS) occurred in 24 (55.8%) patients, with more cases of CRS being reported at higher dose levels of ALLO-715. In 27 patients who received DL3, 19 (70%) experienced CRS. All occurrences of CRS were grade 1 or 2 except for 1 grade 3 CRS reported in a patient at DL3. Median time to onset was 7 d and median duration was 4 d. Ten (23.3%) patients received tocilizumab and six (14%) patients received steroids as treatment for CRS.

Events of potential neurotoxicity were identified in 6 (14%) patients and 2 (5%) had events concurrent with CRS. All cases were grade 1 or 2 and no patients received steroids for events of neurotoxicity. The time to onset of events relative to infusion ranged from 4 d to 56 d with a median time of 8.5 d. No long-term neurotoxicity, no movement disorders or events of parkinsonism were reported. No cases of GvHD were reported. Infusion-related reactions to ALLO-647 were seen in 12 (28%) patients, with all events being grade 1 or 2. No anti-ALLO-715 scFv antibodies were detected in any subject.

Anti-myeloma activity of ALLO-715

The secondary objectives of this trial included evaluating the anti-myeloma activity of ALLO-715 by assessing the response rate. At a median follow-up of 10.2 months (95% confidence interval (CI) 3.8 to not reached), 24 of 43 patients (55.8%; 95% CI: 39.9, 70.9) had a response, with 15 patients (34.9%) experiencing a very good partial response or better (VGPR$^+$). Responses were observed in 0 of 3 patients receiving DL1, 2 of 7 patients receiving DL2 (28.6%), 19 of 27 patients receiving DL3 (70%) and 3 of 6 patients receiving DL4 (50%). Based on clinical responses and cellular kinetics, DL3 (320 × 10^6 CAR$^+$ cells) FCA39, FCA60 or FCA90 LD was expanded to treat additional patients (n = 24; 11 with FCA39 LD, 10 with FCA60 and 3 with FCA90). Among these patients, 17 (70.8%; 95% CI: 48.9, 87.4) achieved a partial response or better whereas 11 (46%) were VGPR$^+$ and 6 (25%) were in complete remission/stringent complete remission (CR/sCR). The median time to response for this cohort was 16 days (range 15–57 days) and the mDOR was 8.3 months (95% CI: 3.4, 11.3) (Fig. 2). The expression of BCMA in patients after relapse is still being analyzed and the data are not yet available.

Responses were observed in patients treated with ALLO-715 who had high-risk cytogenetic abnormalities, penta-refractory disease, a high tumor burden and extramedullary disease (Fig. 3). Efficacy results are summarized in Table 3. Tumor responses to LD using regimens of cyclophosphamide plus ALLO-647 (CA) at total doses of 39 mg

https://doi.org/10.1038/s41591-022-02182-7

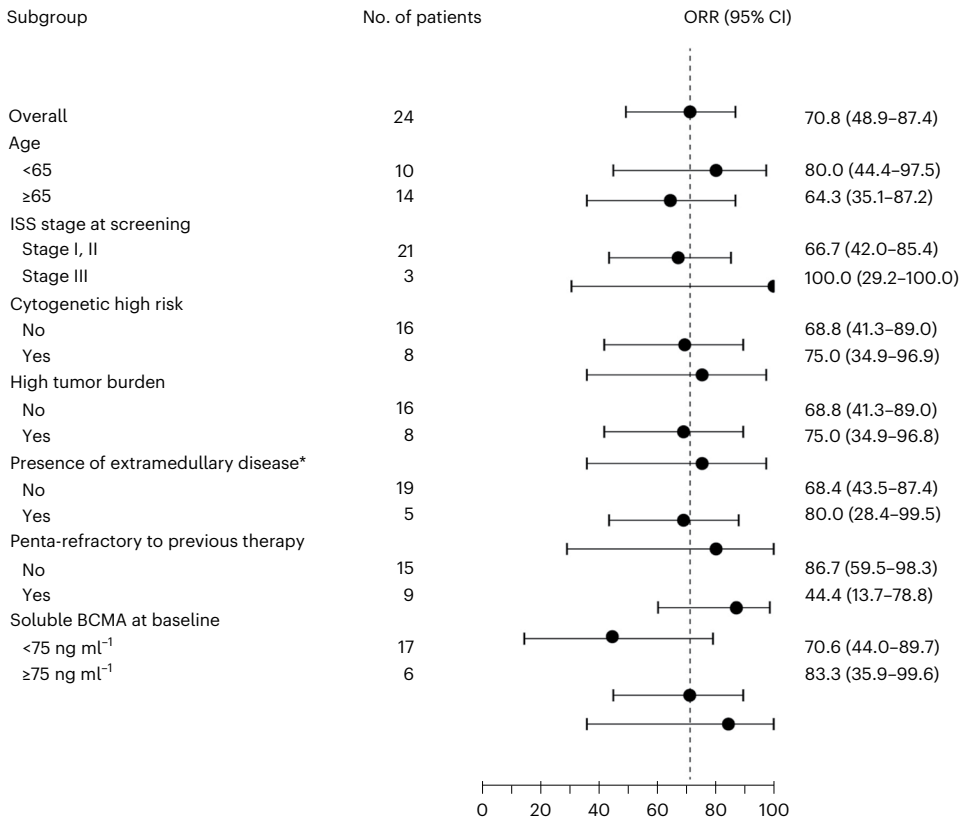


Fig. 3 | Subgroup analysis of response in patients who received 320 × 10^6 CAR^+ T cells with an FCA[a] LD regimen. Overl $n = 24$ independent patients. Data are presented as median values ± the interquartile range. [a]FCA indicates conditioning with fludarabine, cyclophosphamide and varying doses of ALLO-647. *Presence of extramedullary disease at screening. Presence of extramedullary disease 'not applicable' is categorized as 'No'.

(13 mg per day) are presented in Extended Data Table 2. As an exploratory objective, minimal residual disease (MRD) was evaluated in 14 patients at a best response of VGPR or better ($n = 15$) and 13 obtained an MRD-negative status (93%).

Cellular kinetics of ALLO-715

The cellular kinetics of ALLO-715 were characterized as a secondary objective. In the present study, ALLO-715 was derived from three healthy donors with similar levels of expansion observed with all donors. Flow cytometry and an anti-idiotype antibody were used to separate ALLO-715 cells from host lymphocytes (Extended Data Fig. 2) and ALLO-715 displayed in vivo expansion across all dose levels, with a numerically higher expansion seen at DL3 compared with lower dose levels (DL1 and DL2). No apparent increase in expansion was observed at DL4 with DL3 (Extended Data Table 3 and Extended Data Fig. 3). In DL3 FCA cohorts, there was expansion in 20 out of 24 patients and, in those with expansion, the median peak expansion was seen at day 10. No correlation was observed between the occurrence of CRS and the success or failure of expansion. Persistence was variable with 16 of 24 patients (67%) having no detectable CAR T levels by day 28 (Extended Data Fig. 4a,b). CAR T levels were numerically higher in those who had a response compared with those who did not, as measured by vector copy number (Extended Data Fig. 4c).

ALLO-647 pharmacokinetics and immunogenicity

The pharmacokinetic and immunogenic profiles of ALLO-647 were evaluated as a secondary objective. As expected, the concentration of ALLO-647 in the serum of patients was dependent on the dose level, with the highest peak seen in the FCA90 group (Extended Data Fig. 5a). Concentrations decreased in all groups over time and were undetectable in all patients by month 3. Post-treatment, anti-drug antibodies (ADAs) against ALLO-647 were observed in 14% (9 of 64) of patients. Post-treatment ADAs were detected only in patients administered 39 mg of ALLO-647. The population PK model of ALLO-647 did not find the presence of ADAs affecting clearance of ALLO-647.

Host immune cell depletion and reconstitution

Host immune depletion and reconstitution were also evaluated as a secondary objective. The host immune cells trended lower immediately after LD on days 0 and 7 in responders compared with nonresponders, particularly apparent in host T cells on days 0 and 7 in subjects with a VGPR or better response compared with nonresponders (Extended Data Fig. 5b). LD was dose dependent on the treatment regimen, with lower levels of total $CD45^+$ lymphocytes seen at FCA90 compared with FCA39 and FCA60 (Extended Data Fig. 6a). Similar dose-dependent differences were seen in B cells, T cells and natural killer (NK) cells (Extended Data Fig. 6b–d). These differences were maintained during recovery of host immune cells. NK cells rapidly recover to predepletion levels within 1–3 months. Recovery of T cells is slower with the median T cell count across subjects reaching 200 cells per µl by month 3 and B cells recover to a median of 10 cells per µl by month 6.

