A novel BCMA CAR-T-cell therapy with optimized human scFv for treatment of relapsed/refractory multiple myeloma: results from phase I clinical trials

Chimeric antigen receptor (CAR) T cells targeting B-cell maturation antigen (BCMA) demonstrate appealing anti-tumor activity in patients with relapsed/refractory multiple myeloma (RRMM), but toxicity and short-term efficacy limit their clinical usage.[1-4] For recently Food and Drug Administration-licensed idecabtagene vicleucel (murine-derived single-chain variable fragment [scFv]), 128 patients with RRMM achieved 73.4% objective response rate (ORR) and 10.7-month median duration of response (DOR). The study also reported severe toxicities including ≥grade 3 cytokine release syndrome (CRS), neurotoxicity, and treatment-related deaths that occurred within 8 weeks after infusion.[3] In order to improve safety, overcome limited efficacy, and reduce immunogenicity from non-human–derived components, we developed autologous CAR-BCMA T cells (CT053) expressing the fully-human BCMA-specific scFv (25C2). Three investigator-initiated phase I studies investigated CT053's safety, pharmacokinetics, and preliminary efficacy in patients with RRMM (clinicaltrials gov. Identifiers: NCT03302403, NCT03380039, NCT03716856). We found that CT053 demonstrated an acceptable safety, pharmacokinetic, and efficacy profile.

We hypothesized that an optimized, human scFv would avoid immunogenicity and improve safety and efficacy. We identified 25C2 through naïve human scFv phage library screening. Results showed that 25C2 could bind to human and mouse BCMA but not other TNF receptor family members, human TACI and BAFFR, indicating its BCMA-specific binding capacity (*Online Supplementary Figure S1A*). Binding analysis indicated that 25C2 had 603.9 pM binding affinity against recombinant human BCMA and 87% monomer ratio (*Online Supplementary Figure S1B*). CT053 was generated by transducing T cells with lentivirus encoding a CAR comprising the 25C2 scFv, human CD8α hinge domain, CD8α transmembrane domain, 4-1BB co-stimulatory domain and CD3ζ activation domain. In preclinical studies, CT053 showed low tonic signaling and potent *in vitro* MM cell killing (*Online Supplementary Figure S1C-E*). Accordingly, we launched three phase I studies to evaluate CT053 in patients with RRMM.

Thirty patients consented, 27 underwent leukapheresis, and 24 received CT053 infusion (0.5-1.8×10^8 cells) in the three trials (Table 1). Patients met International Myeloma Working Group diagnostic criteria for RRMM and enrollment eligibility criteria.[5] Median age was 60 years (range, 39-70 years) and 37.5% had International Staging System (ISS) stage III disease. Median number of prior systemic regimens was five (range, 2–11). Patients included 41.7% with extramedullary disease (EMD), 50% with high-risk cytogenetics, and 33.3% with Eastern Cooperative Oncology Group (ECOG) scores 2-3. Seven patients received bridging therapy before lymphodepletion (Table 1).

All autologous CT053 products were successfully manufactured at CARsgen's GMP facility. Patients received lymphodepletion comprising fludarabine (median dose, 21 mg/m^2/day [range, 19–27 mg/m^2/day] for 2–4 days) and cyclophosphamide (median dose, 467 mg/m^2/day [range, 192–543 mg/m^2/day] for 1–5 days). Subsequently, patients received one CT053 infusion: P1 received 0.5×10^8 cells, P2 received 1.8×10^8 cells due to their weight (91 kg), and P24's poor clinical condition prompted the 1.0×10^8-cell modified dose. Remaining patients received 1.5×10^8 cells.

All patients experienced ≥grade 3 treatment-related hematological adverse events (AE). Treatment-related hematological toxicities of ≥grade 3, expected lymphodepletion effects, were leukopenia (83.3%), lymphocytopenia (79.2%), neutropenia (75.0%), anemia (33.3%), and thrombocytopenia (25.0%) (Table 2). The median durations for grade 3 or grade 4 neutropenia and thrombocytopenia to recover to ≤grade 2 were 9 days (95% confidence interval [CI]: 5.0-44.0) and 55 days (95% CI: 8.0-not evaluable), respectively. Thirteen SAE were reported in seven patients: eight events were infections, and four events were hematological toxicities. One death occurred: P15 died on day 25 due to bone marrow failure and neutropenic infection related to lymphodepletion and disease progression.

