LETTER TO THE EDITOR

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

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Supplementary Appendix

Supplementary Figure Legends

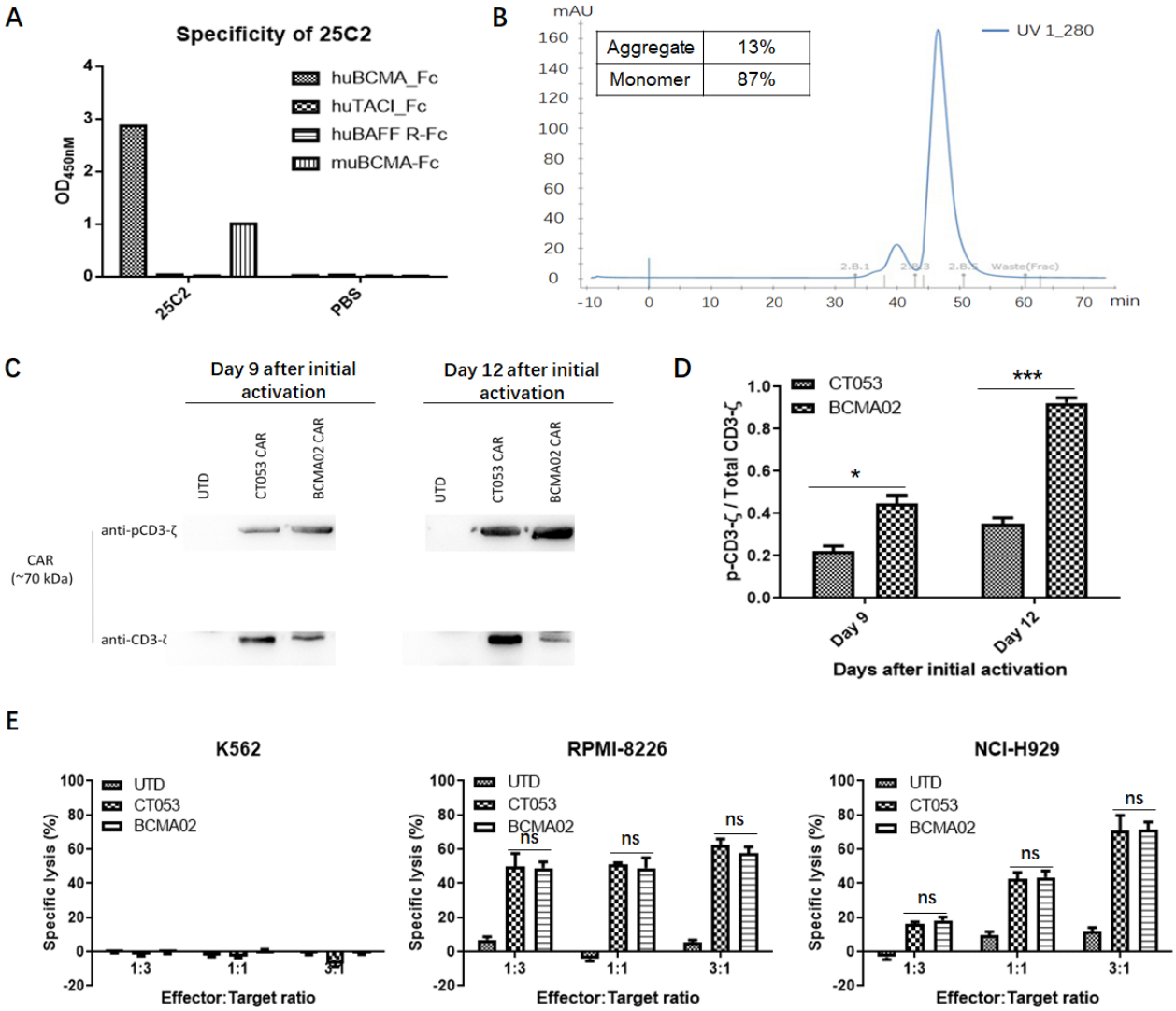
Supplementary Figure S1. Preclinical development of CT053. (A) Specificity and species cross reactivity of 25C2 single-chain variable fragment (scFv). huBCMA_Fc, human B-cell maturation antigen fragment crystallizable region; huTACI_Fc, human transmembrane activator and calcium modulator and cyclophilin ligand interactor Fc; huBAFF-R_Fc, human B-cell activating factor receptor Fc; muBCMA_Fc, murine BCMA_Fc. (B) Aggregation analysis of 25C2 scFv. (C,D) Low tonic signaling/phosphorylation of CT053 CAR in comparison to BCMA02 CAR. (C) Western blot of CAR signaling domain phosphorylation versus total CAR signaling domains using anti-phospho-CD3ζ and anti-CD3ζ, respectively. Auto-phosphorylation of CD3 was evaluated on day 9 and day 12 after initial activation in CT053 and comparator BCMA02 (a CAR comparator with murine anti-BCMA scFv [C11D5.3]). (D) Relative expression of quantified phospho-CD3ζ to total CD3. (E) Effective tumor killing in vitro: in vitro cytotoxicity assay of CAR T cells against BCMA-positive tumor cell lines, RPMI-8226, NCI-H929. Untransduced and transduced CAR T cells were cocultured with indicated cell lines at indicated effector:target ratio for 18 h. Cell lysis was determined using a standard nonradioactive cytotoxic assay.