Discussion

In this first-in-human, phase 1 trial with heavily pretreated MM patients, we demonstrate feasibility, acceptable safety and preliminary evidence of anti-myeloma efficacy for ALLO-715, the first allogeneic BCMA-targeted CAR T therapy. ALLO-715 was successfully administered in 43 patients with a median time from enrollment to start of treatment of 5 days. The trial established an encouraging safety profile for ALLO-715 in line with other anti-BCMA autologous-targeted cell therapies. In the present study, CRS and neurotoxicity were observed at 56% and 14%, respectively, with only one grade 3 case of CRS reported and no

https://doi.org/10.1038/s41591-022-02182-7

Table 3 | Tumor response according to dose of ALLO-715 and LD regimen

Cell dose and LD regimen[a]	40×10^6 CAR$^+$ T cells	160×10^6 CAR$^+$ T Cells	320×10^6 CAR$^+$ T cells				480×10^6 CAR$^+$ T Cells	
	FCA39*n*=3	FCA39*n*=4	FCA39*n*=11	FCA60*n*=10	FCA90*n*=3	All FCA*n*=24	FCA39*n*=3	FCA60*n*=3
ORR[b], *n* (%) (95% CI)	0	2 (50) (6.8, 93.2)	7 (64) (31, 89)	8 (80) (44, 98)	2 (67) (9, 99)	17 (71) (49, 87)	1 (33) (0.8, 91)	2 (67) (9, 99)
VGPR$^+$ rate, *n* (%)	0	1 (25)	5 (46)	5 (50)	1 (33)	11 (46)	0	2 (67)
CR/sCR rate, *n* (%)	0	0	3 (27)	3 (30)	0	6 (25)	0	0
mDOR, months (95% CI)	N/A	5.6 (1.4, 5.6)	8.3 (3.4, 11.3)	NE (5.6, NE)	3.1 (2.4, 3.1)	8.3 (3.4, 11.3)	1.4 (NE, NE)	NE (1.5, NE)
Median follow-up, months (range)[c]	11 (3, 17)	5 (1, 8)	4 (1, 14)	5 (1, 12)	4 (3, 13)	4 (1,14)	3 (1, 13)	10 (2,12)

[a]FCA conditioning with fludarabine, cyclophosphamide and varying doses of ALLO-647 including total doses of 39 mg (13 mg per day; FCA39), 60 mg (20 mg per day; FCA60) and 90 mg (30 mg per day, FCA90). [b]Clinical response evaluation was based on International Myeloma Working Group response criteria[36] and an objective response is defined as a partial response or better. [c]Follow-up time (months) is calculated as the time between ALLO-715 administration and either the end of study date or date of data cutoff. N/A, not applicable; NE, not estimatable.

grade ≥3 neurotoxicity, which is somewhat lower than the rates seen with autologous anti-BCMA CAR T cell therapy[13,14,23]. Likewise, similar rates of prolonged cytopenia (19%) were seen in part A of this trial as with other autologous CAR T therapies. Notably, no GvHD was reported, suggesting that knockout of TRAC provides sufficient mitigation of this potential adverse event with allogeneic products.

The utilization of ALLO-647 as part of an LD regimen was a key component to this trial. ALLO-647 provides prolonged LD with no apparent increase in grade ≥3 infections compared with other anti-BCMA autologous-targeted cell therapies[8,13,24,25]. We observed grade ≥3 infections in 22% of patients including 3 grade 5 events, whereas grade ≥3 infections were reported in 20% of patients treated with cilta-cel in the phase 1b/2 CARTITUDE-1 trial[14] and 22% of patients treated with ide-cel in the phase 2 KarMMa trial[13]. Among anti-CD19 allogeneic CAR T trials, grade ≥3 infections were reported in 27% of patients treated with ALLO-501 in the ALPHA study[26] and 33% of patients in the ANTLER study treated with CB-010 (ref. 27). It should be noted that viral reactivations, in particular CMV viral reactivations, were noted in 33% of patients in UNIVERSAL highlighting the importance of CMV monitoring and consideration of anti-infective prophylaxis. Reassuringly, only two grade 3 CMV reactions requiring the use of intravenous antiviral therapy were reported and no grade 4 or 5 reactivations or infections were reported. Given the small sample size and the lack of a control arm, it is difficult to ascertain what role fewer or shorter courses of prophylaxis may have played at the cohort or even individual level. Per the National Comprehensive Cancer Network (NCCN) guidelines on infectious prophylaxis, patients who are treated with anti-CD52 therapeutic antibodies are considered at high risk for CMV reactivation[28], which may differentiate the UNIVERSAL patient population from those in trials of autologous CAR T therapies. However, most trials for autologous CAR T products to date have categorized infections broadly as bacterial, viral or fungal, which makes it difficult to tease out the individual viral types for comparison to the CMV rates in the UNIVERSAL trial. In addition, CMV monitoring was not standard in the autologous CAR T clinical trials and therefore maybe underreported in published datasets for autologous CAR T products. However, CMV reactivation is an emerging risk that institutional practices are adjusting for and implementing both monitoring and prophylaxis[29]. Although patients were treated on an inpatient basis for this trial, the safety profile of ALLO-715 after FCA LD enables the possibility for outpatient administration that could be explored in the future.

The trial evaluated multiple doses of ALLO-715 and multiple regimens for LD. ALLO-715 displayed in vivo expansion across all dose levels including in 20 out of 24 patients at DL3. A lack of expansion in patients did not correlate with the occurrence of CRS and the probable explanation for the lack of CAR T cell expansion in these patients was insufficient host immune cell depletion. Although responses were observed at doses ranging from 160 × 10^6 cells to 480 × 10^6 cells, most responses were observed at the higher dose levels with no apparent benefit of increasing the cell dose from 320 × 10^6 cells to 480 × 10^6 cells. Responses were also observed at the different ALLO-647-containing, lymphodepleting regimens, with most responses observed with the FCA regimens. In the group of patients treated with FCA LD and 320 × 10^6 cells (n = 24), 71% had an objective response with a median DOR of 8.3 months. In patients treated with the FCA60 and 320 × 10^6 cells (n = 10), the ORR of 80% falls in the range of those observed with other anti-BCMA therapies in a relapsed/refractory MM setting[5,8,9,11,30,31], whereas the median DOR for this group has not yet been reached. Moreover, response rates may further improve in future studies of allogeneic CAR T cells such as those including consolidation dosing[32], addition of the gamma-secretase inhibitor nirogacestat[33] or the next-generation construct ALLO-605, which includes an intrinsic signal 3 designed to recapitulate cytokine signaling selectively in the CAR T cells (TURBO domain) with the aim of improving engraftment and persistence[34].

Autologous CAR T cells targeting BCMA are rapidly evolving with approvals of ide-cel and cilta-cel and multiple additional products under evaluation. Allogeneic CAR T cell therapy has several meaningful advantages over autologous CAR T products, including the ease of manufacture and administration without extended delays or need for leukapheresis or bridging therapy with ALLO-715. For example, in KarMMa, the registrational study for idecabtagene vicleucel, the median time from leukapheresis to product availability was 33 days, with a range of 26–49 days, and 87% of subjects received bridging therapy to control disease during the manufacturing process[8]. Similarly, in CARTITUDE-1, the registrational study for cilta-cel, the median time for leukopheresis to product availability was 32 days with a range of 27–66 days[9]. In contrast, the median time from enrollment to the start of LD in this trial was 5 days and the use of bridging therapy was not needed. In addition, once small dose expansion cohorts were open, all patients who enrolled received treatment, highlighting a key advantage of off-the-shelf therapy. In comparison, both ide-cel and cilta-cel saw a 9% and 14% dropout rate, respectively, between leukapheresis and CAR T infusion[13,14]. As targeted anti-BCMA therapies enter earlier lines of therapy, their availability as an off-the-shelf product will become increasingly important because the potential use case extends beyond areas with ready access to leukapheresis and to a larger patient population.

UNIVERSAL is currently continuing to enroll. Ongoing and future studies will also evaluate the importance of the phenotypic characteristics of T cells obtained from healthy donors versus those obtained from relapsed or refractory patients with multiple previous lines of therapy. In addition, enrollment into a study of ALLO-605, a next-generation, BCMA-targeted, allogeneic TurboCAR product

has begun (NCT05000450). In summary, ALLO-715 is the first allogeneic CAR T cell therapy for myeloma and these initial results from the UNIVERSAL trial provide evidence of feasibility, safety and efficacy for this off-the-shelf cellular therapy as a potential treatment for patients with MM.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-022-02182-7.