Fifteen patients (62.5%) experienced CRS; however, no events were ≥grade 3 (4 grade 1, 11 grade 2). Generally, CRS occurred a median 3 days (range, 1–9 days) after infusion and resolved in a median 6 days (range, 3–9 days). There were no significant differences in peak levels of ferritin, C-reactive protein, and IL-6 within 28 days after infusion between patients with or without CRS (data not shown). Four patients received two tocilizumab doses, and five patients received one dose (4–6 mg/kg). Tocilizumab had no impact on CAR-BCMA copy numbers (data not shown). P11, with no prior convulsive history, experienced grade 3 neurotoxicity with grade 2 CRS, presenting as epilepsy. This event started 6 days after infusion, and resolved within 3 days after treatment with methylprednisolone, diazepam, and sodium valproate.

As of the cutoff date of June 30, 2021, median follow-up

Table 1. Characteristics in patients with or without extramedullary disease.

Characteristics	All patients (N=24)	Without EMD (N=14)	With EMD (N=10)
Age, years[a]	60 (39-70)	58 (39-67)	63 (39-70)
18 to < 65 years, N (%)	18 (75.0%)	12 (85.7%)	6 (60.0%)
≥ 65 years, N (%)	6 (25.0%)	2 (14.3%)	4 (40.0%)
Males, N (%)	13 (54.2%)	7 (50.0%)	6 (60.0%)
BSA (m^2)[a]	1.7 (1.3-2.1)	1.7 (1.4-1.9)	1.6 (1.3-2.1)
Time since diagnosis, years[b, a]	3.5 (0.4-10.8)	4.0 (0.4-10.8)	3.2 (0.8-5.9)
Heavy chain /light chains, N	22/2	13/1	9/1
High-risk cytogenetics,[c] N (%)	12 (50%)	4 (29%)	8 (80%)
ECOG,[d] N (%)			
0-1	16 (66.7%)	11 (78.6%)	5 (50.0%)
2	6 (25.0%)	3 (21.4%)	3 (30.0%)
3	2 (8.3%)	0 (0.0%)	2 (20.0%)
ISS, N (%)			
I & II	15 (62.5%)	7 (50.0%)	8 (80.0%)
III	9 (37.5%)	7 (50.0%)	2 (20.0%)
BCMA expression in BM, N (%)[a]	91.3 (30.4-99.8)	90.8 (58.5-99.8)	93.5 (30.4-99.5)
N of prior anti-MM regimens,[a] N (%)	5 (2-11)	6 (2-11)	4 (2-8)
Proteasome inhibitors	24 (100%)	14 (100%)	10 (100%)
Bortezomib	24 (100%)	14 (100%)	10 (100%)
Ixazomib	2 (8.3%)	1 (7.1%)	1 (10.0%)
Carfilzomib	3 (12.5%)	2 (14.3%)	1 (10.0%)
Immunomodulatory drugs	22 (91.7%)	13 (92.9%)	9 (90.0%)
Lenalidomide	18 (75%)	11 (78.6%)	7 (70.0%)
Pomalidomide	3 (12.5%)	3 (21.4%)	0 (0.0%)
Thalidomide	11 (45.8%)	7 (50%)	4 (40.0%)
Anti-CD38 monoclonal antibody (daratumumab)	5 (20.8%)	4 (28.6%)	1 (10.0%)
Stem cell transplantation	10 (41.7%)	7 (50.0%)	3 (30.0%)
Refractory to last regimen			
Yes	22 (91.7%)	13 (92.9%)	9 (90.0%)
Unknown	2 (8.3%)	1 (7.1%)	1 (10.0%)
Bridging therapy,[e] N (%)	7 (29.2%)	2 (14.3%)	5 (50%)

BCMA: B-cell maturation antigen; BM: bone marrow; BSA: body surface area; EMD: extramedullary disease; ISS: International Staging System; MM: multiple myeloma. Thirty patients consented for the 3 studies. Three patients who consented were ineligible to receive CT053 because of their blood test results (creatinine increased beyond range of eligibility). Twenty-seven patients underwent leukapheresis. Of these, patients were excluded because of intracranial hemorrhage (1), intracranial infiltration (1), and low platelet count (1). The 24 patients who received CT053 infusion are included in the table. [a]Median (range), [b]the time between the initial diagnosis and the study screening visit. [c]Results of cytogenetics obtained by fluorescence *in situ* hybridization from bone marrow aspirate performed at any point before treatment with CT053. High-risk cytogenetics were defined as gain(1q), del(13), del(17p), t(4;14), t(14;16), t(14;20). [d]Eastern Cooperative Oncology Group (ECOG) performance status scores range from 0 to 5, with higher scores indicating greater disability; a score of 5 indicates death. [e]Bridging therapy was administered in seven patients after leukapheresis and before lymphodepletion. Five patients received a bortezomib-based regimen, 1 patient received a lenalidomide-based regimen, and 1 patient received etoposide-, cyclophosphamide- and cisplatin-based combination chemotherapy.

