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Corresponding author(s): Maximilian Merz

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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 specific code and software were used for data collection. The data depicted in the manuscript was generated by the authors.
Data analysis	Cell Ranger (v7.1.0), souporcell (v2.0 ,R (v4.3.2), survival (v3.5.7), survminer (v0.4.9), Seurat (v5.1.0 scDblFinder (v1.16.0), scds (v1.18.0), scRepertoire (v2.0.0), Azimuth (v5.0.0), Harmony (v1.2), ProjecTILs (v3.3.1), UCell (v2.6.2), scGATE (v1.6.0), clustifyr (v1.14), fgsea (v1.28.0), speckle (v1.20), scRepertoire (v2.0.0), STARTRAC (v0.10.0), destiny (v3.16.0), slingshot (v2.10.0), iTalk, LIANA (v0.1.12), Spectra (v0.1.0) Processing and analysis code related to this study is deposited in a GitHub repository at https://github.com/fraunhofer-izi/Rade_et_al_CAR_2025

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

Accession codes will be available before publication.

Single-cell sequencing data (RNA, BCR, TCR and ADT) supporting the study's findings have been deposited in the Gene Expression Omnibus under accession code GSE234261 for sequencing run 1. RNA/ADT Cell ranger outputs for sequencing run 2-4, sopourcell outputs and processed Seurat objects for the single-cell data were deposited at figshare or zenodo (XXXXXXXXX) with restricted access. Raw data and TCR/BCR Cell ranger outputs for sequencing run 2-4 and are available in the European Genome-Phenome Archive (accession code EGAXXXXXXXX) with restricted access.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	We have included consecutive patients treated with anti-BCMA CAR T-cells for relapsed/refractory multiple myeloma at our institution regardless of sex and gender. Both sexes are equally represented in our study and reflect the general population of multiple myeloma in Germany.
Reporting on race, ethnicity, or other socially relevant groupings	Since we included all consecutive patients treated with anti-BCMA CAR T-cells for relapsed/refractory multiple myeloma between 01/06/2022 and 01/04/2024 at our institution regardless of race, ethnicity or social status, we can rule out selection bias towards any of the mentioned factors. No patients were excluded from the analyses.
Population characteristics	The median age at CAR-T infusion was 64 (range 31-75) years and 44% of patients were female. All patients were of Non-Hispanic European origin.
Recruitment	All patients treated with anti-BCMA directed CAR T-cells for multiple myeloma at the university of Leipzig provided written informed consent to provide clinical data and samples for this study. Patients not treated with anti-BMCA CAR T cells or without the diagnosis of multiple myeloma were excluded.
Ethics oversight	The study was approved by the local ethics review committee and performed in accordance with the declaration of Helsinki.

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	Mononuclear cells from 61 patients from peripheral blood (PBMCs) were obtained from LP, on days 21–60 (average 31) and on days 79–169 (average 101) after CAR-T infusion for single cell multi-omics sequencing. PBMC for flow cytometry and blood sera were obtained on the day of LP, and on days 0, 7, 14, 30 and 100. Samples were cryopreserved and stored by trained staff of the Leipzig Medical Biobank according to standard operating procedures.
Data exclusions	No data were excluded.
Replication	By sequencing hundreds-of-thousands of single cells from different patients using a commercialized kit, we ensured reproducibility of our single cell and flow cytometry experiments. Details on sample handling, sequencing and downstream analyses can be found in the patients and methods section to allow reproduction of results. All attempts to replicate results were successful.
Randomization	No randomization was performed.
Blinding	Since patients were not randomized to certain groups or interventions, no blinding was performed. Authors involved in the wet lab experiments and primary bioinformatic analyses were blinded to outcome.

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

For flow cytometry, the following antibodies were used:
anti-biotin antibody APC (Miltenyi Biotec)
CD4 BV786 CD45 V500
CD8 APC-H7 (all from Becton Dickinson)
biotin labeled BCMA CAR Detection Reagent (Miltenyi Biotec)

Validation

All used antibodies are commercially available and validated for the respective experiments. : Details on validation of all primary antibodies for the species and application can be found on the manufacturers' website.

Plants

Seed stocks

na

Novel plant genotypes

na

Authentication

na

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

Blood samples of MM patients taken before CAR T cell treatment (day 0) as well as 7, 14, 30 and 100 days after treatment were analyzed by flow cytometry. For analysis of the CAR immune status, biotin labeled BCMA CAR Detection Reagent (2|JI; Miltenyi Biotec) was added directly to fresh 100 II EDTA-anti-coagulated blood and incubated for 5 min at RT. After addition of 2 ml PBS and one washing step (500g, 5 min, RT), cell pellets were resuspended in 100 II PBS and following antibodies were added: 2 II 5 µl anti-biotin antibody APC (Miltenyi Biotec), 1 II 5 µl CD4 BV786; 2.5 II (each) 5µl CD4S V500, 5 µl CD8 APC-H7, (all from Becton Dickinson). After incubation for 15 min at RT, red blood cells were lysed by addition of 2 ml of lyse solution (Becton Dickinson) for 10 min. The cells were centrifuged (500g, 5 min, RT), cell pellets were resuspended and washed with 2 ml PBS (500g, 5 min, RT) before flow cytometer.

Instrument	Samples were analyzed using a FACSLyric flow cytometer (Becton Dickinson)
Software	Data analysis was performed using the FACSSuite software (Becton Dickinson).
Cell population abundance	Cell population and abundance are described in the results section
Gating strategy	Mononuclear cells were seperated into CD4+/CD8+ CAR-positive and negative cells starting from the fraction of CD3+ cells. We have added the gating strategy in the supplemental material

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