References

1. Kumar, S. K. et al. Risk of progression and survival in multiple myeloma relapsing after therapy with IMiDs and bortezomib: a multicenter international myeloma working group study. *Leukemia* **26**, 149–157 (2012).
2. Carpenter, R. O. et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin. Cancer Res.* **19**, 2048–2060 (2013).
3. Moreaux, J. et al. BAFF and APRIL protect myeloma cells from apoptosis induced by interleukin 6 deprivation and dexamethasone. *Blood* **103**, 3148–3157 (2004).
4. Novak, A. J. et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood* **103**, 689–694 (2004).
5. Blenrep (belantamab mafodotin-blmf) [package insert] (GlaxoSmithKline Intellectual Property Development Ltd, 2022).
6. Shah, N., Chari, A., Scott, E., Mezzi, K. & Usmani, S. Z. B-cell maturation antigen (BCMA) in multiple myeloma: rationale for targeting and current therapeutic approaches. *Leukemia* **34**, 985–1005 (2020).
7. Usmani, S. Z. et al. Teclistamab, a B-cell maturation antigen × CD3 bispecific antibody, in patients with relapsed or refractory multiple myeloma (MajesTEC-1): a multicentre, open-label, single-arm, phase 1 study. *Lancet* **398**, 665–674 (2021).
8. Abcema (idecabtagene vicleucel) [package insert] (Celgene Corporation, 2021).
9. Carvykti (ciltacabtagene autoleucel) [package insert] (Janssen Biotech, Inc., 2022).
10. Lonial, S. et al. Longer term outcomes with single-agent belantamab mafodotin in patients with relapsed or refractory multiple myeloma: 13-month follow-up from the pivotal DREAMM-2 study. *Cancer* **127**, 4198–4212 (2021).
11. Moreau, P. et al. Teclistamab in relapsed or refractory multiple myeloma. *N. Engl. J. Med.* **387**, 495–505 (2022).
12. Mailankody, S. & Landgren, O. T-cell engagers—modern immune-based therapies for multiple myeloma. *N. Engl. J. Med.* **387**, 558–561 (2022).
13. Munshi, N. C. et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N. Engl. J. Med.* **384**, 705–716 (2021).
14. Berdeja, J. G. et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. *Lancet* **398**, 314–324 (2021).
15. Garcia-Guerrero, E., Sierro-Martinez, B. & Perez-Simon, J. A. Overcoming chimeric antigen receptor (CAR) modified T-cell therapy limitations in multiple myeloma. *Front. Immunol.* **11**, 1128 (2020).
16. Rafiq, S., Hackett, C. S. & Brentjens, R. J. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat. Rev. Clin. Oncol.* **17**, 147–167 (2020).
17. Chen, A. 'How do you decide?': Cancer treatment's CAR-T crisis has patients dying on a waitlist. *STAT* https://www.statnews.com/2022/06/02/car-t-crisis-cancer-patients-die-waiting (2022).
18. Dhakal, B., Chhabra, S., Savani, B. N. & Hamadani, M. Promise and pitfalls of allogeneic chimeric antigen receptor therapy in plasma cell and lymphoid malignancies. *Br. J. Haematol.* **197**, 28–40 (2022).
19. Benjamin, R. et al. Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. *Lancet* **396**, 1885–1894 (2020).
20. Hua, J. et al. Donor-derived anti-CD19 CAR T cells compared with donor lymphocyte infusion for recurrent B-ALL after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transpl.* **56**, 1056–1064 (2021).
21. Sommer, C. et al. Preclinical evaluation of allogeneic CAR T cells targeting BCMA for the treatment of multiple myeloma. *Mol. Ther.* **27**, 1126–1138 (2019).
22. Sommer, C. et al. Allogeneic FLT3 CAR T cells with an off-switch exhibit potent activity against AML and can be depleted to expedite bone marrow recovery. *Mol. Ther.* **28**, 2237–2251 (2020).
23. Martin, T. et al. Ciltacabtagene autoleucel, an anti-B-cell maturation antigen chimeric antigen receptor T-cell therapy, for relapsed/refractory multiple myeloma: CARTITUDE-1 2-year follow-up. *J. Clin. Oncol.* https://doi.org/10.1200/jco.22.00842 (2022).
24. Harrison, S. J. et al. A phase 1 first in human (FIH) study of AMG 701, an anti-B-cell maturation antigen (BCMA) half-life extended (HLE) BiTE® (bispecific T-cell engager) molecule, in relapsed/refractory (RR) multiple myeloma (MM). *Blood* **136**, 28–29 (2020).
25. Rafae, A. et al. Recent updates on bispecific T-cell engager (BiTE) antibodies in relapsed and refractory multiple myeloma. *J. Clin. Oncol.* **39**, e20034–e20034 (2021).
26. Neelapu, S. S. et al. ALPHA Study: ALLO-501 produced deep and durable responses in patients with relapsed/refractory non-Hodgkin's lymphoma comparable to autologous CAR T. *Blood* **138**, 3878–3878 (2021).
27. Nastoupil, L. J. et al. P1455: first-in-human trial of CB-010, a crispr-edited allogeneic anti-CD19 CAR-T cell therapy with a PD-1 knock out, in patients with relapsed or refractory B cell non-Hodgkin lymphoma (ANTLER STUDY). *HemaSphere* **6**(Suppl), 1337–1338 (2022).
28. *Prevention and Treatment of Cancer-Related Infections (Version 3.2022)*. NCCN Clinical Practice Guidelines in Oncology, Vol. 2022 (National Comprehensive Cancer Network, 2022).
29. Sassine, J. et al. Refractory and resistant cytomegalovirus after hematopoietic cell transplant in the letermovir primary prophylaxis era. *Clin. Infect. Dis.* **73**, 1346–1354 (2021).
30. Lancman, G. et al. Bispecific antibodies in multiple myeloma: present and future. *Blood Cancer Discov.* **2**, 423–433 (2021).
31. Lonial, S. et al. Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. *Lancet Oncol.* **21**, 207–221 (2020).
32. Maybury, B., Cook, G., Pratt, G., Yong, K. & Ramasamy, K. Augmenting autologous stem cell transplantation to improve outcomes in myeloma. *Biol. Blood Marrow Transpl.* **22**, 1926–1937 (2016).
33. Nooka, A. K. et al. Belantamab mafodotin in combination with novel agents in relapsed/refractory multiple myeloma: DREAMM-5 study design. *Future Oncol.* **17**, 1987–2003 (2021).
34. Sommer, C. et al. Preclinical evaluation of ALLO-605, an allogeneic BCMA turbocar TTM cell therapy for the treatment of multiple myeloma. *Blood* **136**, 8–8 (2020).

35. Lee, D. W. et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol. Blood Marrow Transpl.* **25**, 625–638 (2019).
36. Kumar, S. et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol.* **17**, e328–e346 (2016).

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.

© The Author(s), under exclusive licence to Springer Nature America, Inc. 2023

[1]Myeloma Service and Cellular Therapy Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA. [2]Department of Medicine, Weill Cornell Medical College, New York, NY, USA. [3]Colorado Blood Cancer Institute, Denver, CO, USA. [4]Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA. [5]Division of Hematology, Department of Medicine, Stanford University, Stanford, CA, USA. [6]Division of Bone Marrow Transplantation and Cellular Therapy, Stanford University Hospital, Stanford, CA, USA. [7]Vanderbilt University Medical Center, Nashville, TN, USA. [8]Bone Marrow Transplant and Cellular Therapy Program, Sarah Cannon Blood Cancer Center at St. David's South Austin Medical Center, Austin, TX, USA. [9]Banner MD Anderson Cancer Center, Gilbert, AZ, USA. [10]Taussig Cancer Institute, Department of Hematology and Medical Oncology, Cleveland Clinic, Cleveland, OH, USA. [11]Texas Transplant Institute, San Antonio, TX, USA. [12]Department of Hematology/HCT, City of Hope National Medical Center, Duarte, CA, USA. [13]Allogene Therapeutics, South San Francisco, CA, USA. [14]Mayo Clinic, Rochester, MN, USA. ✉e-mail: mailanks@mskcc.org

Methods

Study design and participants

UNIVERSAL is a phase 1, single-arm, open-label trial (NCT04093596) conducted at 13 sites in the United States of America. For this unplanned, post-hoc analysis, 48 patients were enrolled in the study and 43 received treatment as shown in Fig. 1, including 16 women (37%), 18 patients (52%) aged <65 years, 18 patients (42%) aged 65–75 years and 3 (7%) aged ≥75 years. Eligible patients were men or women who had relapsed/refractory MM, were aged ≥18 years and had received at least three previous lines of therapy, including a PI, IMiD and anti-CD38 monoclonal antibody. Patients were also required to be refractory to their last line of treatment (progression during or within 60 d of their last dose), and have measurable disease, an ECOG PS of 0 or 1, adequate hematological, renal and liver function and a left ventricular ejection fraction ≥50%, with no clinically significant pericardial or pleural effusion at screening. All patients were screened for the presence of DSAs and those with positive DSA tests ($n = 6$) were excluded. Patients were also required to have normal blood oxygen saturation levels. Patients who had received pevious non-CAR T BCMA-targeted therapy (bispecific, ADC or other) were allowed. Previous exposure to a BCMA-directed CAR T was excluded. The acute effects of any pevious therapy had to be resolved and any patients who had a grade ≥2 AE or serious (S)AE before LD were discussed with the sponsor before inclusion. Finally, eligible patients had to be seronegative for hepatitis B antigen and hepatitis C antibody, and women of childbearing potential had to have a negative serum pregnancy test.