time was 17.4 months (range, 0.9–38.7 months), and ORR (partial response [PR] or better) was 87.5%, with 79.2% patients experiencing complete reponse (CR) (12.5%) or stringent complete response (sCR, 66.7%) (Figure 1A). Seven patients died due to disease progression, including four who relapsed from sCR, in addition to P15 who died due to SAE. Responses occurred early, with median 4.1 weeks (range, 1.9–12.7 weeks) to first PR or better after infusion. Median time to best response was 8.3 months (range, 1.0–16.5 months). Nine patients (37.5%) had persistent CR/sCR and completed 24-month follow-up, including seven who had minimal residual disease (MRD)-negative status through the last follow-up visit at 24 months.[5]

The CR/sCR rate was 70% for patients with EMD and 86% in patients without EMD. Notably, P21 presented with thoracic cutaneous plasmacytomas that significantly shrank after infusion, and computed tomography (CT) showed 80% reduction at day 12 after infusion (Figure 1B and C). The lesions were confirmed eliminated by CT at day 64. P21 remained in sustained remission at data cutoff.

Median progression-free survival (PFS) was 18.8 months (95% CI: 10.1–not evaluable [NE]) in all patients, and there was no statistical difference in patients with or without EMD (*Online Supplementary Figure S2*). Median overall sur-

Table 2. Incidence of treatment-related and treatment-emergent adverse events in ≥2 patients (N=24).

Preferred term[a], N (%)	Treatment-related adverse events[b]		Treatment-emergent adverse events[c]	
	All grades	Grade ≥3	All grades	Grade ≥3
Cytokine release syndrome	15 (62.5)	0	15 (62.5)	0
Hematologic adverse events				
Neutrophil count decreased	23 (95.8)	18 (75.0)	22 (91.7)	16 (66.7)
White blood cell count decreased	21 (87.5)	20 (83.3)	20 (83.3)	14 (58.3)
Platelet count decreased	14 (58.3)	6 (25.0)	14 (58.3)	8 (33.3)
Lymphocyte count decreased	19 (79.2)	19 (79.2)	13 (54.2)	6 (25.0)
Anemia	10 (41.7)	8 (33.3)	12 (50.0)	7 (29.2)
Non-hematologic adverse events				
Pyrexia	18 (75.0)	6 (25.0)	18 (75.0)	6 (25.0)
Hypokalemia	6 (25.0)	2 (8.3)	14 (58.3)	3 (12.5)
Hypocalcemia	4 (16.7)	0	10 (41.7)	0
Aspartate aminotransferase increased	4 (16.7)	1 (4.2)	8 (33.3)	1 (4.2)
Alanine aminotransferase increased	2 (8.3)	0	8 (33.3)	0
Upper respiratory tract infection	2 (8.3)	1 (4.2)	7 (29.2)	2 (8.3)
Immunoglobulins decreased	5 (20.8)	0	6 (25.0)	0
Decreased appetite	6 (25.0)	0	5 (20.8)	0
Asthenia	5 (20.8)	0	5 (20.8)	0
Diarrhea	3 (12.5)	0	5 (20.8)	0
Pneumonia	3 (12.5)	3 (12.5)	4 (16.7)	4 (16.7)
Mouth ulceration	0	0	4 (16.7)	0