Supplementary Figure S2. **Kaplan-Meier plot of progression-free survival and overall survival.** (A) Progression-free survival (PFS) in all treated patients. The median PFS was 18.8

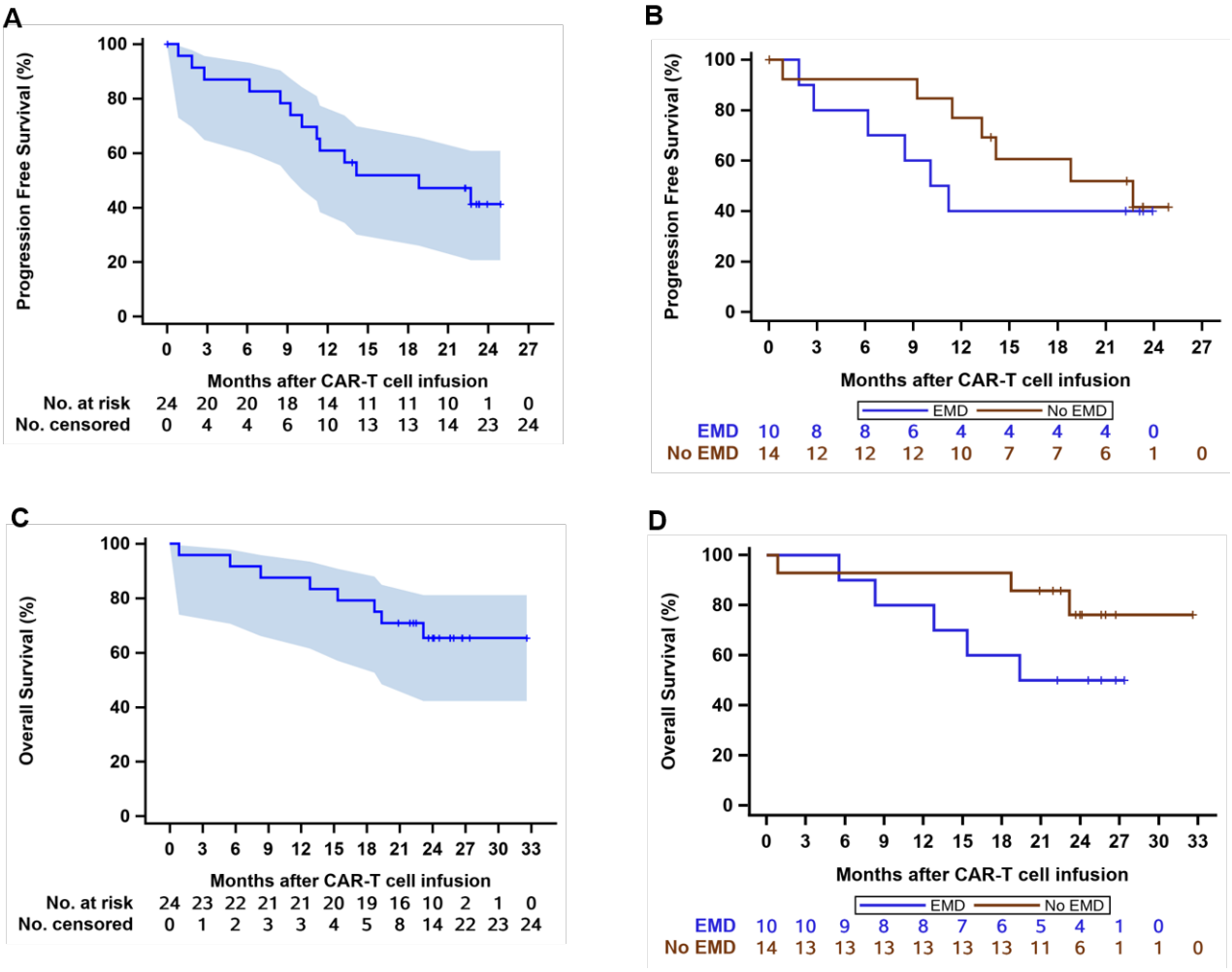
[95% CI, 10.1–not estimable (NE)]. (B) PFS in patients with or without extramedullary disease (EMD). The median PFS in patients with EMD or without EMD was [10.6 (95% CI, 1.9–NE)] vs [22.7 months (95% CI, 11.4–NE)], respectively. (C) Overall survival (OS) in all treated patients. The median OS was not reached. (D) OS in patients with or without EMD. The median OS was not reached in either group. Shaded regions indicate 95% CI.

Supplementary Figure S3. CT053 pharmacokinetics and correlation of CT053 expansion with response. (A) Cellular kinetics as measured by CAR-BCMA vector transgene copies per microgram of genomic DNA in peripheral blood for all patients over time after infusion. Dashed line denotes the lower limit of quantification (LLOQ). Data shown from the day of infusion to the last detectable value after infusion. (B) Correlation between CT053 expansion (peak vector transgene copies per microgram of genomic DNA) after infusion and tumor antigen exposure (BCMA-positive plasma cells in the bone marrow) at baseline. Empty diamonds represent individual patients. The P-value is based on Spearman's test. (C) Correlation between peak vector transgene copies per microgram of genomic DNA after infusion and concentration of IL-6 (pg/mL) in peripheral blood. Error bars represent interquartile range. (D) Difference in peak vector copies per microgram of genomic DNA in patients with at least 1-month assessment with the tumor response ≥ VGPR vs < VGPR. The left panel shows the tumor response at month 4 after infusion and the right panel shows the best response across the study. The P-value is based on two-sided Mann-Whitney test.

Online Supplementary Figure S1. Preclinical development of CT053.



Online Supplementary Figure S2. Kaplan-Meier plot of progression-free survival and overall survival.



Online Supplementary Figure S3. Detection of CAR-BCMA T cells and correlation of CAR-BCMA T-cell expansion with response.