Patients with known active or history of central nervous system (CNS) or leptomeningeal involvement of myeloma or plasma cell leukemia were excluded, as were patients with significant CNS dysfunction or any autoimmune disease with CNS involvement. Patients with current or a history of thyroid disorder, except hypothyroidism controlled on a stable dose of hormone replacement therapy, were excluded as well as those with active malignancies that required systemic treatment within 3 years before enrollment. Patients were excluded if they had major surgery within 3 months before the start of LD, radiation therapy within 2 weeks before the start of LD or autologous stem-cell transplantation within 6 weeks before the start of LD. Eligible patients were not permitted to have any previous allogeneic hematopoietic stem-cell transplantation and those with systemic anticancer therapy within 2 weeks before conditioning regimen or rituximab within the past 2 years were also excluded. Patients were excluded if they participated in other studies of investigational drugs within 28 days before lymphodepletion, had previous treatment with any gene therapy (CAR T cell therapy was permitted) or previous treatment with anti-CD52 monoclonal antibody (ALLO-647 was permitted). Ongoing treatment with immunosuppressive agents was not permitted, including corticosteroid use within 1 week before first dose of ALLO-715 (except an inhaled steroid for asthma, topical steroid use or another local corticosteroid administration), and infliximab had to be stopped at least 45 days before administration of ALLO-715. Active and clinically significant autoimmune disease within the last 2 years was exclusionary. as was any active uncontrolled bacterial, fungal or viral infection at screening and the presence of positive blood cultures within 7 days before ALLO-715 infusion. Patients known to be refractory to platelet or red blood cell transfusions were excluded as were those with any form of primary or acquired immunodeficiency. Patients with any indwelling line or drain were excluded, although dedicated central venous access catheters such as a Port-a-Cath or Hickman catheter were permitted. Patients were excluded if they had any of the following in the previous 6 months: myocardial infarction, congenital long QT syndrome, torsade de pointes, arrhythmias, left anterior hemiblock, unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident, transient ischemic attack, pulmonary embolism, deep vein thrombosis or other clinically significant episode of thromboembolic disease. Patients with ongoing cardiac dysrhythmias of NCI CTCAE (National Cancer Institute Common Terminology Criteria for Adverse Events) grade ≥2 or atrial fibrillation of any grade and those with cardiac amyloidosis were also ineligible. A history of hypertension crisis or hypertensive encephalopathy within 6 months before screening was exclusionary, as was a known or suspected hypersensitivity to murine or bovine products. Fertile male subjects and female subjects of childbearing potential were excluded if they were unwilling or unable to use a highly effective method of contraception for at least 12 months (6 months for males) after the last dose of cyclophosphamide or 6 months for females and males after the last dose of ALLO-647, whichever was later. Finally, patients with other acute or chronic medical or psychiatric conditions, including recent or active suicidal ideation or behavior, laboratory abnormality that could increase the risk associated with study participation or investigational product administration or could interfere with the interpretation of study results, were excluded as were those unwilling to participate in an extended safety monitoring period.

ALLO-715 was manufactured by Allogene from peripheral blood mononuclear cells obtained by leukapheresis from three healthy volunteer donors and was supplied for infusion as a frozen cell suspension in four different good manufacturing practice lots. Patients received LD followed by ALLO-715 at one of four dose levels (DLs) in a 3 + 3 dose escalation design: 40 (DL1), 160 (DL2), 320 (DL3) and 480 (DL4) $\times 10^6$ CAR^+ T cells. The first subject was treated and observed for 28 d before treating subsequent subjects with ALLO-715 and, if no DLT occurred in 3 subjects or if there was 1 DLT within 6 subjects at a given DL, enrollment to the next higher DL commenced in a staggered fashion.

Several LD regimens were evaluated: FCA39, FCA60, FCA90 and CA39, with fludarabine (F) 90 mg m^{-2}, cyclophosphamide (C) 900 mg m^{-2} and ALLO-647 (A) 39, 60 or 90 mg divided over 3 days. The starting dose of ALLO-647 (39 mg) was determined based on preclinical pharmacokinetic results and FCA39 was the first LD tested. ALLO-647 was infused over 3 days at a concentration of 10 mg ml^{-1}. The study protocol allowed for alternative doses of ALLO-647 to be tested for LD in DL2, DL3 or DL4 at the same or lower dose of ALLO-715, following the same 3 + 3 design used for the ALLO-715 escalation: escalate if no DLT occurred in 3 subjects or if there was 1 DLT within 6 subjects at a given dose level. There was no simultaneous escalation with ALLO-715 and ALLO-647. In the dose escalation phase, subjects were enrolled sequentially into FCA (at least three subjects), then into other available cohorts when open, until three to six subjects are treated in each cohort. Only one LD tested at a time and other LD doses were opened after a DLT period of the previous LD regimen. The FCA60 regimen was chosen for expansion cohorts based on its favorable safety efficacy and tolerability profile.

During the study, patients received prophylaxis for infection with pneumocystis pneumonia, herpesvirus and fungal infections according to NCCN guidelines for patients at high risk of infection[37] or standard institutional practice. NCCN recommendations included microbial prophylaxis during neutropenia using fluoroquinolone, fungal prophylaxis during neutropenia, antiviral therapy for 2 months after anti-CD52 therapy and until $CD4^+$ ≥200 cells μl^{-1} and prophylaxis for *Pneumocystis jirovecii* with trimethoprim/sulfamethoxazole (or atovaquone, dapsone, pentamidine if trimethoprim/sulfamethoxazole intolerant) for at least 2 months after ALLO-647 and until $CD4^+$ ≥200 cells μl^{-1}. Letermovir was recommended for CMV prophylaxis, especially in CMV-seropositive subjects, and patients were monitored weekly by PCR for a minimum of 2 months after ALLO-647 treatment. On confirmation of CMV viremia, recommended pre-emptive therapy was oral valganciclovir or intravenous ganciclovir for 2 weeks and until CMV was no longer detectable. CMV PCR antigen was monitored weekly. There was no protocol-defined level at which treatment was recommended, sites and investigators using their clinical judgment. For most subjects, the CMV antigen PCR level at which treatment was started was between 170 IU ml^{-1} and 2,200 IU ml^{-1} with a median of

800 IU ml^{-1}. Patients were to be followed for at least 54 months and then asked to participate in a separate long-term follow-up study.

Study oversight

Allogene sponsored the study, provided ALLO-715 and ALLO-647, and collaborated with academic investigators on study design, data analysis/interpretation and manuscript writing. The trial was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines, Declaration of Helsinki and all applicable regulatory requirements. The trial protocol was approved by an independent institutional review board (IRB) at each site before initiation, including Medical College of Wisconsin IRB, Memorial Sloan Kettering, Cancer Center IRB, Vanderbilt IRB, Stanford IRB, Advarra IRB, Integ Review IRB, Dana-Farber Cancer Institute IRB, Mayo Clinic IRB, WCG IRB, Cleveland Clinic IRB, Mt. Sinai IRB and City of Hope IRB. A data safety monitoring board was not used in this part of the study. All patients gave written informed consent. All authors confirm the accuracy of the data and adherence of the trial to the protocol.

Endpoints and study procedures

The primary objectives of the present study were to evaluate the safety and tolerability of ALLO-715 and ALLO-647. All AEs were collected for 3 months (90 days) after the dose of ALLO-715 or until the subject began a new anticancer therapy, whichever happened first. After 3 months, only SAEs were collected until disease progression or initiation of new anticancer therapy, whichever happened first. SAEs that were assessed as related to ALLO-715 or ALLO-647 by the investigator were collected regardless of the time of occurrence. Severity was graded according to the NCI CTCAE v.5.0. CRS was defined and graded according to the American Society of Transplantation and Cellular Therapy (ASTCT) grading criteria[35]. Neurological toxicity was evaluated using a broad standardized MedDRA query (SMQ) of noninfectious encephalopathy/delirium with adjudication by clinical review using ASTCT grading criteria for events reported as immune effector cell-associated neurotoxicity syndrome and CTCAE v.5.0 criteria for all other neurological toxicities. Infusion-related reactions (IRRs) were identified by review of AEs that occurred within 24 hours of ALLO-647 infusion and deemed related to ALLO-647. Potential symptoms of IRRs were adjudicated by clinical review. Events were graded according to the NCI CTCAE on the basis of the highest individual symptom grade. Prolonged cytopenias were defined as AEs of neutropenia and/or thrombocytopenia and/or anemia of grade ≥3 present or persisting at study day 56 after ALLO-715 infusion and which had been ongoing for the preceding 21 days. Lymphopenias were defined as events reported by investigators using the Preferred Terms lymphopenia, lymphocyte count decreased, T lymphocyte count decreased or B lymphocyte count decreased in Medical Dictionary for Regulatory Activities. The definition was per the individual sites using CTCAE v.5.0 and most of the events were reported as lymphocyte count decreased.

Key secondary endpoints were response and DOR. Clinical response and disease progression were assessed by the investigator according to the International Myeloma Working Group consensus criteria[36]. In addition, ALLO-715 cellular kinetics, ALLO-647 pharmacokinetics, immunogenicity of ALLO-715 and ALLO-647, and evaluation of host immune depletion and reconstitution by T and B lymphocyte and NK (TBNK) cell subsets were also evaluated. For TBNK and cellular kinetics evaluations, patient peripheral blood samples were assayed by multi-parameter flow cytometry before and after infusion to detect TBNK cell subsets, with validated antibody panels or BCMA CAR$^+$ T cells using an anti-idiotypic antibody with a lower limit of 0.01% of leukocytes (CellCarta Precision Medicine). Whole-blood samples were collected into Cyto-Chex (Streck) and shipped on ice overnight to CellCarta. Samples were stained with antibodies to CD45 and CD3 (Becton Dickenson) and anti-Allo-715 CAR idiotype (Allogene Therapeutics) and run on an LSR-II cytometer (Becton Dickenson). Data analysis was done using FlowJo software (FlowJo LLC). After gating out dead cells and debris, single cells were defined using side scatter peak height versus area. Lymphocytes were gated using a combination of side scatter and CD45. CAR T cells were separated from host lymphocytes using an anti-idiotype antibody developed at Allogene. A quantitative (q)PCR assay was also used to determine lentiviral vector transgene copy number to enable quantitative tracking of BCMA CAR$^+$ T cells to a minimum of 50 copies of transgene per microgram of DNA (Navigate BioPharma). The concentration versus time profile of ALLO-647 was adopted from a population PK model, which used post-hoc concentrations for all subjects enrolled in the UNIVERSAL study. The model-predicted exposure of ALLO-647 increased with administered dose and appeared to be more than dose proportional.