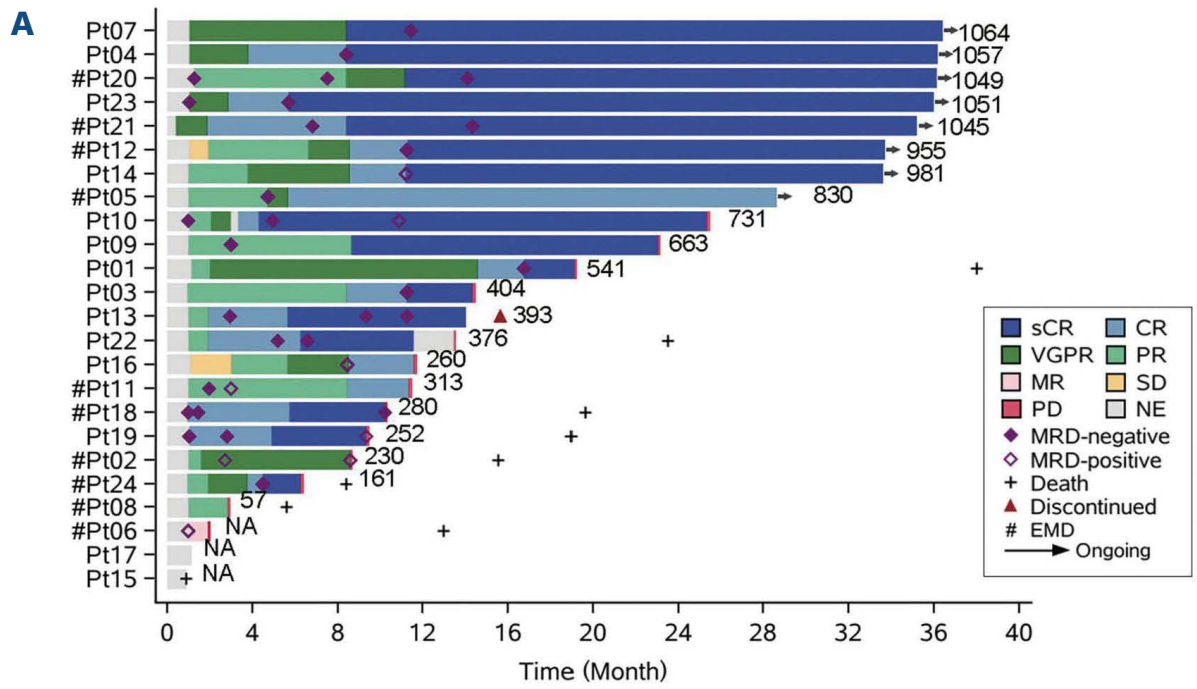
[a]Medical Dictionary for Regulatory Activities (version 24.0), graded according to National Cancer Institute Common Terminology Criteria for Adverse Event version 4.01. [b]Treatment-related adverse event indicates lymphodepletion-related and/or CT053-related adverse event. [c]Treatment-emergent adverse event is defined as any adverse event starting from CT053 infusion to 24 months after infusion.

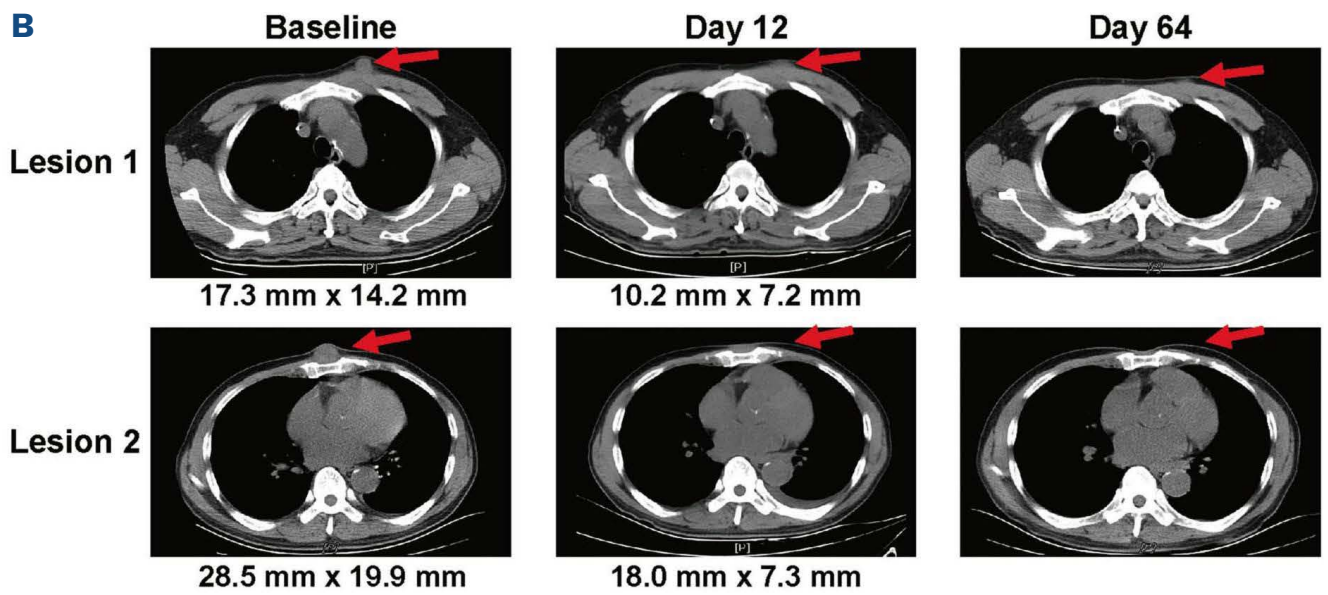
vival (OS) was not reached. Median DOR was 21.8 months (95% CI: 9.2–NE) in all patients. Numerically higher median DOR was observed in patients achieving MRD-negativity than those with MRD-positivity, though not statistically significant (24.0 months, 95%CI: 10.3–NE *vs*. 8.5 months, 95%CI: 7.6–NE, respectively).