Exploratory endpoints included the evaluation of MRD by either next-generation sequencing (clonoSEQ, Adaptive Biotechnologies) or EuroFlow Next Generation Flow (Covance) with a minimum sensitivity of 10^{-5} in patients who obtained VGPR$^+$. In situations where central MRD could not be obtained, local MRD calculations were considered. Other exploratory endpoints included the measurement of select cytokines and quantification of soluble BCMA at select timepoints.

Statistical analysis

This was an unplanned post-hoc analysis. The dose escalation part of this phase 1 study was governed by a 3 + 3 design and 4 DLs, including 3 predefined DLs (DL1, DL2 and DL3) and 1 additional DL (DL4) or intermediate dose. Up to 6 subjects could be tested in each cohort at each dose level with 1 cohort at DL1, 2 cohorts at DL2, 3 cohorts at DL3 and up to 3 cohorts at DL4 or intermediate dose. To characterize the dose of ALLO-647, the DL3 cohorts were utilized to evaluate higher doses of ALLO-647. No sample size calculation was used because the size of each dose cohort was defined by the nature of the 3 + 3 design. The backfill option added up to three subjects per cohort within a DL to a maximum of six per cohort. All patients who enrolled were used for summaries related to subject demographics. All patients who received any amount of study drug (either ALLO-647 or ALLO-715) were included in safety analyses. All patients who received any amount of ALLO-715 were included in a modified intention to treat population for efficacy analyses.

Descriptive statistics include medians with minima and maxima for continuous variables and counts and percentages for categorical variables. Investigator-assessed responses are reported as ORRs with CIs assessed by exact methods (Clopper–Pearson 95% CIs). DOR was estimated using Kaplan–Meier methods. Censoring of data for DOR was based on FDA-censoring recommendations. Follow-up time was calculated using the reverse Kaplan–Meier method. Given the exploratory nature of the present study, no adjustments for multiple comparisons were made. Analyses were performed with SAS 8.2. All data presented utilize a data cutoff of 14 October 2021.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

This trial is currently ongoing. Subject to patient privacy and confidentiality obligations, access to patient-level data and supporting clinical documents may be available upon request and subject to review by the study sponsor on completion of the trial. Such requests can be made to Allogene Therapeutics, Inc., 210 East Grand Avenue, South San Francisco, CA 94080, USA or by email at info@allogene.com. A material transfer and/or data access agreement with the sponsor will be required to access the data.

References

37. *Prevention and Treatment of Cancer-Related Infections (Version 1.2021)*. NCCN Clinical Practice Guidelines in Oncology, July 2 (National Comprehensive Cancer Network, 2021).

Acknowledgements
The study was sponsored and funded by Allogene Therapeutics. We thank the patients who participated in this trial and the family and friends who supported them through the process as well as S. Ghatta (Allogene) and Medical Leverage and E. MacLaren who assisted in the preparation of this manuscript. TALEN gene-editing is a technology owned by Cellectis. S.M. acknowledges funding support from the NCI Memorial Sloan Kettering Core grant (P30 CA008748).

Author contributions
E.E.K., W.L., W.Y., A.B. and S.K.K. provided the overall trial design. E.E.K., W.L. and W.Y. did the clinical analysis. S.M., J.V.M., S.C., M.L., S.S., O.O.O., S.A.M., R.N., F.A., J.C.C., M.H., E.E.K. and S.K.K. carried out the clinical interpretation. M.D. and E.B. did the translational experiments, planning, execution and analysis. S.M., M.L., S.S., R.N., F.A., E.E.K., E.B., M.D. and W.Y. drafted the manuscript. A.B. and E.E.K. ensured the integrity of the work as a whole.

Competing interests
S.M. received consulting fees from Evicore, Optum, BioAscend, Janssen Oncology and Legend Biotech. Memorial Sloan Kettering Cancer Center receives research funding from the NCI, Janssen Oncology, Bristol Myers Squibb, Allogene Therapeutics, Fate Therapeutics and Takeda Oncology for conducting research. S.M. received honoraria from OncLive, Physician Education Resource, MJH Life Sciences and Plexus Communications. C.S. received institutional research funding from Janssen, Bristol Myers Squibb, Amgen, Novartis, Syndax, Ionis, Allogene and Sanofi GSK and honoraria for advisory boards/speakers bureau from Sanofi, GSK and Omeros. J.V.F., S.A.M., J.C.C. and M.H. report no competing interests. L.M. received honoraria from Pfizer and clinical trial funding from Caelum. L.M. is a member of the Board of Directors or other advisory committees at Alnylam, Bristol Myers Squibb, Caelum, Celgene, GlaxoSmithKline, Janssen Pharmaceuticals, Karyopharm, Kite, Kura Oncology, Oncopeptides, Pfizer, Sanofi and Takeda. S.S. reports consultancy fees from Janssen, Magenta Therapeutics and Bristol Myers Squibb, as well as research funding from Magenta Therapeutics and Allogene. O.O.O. held consultancy and advisory board roles for Pfizer, Kite, Gilead, AbbVie, Janssen, TGR therapeutics, Novartis, curio science and Nekktar, and received institutional funding from Kite, Pfizer, Daichi Sankyo and Allogene and honoraria from Pfizer and Gilead. R.N. reports consultancy fees and honoraria from Actinium and Incyte. F.A. received personal fees from Bristol Myers Squibb as a speaker and a fee from Janssen pharmaceutical as an advisory board member. F.A. consulted or held an advisory role for Seattle Genetics, Incyte Corporation Speakers' Bureau and Company: Incyte Corporation and received travel and accommodation expenses from Seattle Genetics and Incyte and honoraria from Incyte. Without receiving direct funding, F.A. served as the local principal investigator for Allogene Therapeutics, Celgene, GlaxoSmithKline, Bristol Myers Squibb, Seattle Genetics, Acetylon Pharmaceuticals, Millennium, Astellas Pharma and AbbVie. E.E.K., W.L., M.D. and A.B. are employees of Allogene Therapeutics. E.B. and W.Y. are consultants with Allogene Therapeutics who do not own stock. S.K.K. receives institutional funding for clinical trials from Abbvie, Amgen, Allogene, AstraZeneca, Bristol Myers Squibb, Carsgen, GlaxoSmithKline, Janssen, Novartis, Roche-Genentech, Takeda, Regeneron and Molecular Templates. S.K.K. also participated in consulting/advisory boards without personal payment for Abbvie, Amgen, Bristol Myers Squibb, Janssen, Roche-Genentech, Takeda, AstraZeneca, Bluebird Bio, Epizyme, Secura Biotherapeutics, Monterosa therapeutics, Trillium, Loxo Oncology, K36, Sanofi and ArcellX, and with personal payment for Oncopeptides, Beigene, Antengene and GLH Pharma.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41591-022-02182-7.

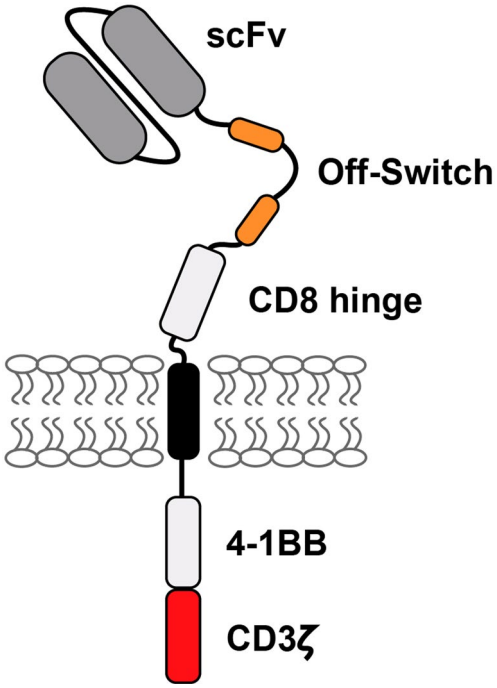
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-022-02182-7.

Correspondence and requests for materials should be addressed to Sham Mailankody.

Peer review information *Nature Medicine* thanks Katayoun Rezvani, Ying Yuan and Jay Spiegel for their contribution to the peer review of this work. Primary Handling Editors: Saheli Sadanand and Joao Monteiro, in collaboration with the *Nature Medicine* team

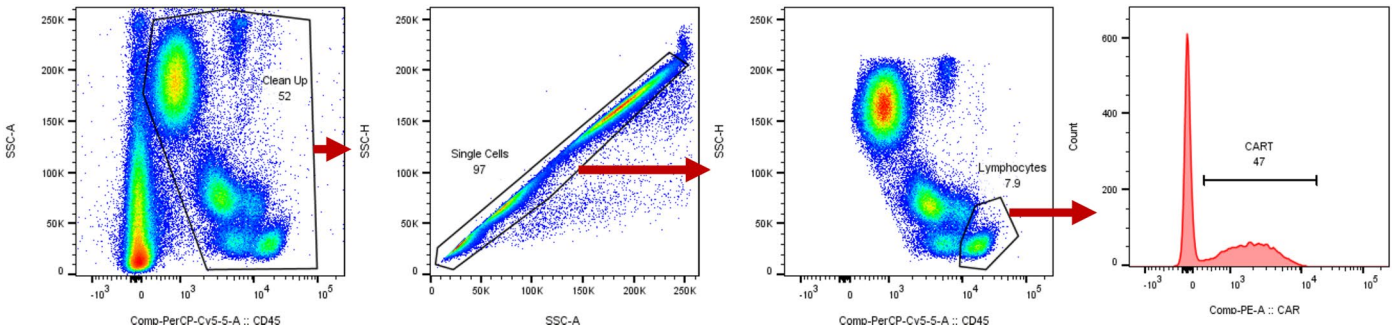
Reprints and permissions information is available at www.nature.com/reprints.

https://doi.org/10.1038/s41591-022-02182-7



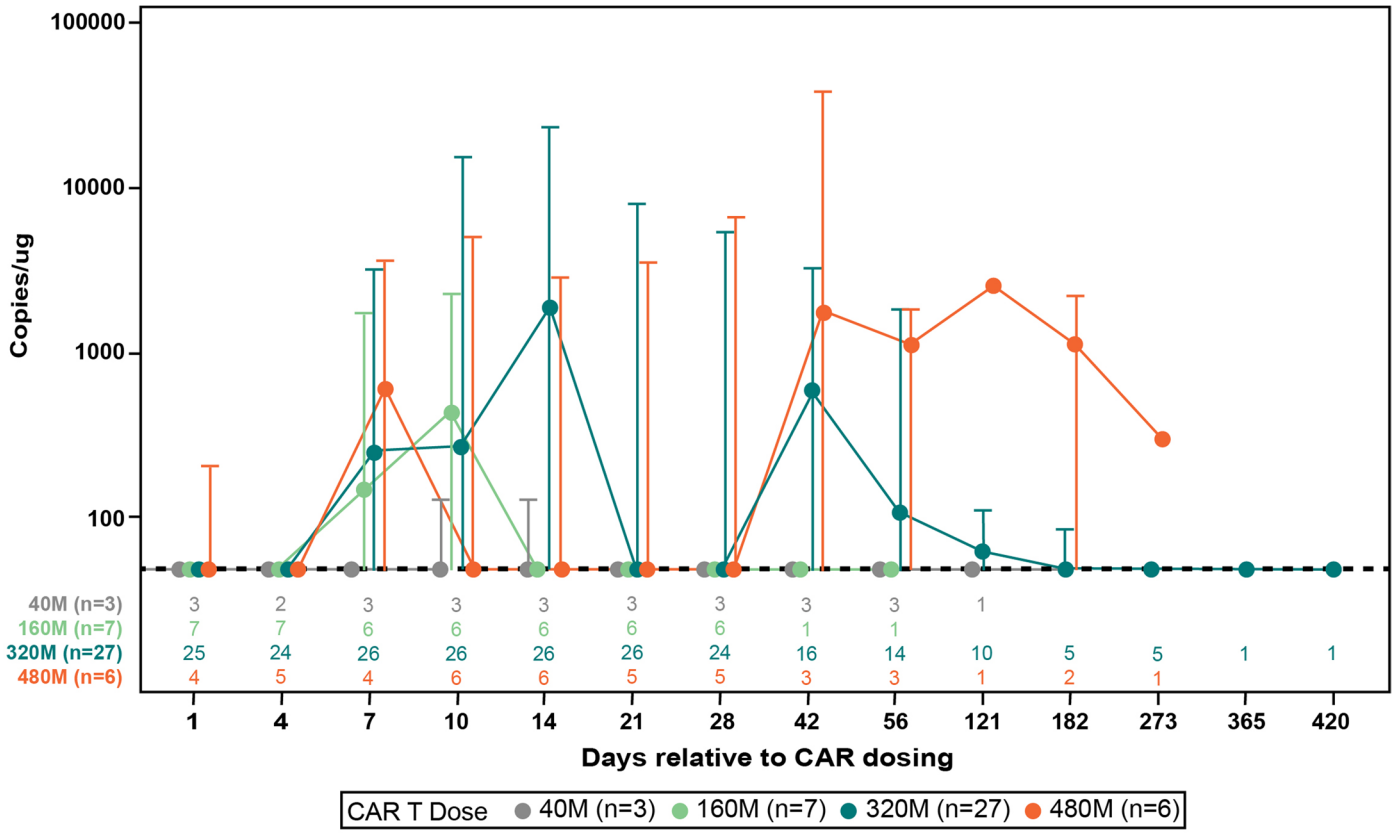
Extended Data Fig. 1 | Diagram of the scFv in ALLO-715. The major domains in ALLO-715 are shown schematically.

https://doi.org/10.1038/s41591-022-02182-7



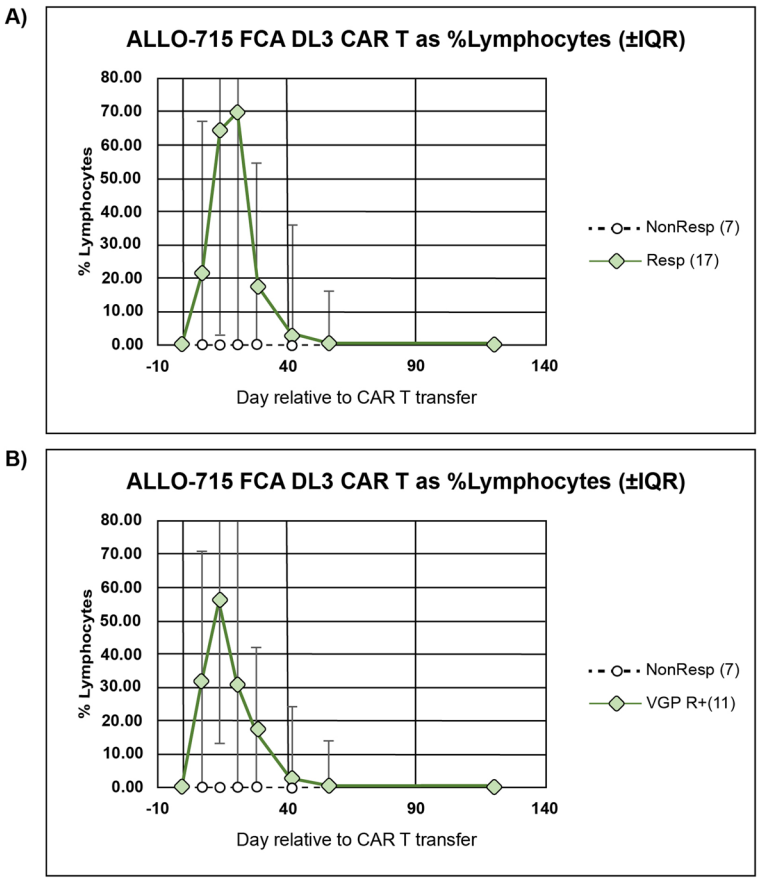
Extended Data Fig. 2 | Flow Cytometry Gating Strategy. After gating out dead cells and debris, single cells were defined using SSC peak height vs area. Lymphocytes were gated using a combination of side scatter and CD45. CAR T cells were separated from host lymphocytes using an anti-idiotype antibody developed at Allogene.

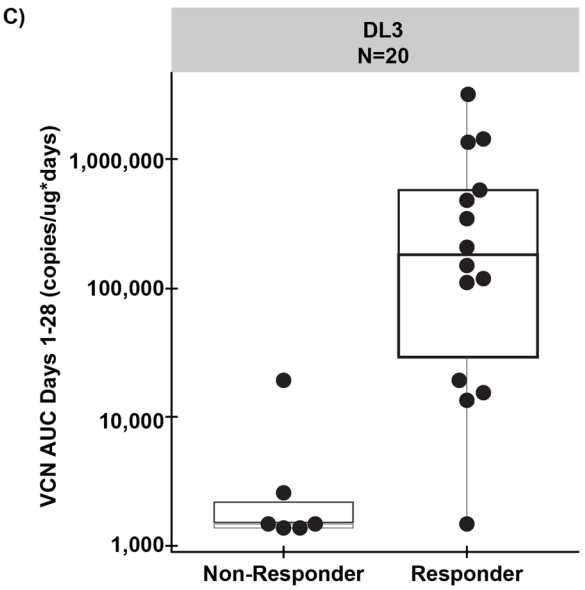
https://doi.org/10.1038/s41591-022-02182-7



Extended Data Fig. 3 | ALLO-715 in-vivo expansion by Dose Level in Patients Who Received a Fludarabine/Cyclophosphamide/ALLO-647 (FCA) Containing Lymphodepletion Regimen by Days Relative to CAR Dosing. Data are presented as median values ± interquartile range. N = 43 independent study subjects. Results reported as 'Not Quantifiable,' 'Reported and Unreliable,' or below the lower limit of quantitation (LLOQ) value have been replaced with LLOQ value (50 Copies/ug).