After CT053 infusion, CAR-BCMA transgene copies became detectable at days 1–7 in all patients. Median peak value of transgene copies was 92,621 copies/μg genomic DNA (15,047–449,369 copies/μg genomic DNA), and median time to peak value was 13.5 days (range, 7–21 days). CT053 was detectable in nine of 20 patients at 6 months, and three of seven patients had detectable CT053 at 12 months (*Online Supplementary Figure S3A*).

CT053 expansion correlated with tumor antigen exposure. Peak transgene copy numbers significantly correlated with the burden of BCMA-positive plasma cells (r=0.7684, *P*<0.001) (*Online Supplementary Figure S3B*). However, IL-6 levels stayed relatively low (median 17.23 pg/mL) regardless of transgene copy numbers (*Online Supplementary Figure S3C*). Median peak values of transgene copy numbers were significantly higher in patients with very good partial response (VGPR) or better *versus* those who had not reached VGPR at month 4 (164,380 copies/μg genomic DNA [gDNA] *vs*. 60,547 copies/μg gDNA, respectively) and across the study (111,214 copies/μg gDNA *vs*. 17,301 copies/μg gDNA, respectively) (*Online Supplementary Figure S3D*). Results indicated that early tumor responses and best responses were associated with CT053 expansion levels. Nevertheless, we observed little difference in CAR-T-cell expansion and persistence between CT053 and non-human BCMA CAR-T cells.[3]

Anti-drug antibody (ADA) was not detected in patients after infusion, demonstrating no obvious immunogenicity for CT053. ADA is a risk factor for suppressed CAR-T-cell expansion.[4] Given fully-human CT053's lack of immunogenicity, we plan to explore repeat dose efficacy in ongoing trials. Our results may reflect CT053's scFv optimization. We selected CT053's novel fully-human scFv for its high affinity, stability, and favorable preclinical efficacy with reduced toxicity. Higher affinity scFv improve tumor recognition and enhance antitumor efficacy *in vitro* and *in vivo*.[6] However, the highest affinities can inversely correlate with efficacy and cause on-target off-tumor toxicity. Fine-tuning scFv af-