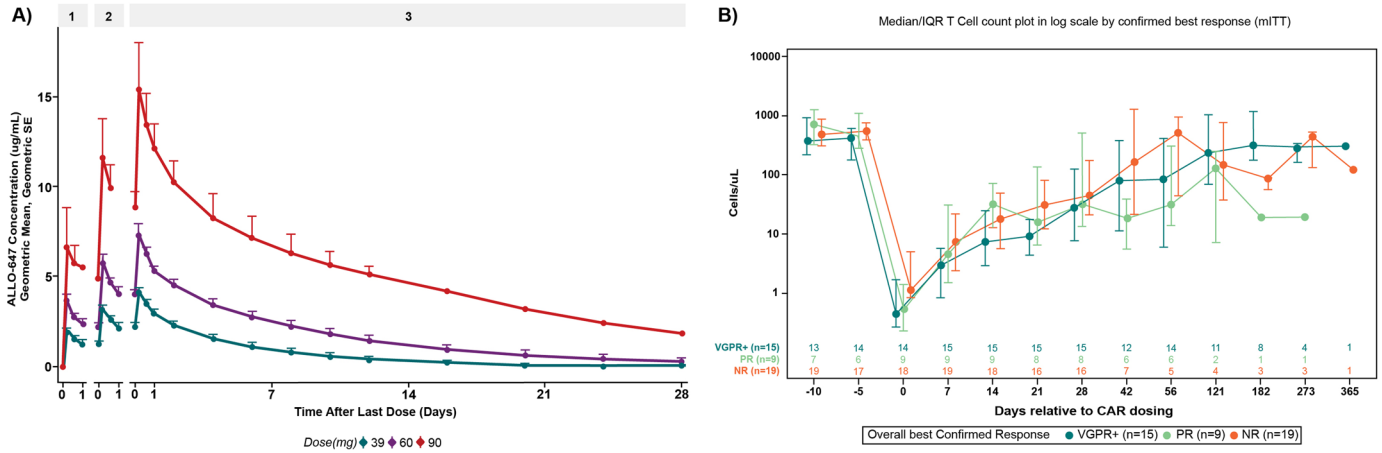
https://doi.org/10.1038/s41591-022-02182-7





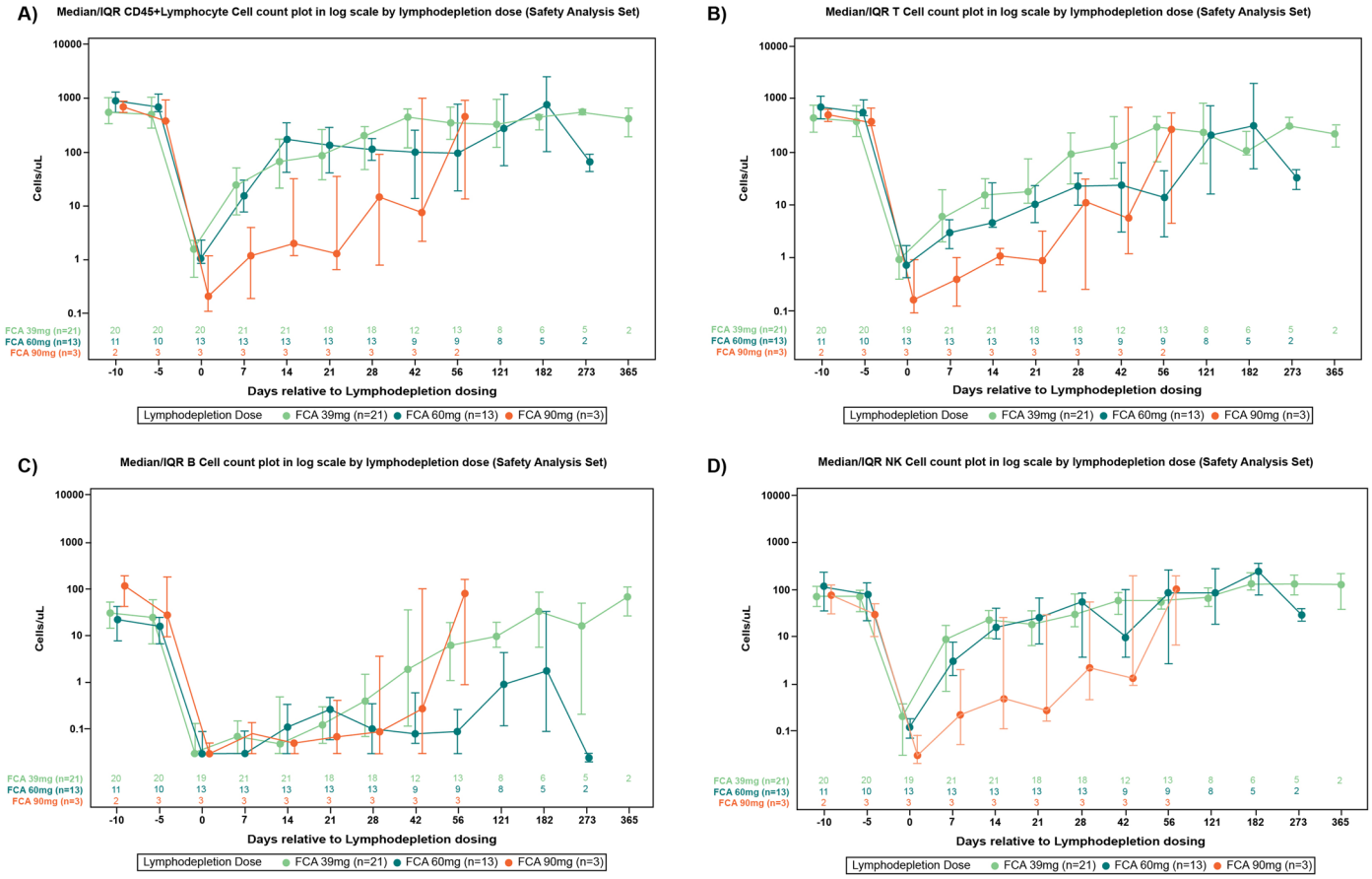
Extended Data Fig. 4 | CAR T Cell Concentrations Stratified by Response. (**a**) CAR T cell concentrations among ALLO-715 DL3 patients that received any fludarabine/cyclophosphamide/ALLO-647 (FCA) lymphodepletion are shown in responders and non-responders. Error bars show the interquartile range (IQR) for each data point. (**b**) CAR T cell concentrations among ALLO-715 DL3 patients that received any FCA lymphodepletion in non-responders and those with very good partial responses or better (VGPR+). Error bars show the IQR for each data point. (**c**) Box plot of vector copy number (VCN) responders and non-responders for all patients with assay results between days 1-28. The boxes represent the first quartile (Q1), median, and third quartile (Q3). The lower and the upper whiskers extend to the most extreme points within 1.5 × IQR of Q1 and Q3 respectively, where IQR = Q3-Q1. N = 20 independent study subjects (N = 6 non-responders; N = 14 responders).

https://doi.org/10.1038/s41591-022-02182-7



Extended Data Fig. 5 | ALLO-647 Pharmacokinetics and Host Immune Cell Depletion and Reconstitution. (**a**) Concentration vs time profile of ALLO-647 adopted from a population PK model, which used post-hoc concentrations for all subjects enrolled. The model-predicted exposure of ALLO-647 increased with administered dose and appeared to be greater than dose proportional. The geometric mean is shown, and the error bars represent the geometric standard error. N = 96 independent subjects including N = 13 from ALPHA2, N = 41 from ALPHA, and N = 42 from UNIVERSAL. (**b**) Host T cell counts per ul of whole blood relative to study day. Data are presented as median values ± interquartile range, and N = 43 independent study subjects. VGPR+= very good partial response or better. PR = partial response. NR=no response.

https://doi.org/10.1038/s41591-022-02182-7



Extended Data Fig. 6 | Host Immune Cell Counts Per µl Whole Blood Relative to Study Day. Counts for the following immune cell types are shown: (**a**) CD45+ cells; (**b**) T cells; (**c**) B cells; (**d**) and NK cells. Data are presented as median values ± interquartile range. N = 43 independent study subjects. FCA = Fludarabine/Cyclophosphamide/ALLO-647.