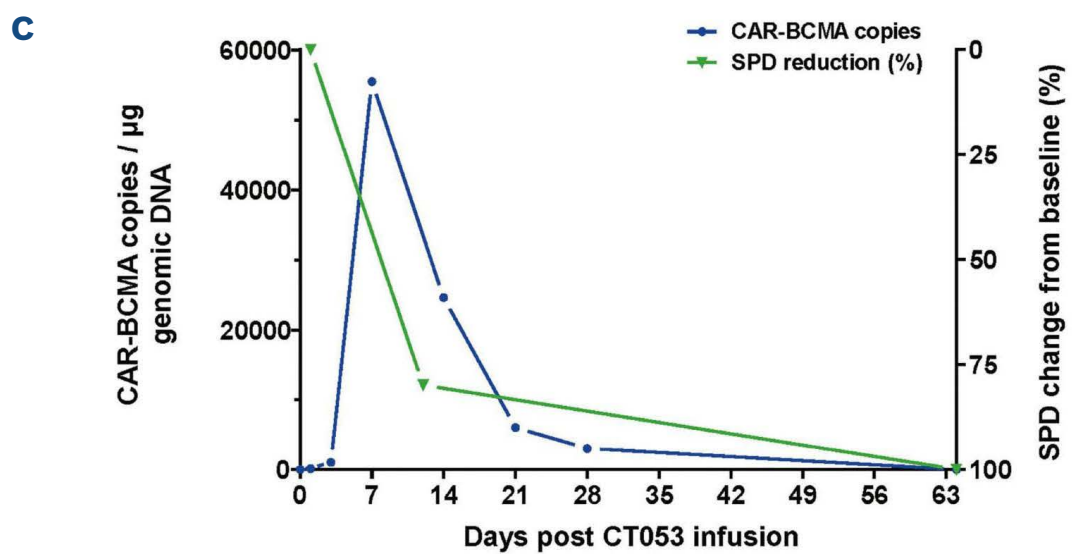


Figure 1. Patient-level responses to CT053. (A) Duration of patient response after CT053 infusion. Numbers to the right of the lanes represent duration of response (days). CR: complete response; MR: minimal response; MRD: minimum residual disease; NE: not evaluable; PD: partial response; sCR: stringent complete response; SD: stable disease; VGPR: very good partial response; # patients with extramedullary disease (EMD) at baseline. (B) Computed tomography images showing rapid regression of subcutaneous plasmacytomas (extramedullary myeloma) in patient 21 at baseline, day 12 and day 64 after CT053 infusion. The size of two lesions significantly reduced at day 12 and completely disappeared at day 64. (C) The expansion of patient 21's CT053 related to the reduction of the sum of the products of maximal perpendicular diameter (SPD). The lesion SPD changes are presented as percentage of baseline value. Blue line with dots represents the transgene vector copy numbers (left *y*-axis), and green line with triangles represents the percentage change of SPD (right *y*-axis).

finity could increase CAR-T cells' ability to distinguish tumors from normal tissues with low-level target antigen expression while retaining robust anti-tumor efficacy.[6-8] High binding affinity may have helped CT053 recognize MM cells with low-level BCMA expression and resulted in a high CR/sCR rate.

CT053's 25C2 scFv was 87% monomeric, suggesting that its high stability could have limited CD3 autophosphorylation and subsequent IL-6 secretion. Lower-grade CRS events in this study may have resulted from lower IL-6 levels, ~10 pg/mL, compared to reported grade 3-5 neurotoxicity events with IL-6 levels ≥100 pg/mL.[9] In patients, CT053 showed a better safety profile with lower CRS severity *versus* other BCMA CAR-T-cell programs reporting 6-41% of ≥grade 3 CRS.[1,2,4,10-12]

Despite enrolling 41.7% patients with EMD, ECOG scores ≥2 (33.3%), and/or high-risk cytogenetics (50%), we obtained 79.2% CR/sCR rate with sustained 21.8-month DOR compared to 33% CR/sCR rate and 10.7-month DOR reported in a trial with non-human scFv.[3] Also, this study reports significant improvement in clinical outcomes for patients with EMD compared to previous BCMA CAR-T-cell therapies.[4,13] Four of ten patients with EMD at baseline were still in sustained CR/sCR (range, 27.3–34.5 months). However, the study's non-Western population mostly lacked exposure to anti-CD38 antibody. In order to address this limitation, patients with RRMM and prior anti-CD38 treatment are actively enrolling in the North American pivotal phase II LUMMICAR STUDY 2.

Taken together, we generated CT053 with an optimized, fully-human scFv, and we demonstrated that CT053 had strong efficacy and a good safety profile when administered to RRMM patients. Our study indicated CT053's promise for treatment of RRMM patients and showed that scFv selection in CAR-T cells is critical to achieving better clinical outcomes.

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ZL, PW, and HW have submitted patent applications related to this work. The other authors declare no potential conflicts of interest. Preliminary results from this report were presented in a virtual oral session at the 62nd annual meeting of the American Society of Hematology (ASH) held December 5-8, 2020. A related high-risk group integrated analysis of this study combined with LUMMICAR STUDY 1 was presented in a poster at the ASH annual meeting, December 11-14, 2021.

Contributions

ZL and JJ designed the overall project; PW and HJ performed preclinical studies and analysis; JJ, SJ and SH were responsible for the clinical design, supervision, data analysis and interpretation; Patient care: MY, WZ, KY, LC, HM, YW, RT, XH, CX, JW, SW, LD, SH, JJ, and SJ took care of patients; HW manufactured CT053; ZL, HM, AYH, WW, JX, SH, SJ, and JJ were responsible for medical oversight, data analysis, drafting or revision of the manuscript. All authors reviewed and approved the manuscript.

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Data-sharing statement

All data generated or analyzed during this study are included in this published article and its *Online Supplementary Appendix*. Further information is available from the corresponding author on reasonable request.

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