https://doi.org/10.1038/s41591-022-02182-7

Extended Data Table 1 | Summary of infections by maximum CTCAE grade

Preferred Term	Grade 1 n (%)	Grade 2 n (%)	Grade 3 n (%)	Grade 4 n (%)	Grade 5 n (%)	All Grades n (%)
Number of patients with any infections TEAE	3 (7.0%)	10 (23.3%)	7 (16.3%)	0 (0.0%)	3 (7.0%)	23 (53.5%)
Viral infections	5 (11.6%)	9 (20.9%)	3 (7.0%)	0 (0.0%)	1 (2.3%)	18 (41.9%)
Cytomegalovirus viraemia	5 (11.6%)	7 (16.3%)	2 (4.7%)	0 (0.0%)	0 (0.0%)	14 (32.6%)
Adenovirus reactivation	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	2 (4.7%)
BK virus infection	2 (4.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (4.7%)
Human herpesvirus 6 infection reactivation	2 (4.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (4.7%)
Adenoviral hepatitis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	1 (2.3%)
Adenovirus infection	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (2.3%)
Human herpesvirus 6 infection	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (2.3%)
Colitis herpes	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Herpes simplex	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Parvovirus B19 infection	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Oral herpes	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Pneumonia respiratory syncytial viral	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Respiratory syncytial virus infection	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Influenza	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Other infections	0 (0.0%)	2 (4.7%)	5 (11.6%)	0 (0.0%)	1 (2.3%)	8 (18.6%)
Pneumonia	0 (0.0%)	1 (2.3%)	3 (7.0%)	0 (0.0%)	0 (0.0%)	4 (9.3%)
Urinary tract infection	0 (0.0%)	2 (4.7%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	3 (7.0%)
Sepsis	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	1 (2.3%)	2 (4.7%)
Folliculitis	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Enterocolitis infectious	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Fungal infections	2 (4.7%)	2 (4.7%)	1 (2.3%)	0 (0.0%)	2 (4.7%)	6 (14.0%)
Candida infection	1 (2.3%)	2 (4.7%)	1 (2.3%)	0 (0.0%)	1 (2.3%)	4 (9.3%)
Pneumonia fungal	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)	1 (2.3%)
Oral candidiasis	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Bacterial infections	0 (0.0%)	1 (2.3%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	2 (4.7%)
Staphylococcal bacteraemia	0 (0.0%)	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	1 (2.3%)
Enterocolitis bacterial	0 (0.0%)	1 (2.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (2.3%)

TEAE = Treatment Emergent Adverse Event

https://doi.org/10.1038/s41591-022-02182-7

Extended Data Table 2 | Efficacy in patients who received CA LD regimens

Cell Dose & Lymphodepletion Regimen¶	160 x 10^6 CAR+ T Cells	320 x 10^6 CAR+ T Cells
	CA39 (N=3)	CA39 (N=3)
ORR*, n (%) (95% CI)	0	2 (67) (9.4, 99.2)
VGPR+ rate, n (%)	0	1 (33)
CR/sCR rate, n (%)	0	1 (33)
mDOR, months (95% CI)	N/A	8.5 (1, 8.5)
Median follow-up, months (range)†	1 (0, 1)	3 (1, 9)

https://doi.org/10.1038/s41591-022-02182-7

Extended Data Table 3 | ALLO-715 in vivo expansion by DL in patients who received an FCA-containing LD regimen

	DL1 (N=3)	DL2 (N=4)	DL3 (N=24)	DL4 (N=6)
C_{max} (copies/µg), median (IQR)	50 (50, 130.8)	833.1 (109.5, 1556.7)	6419.4 (99.1, 34669.3)	621.9 (50.0, 6615.3)
T_{max} (days), median (IQR)	1 (1, 14)	5.5 (1.0, 10.0)	10 (7, 12)	5.5 (1, 10)
AUC28 (copies/µg*days), median (IQR)	1411.4 (1390.2, 2119.6)	3115.8 (1470.2, 7335.7)	65839.4 (2010.0, 420363.6)	53591.8 (1335.7, 427530.8)

nature portfolio

Corresponding author(s): Sham Mailankody

Last updated by author(s): Dec 2, 2022

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No software was used in the collection of data.
Data analysis	Analyses were performed with SAS 8.2.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

This trial is currently ongoing. Subject to patient privacy and confidentiality obligations, access to patient-level data and supporting clinical documents may be available upon request and subject to review by the study Sponsor upon completion of the trial. Such requests can be made to Allogene Therapeutics, Inc., 210 East Grand Avenue, South San Francisco, CA 94080. A material transfer and/or data access agreement with the Sponsor will be required to access the data.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Sex and gender were not considered in study design. Sex/gender analysis carried out are described in the publication.
Population characteristics	Eligible patients were ≥ 18 years of age and must have received at least 3 prior lines of therapy including a proteasome inhibitor, immunomodulatory agent, and an anti-CD38 monoclonal antibody. Patients were also required to be refractory to their last line of treatment (progression during or within 60 days following their last dose); have measurable disease; an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and have adequate organ function.
Recruitment	Participants were recruited by investigators pursuant to IRB approved methods to reduce the possibility of biased recruiting.
Ethics oversight	Local IRBs approved all study protocols and ICFs, including Medical College of Wisconsin IRB, Memorial Sloan Kettering, Cancer Center IRB, Vanderbilt IRB, Stanford IRB, Advarra IRB, Integ Review IRB, Dana-Farber Cancer Institute IRB, Mayo Clinic IRB, WCG IRB, Cleveland Clinic IRB, Mt. Sinai IRB, and City of Hope IRB.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	A total of 48 patients were enrolled into Part A of UNIVERSAL, and the sample size was governed by a standard 3+3 study design and 4 dose levels (DL), including 3 predefined DLs (DL1, DL2, and DL3) and 1 additional DL (DL4) or intermediate dose. Up to 6 subjects could be tested in each cohort at each dose level with 1 cohort at DL1, 2 cohorts at DL2, 3 cohorts at DL3, and up to 3 cohorts at DL4 or intermediate dose. No sample size calculation was used because the size of each dose cohort was defined by the nature of the 3+3 design. The backfill option added up to 3 subjects per cohort within a dose level to a maximum of 6 per cohort.
Data exclusions	All patients were screened for the presence of donor (product)-specific anti-HLA antibodies (DSA) and those with positive DSA were excluded.
Replication	This is an unplanned, ad hoc analysis. Sample sizes of independent patients are noted to show the degree of replication in the results.
Randomization	This was a single-arm trial.
Blinding	Blinding was not applied to this study because it was a single-arm trial in a setting of high unmet medical need.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	ALLO-647, a proprietary humanized anti-CD52 mAb provided by Allogene. ALLO-647 was infused over 3 days (13 to 30 mg/day at a concentration of 10 mg/mL). Anti-CD38 antibodies are referred to in the manuscript in the context of previous patient treatments and were not directly involved in the current study, therefore the dilutions and details are not provided.
Validation	All validation of this antibody is proprietary information and has been included in IND application and has been reviewed by the FDA in clearance of the IND.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT04093596
Study protocol	Copies of the protocol may be reasonably requested from Sponsor, in accordance with the terms of an executed NDA.
Data collection	Patients were enrolled from September 10, 2019 through October 14, 2021, and data were collected at 13 clinical study sites in the United States.
Outcomes	The primary outcomes of this study were to evaluate the safety and tolerability of ALLO-715 and ALLO-647. These outcomes were assessed by collecting all adverse event (AE) data through 90 days of follow-up after the dose of ALLO-715. After 90 days, serious AEs were collected until disease progression or initiation of new anti-cancer therapy, whichever occurred first. The secondary outcomes of the study included evaluating the preliminary anti-tumor activity of ALLO-715, characterizing the cellular kinetics of ALLO-715, characterizing the pharmacokinetic parameters of ALLO-647, evaluating the immunogenicity of ALLO-715 and ALLO-647, and evaluating host immune cell depletion and reconstitution resulting from lymphodepletion with ALLO-647 in combination with Flu and/or Cy, or ALLO-647 alone, prior to ALLO-715. The anti-tumor activity of ALLO-715 was assessed by collecting the overall response rate (ORR), stringent complete response (sCR), complete response (CR), very good partial response (VGPR), and partial response (PR), according to the International Myeloma Working Group (IMWG) consensus criteria for response and minimal residual disease assessment in multiple myeloma, and time-to-event endpoints including time to response, duration of response (DOR), progression-free survival (PFS), overall survival (OS). The cellular kinetics of ALLO-715 were characterized by assessing peak expansion (Cmax), persistence (Clast and Tlast, t1/2), area under the curve (AUC28d and AUClast). The pharmacokinetics of ALLO-647 were characterized by assessing of maximum concentration (Cmax), area under the concentration versus time curve from time zero to the last quantifiable time point (AUClast). The immunogenicity of ALLO-715 and ALLO-647 was assessed by the incidence and levels of anti-ALLO-715 antibodies and anti-TALEN® antibodies, donor-specific anti-human leukocyte antigen (HLA) antibodies directed against ALLO-715, and the incidence and level of anti-ALLO-647 and neutralizing antibodies against ALLO-647. Finally, host immune cell depletion and reconstitution were evaluated by enumerating host peripheral T-cell, B-cell, and NK-cell subsets and assessing serum immunoglobulins.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Whole blood samples were collected into Cyto-Chex (Streck, Omaha, NE, USA) and shipped on ice overnight to CellCarta (Montreal, Canada). Samples were stained with antibodies to CD45 and CD3 (Becton Dickenson, San Jose, CA, USA) and anti-Allo-715 CAR idiotype (Allogene Therapeutics, South San Francisco, CA, USA).
Instrument	Samples were run on an LSR-II cytometer (Becton Dickenson).
Software	Data analysis was done using FlowJo software (FlowJo LLC, Ashland OR, USA).
Cell population abundance	After gating out dead cells and debris, single cells were defined using SSC peak height vs area.
Gating strategy	Lymphocytes were gated using a combination of side scatter and CD45. CAR T cells were separated from host lymphocytes using an anti-idiotype antibody developed at Allogene.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.