nature portfolio

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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	Flow cytometry was collected using BD FACSDiva software v8.0.1.
Data analysis	Raw gene expression and TCR-sequencing data was processed using cellranger v6.0.1. Souporcell v2020.7 was used to demultiplex pooled samples. Ambient contamination was filtered using cellbender v0.2.0. Standard single-cell analyses were performed using scanpy v1.8.1. Doublets were filtered using scrublet v0.2.1. In vivo images were analyzed using Aura software v4.0.0. Validation analyses for in vitro and in vivo experiments utilized GraphPad Prism 9.0 software. Flow cytometry data with analyzed using Flowjo v10.8.1. The swimmers plot was generating using RStudio v2021.09.1 and the package swimplot v1.2.0. Our tool for CD45 isoform quantification is available at https://github.com/getzlab/10x-cd45-isoform-quantification. Other custom code used in this work is available at https://github.com/getzlab/Haradhvala_et_al_2022.

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

Gene expression matrices from the scRNA data have been deposited to the Gene Expression Omnibus (GEO accession GSE197268). Raw sequencing data have been deposited to the database of Genotypes and Phenotypes (phs002922.v1.p1). Data from a prior study of axi-cel infusion products is available at GSE151511.

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	No statistical methods were used to pre-determine sample size. The study was designed to make a minimum of 3 patients of each response category for each treatment product.
Data exclusions	No samples were excluded from this study.
Replication	Findings in scRNA data were, when possible, explored in other publicly available datasets (Deng et al. 2020) or supported with in vitro and in vivo experiments. Experiments were performed a single time with multiple technical replicates as noted in the figure legends.
Randomization	No randomization was performed for patient studies as this was an observational study of response. Mice were randomized prior to CAR-T treatment to ensure equivalent tumor burden among groups.
Blinding	Blinding was not possible for the analyses of patient data as this study involved data exploration without predetermined knowledge of the cellular features that would be relevant to test. For animal experiments one veterinary technician was in charge of injecting all mice for bioluminescent readings and was blinded to the expected outcomes.

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
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Antibodies

Antibodies used	The following antigens were stained using the indicated antibody clones for flow cytometry analysis CD45 (BV786 BD HI30, 563716). Used at dilution of 1 in 167. CD3 (APC BD Biosciences, SK3, 340661). Used at dilution of 1 in 83.5. CD4 (V450 BD Bioscience SK3, 651850). Used at dilution of 1 in 83.5. CD8 (V500c BD Bioscience SK1, 647458). Used at dilution of 1 in 83.5. CD14 (FITC BD Bioscience MϕP9, 347493). Used at dilution of 1 in 83.5. Human CD19 Fc Chimera (carrier free) (PE, BioLegend custom conjugation , 900002598, lot B324342) Used at dilution of 1 in 55.6. CD4 (BV510 BD Biosciences SK3 562970) used at a dilution 1 in 20, CD8 (APC-H7 BD Biosciences SK1 560273) used at dilution 1:40, CD127 (BV711 BD Biosciences HIL-7R-M21 561028) used at dilution of 1 in 20,

FOXP3 (APC Invitrogen PCH101 17-4776-42) used at dilution 1 in 20.

Validation

All antibodies were independently titrated and validated using healthy PBMC followed by Axi-cel and Tisa-cel infusion product prior to use in experiments in this manuscript.

All BD antibodies were provided with a certificate of analysis and were used within their expiry dates.
"This product complies with all BDB release criteria. BD Biosciences San Jose is a registered facility with the US
Food and Drug Administration subject to the requirements of 21CFR Part 820 for the manufacture of in vitro diagnostic products. BD Biosciences San Jose also holds the following international standard certifications for
Medical Device
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Quality Management Systems:
ISO 9001:2015, Certificate
FM 32438
ISO 13485:2016, Certificate MDSAP 690529
EN ISO 13485:2016, Certificate MD 605599

Invitrogen antigen statement from manufacturer "This Antibody was verified by Relative expression to ensure that the antibody binds to the antigen stated."

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	JeKo-1 and HEK-293T were acquired from American Type Culture Collection (ATCC).
Authentication	Cell lines were authenticated using STR profiling
Mycoplasma contamination	All cell lines tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	NSG (NOD.Cg-PrkdcscidIl2rgtm1Wjl/SzJ) mice were purchased from Jackson Laboratory and bred in pathogen-free conditions at the MGH Center for Cancer Research. 6 week old female NSG mice were engrafted for experiments.
Wild animals	This study did not involve wild animals
Field-collected samples	This study did not involve samples collected from the field
Ethics oversight	Mice were maintained at the MGH Center for Cancer Research and all care and conducted experiments were carried out using protocols approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee.

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Age, gender, and information about prior treatment for all patients contributing samples for scRNA-seq are detailed in Supplementary Table 1. Healthy donor T-cells were obtained from purchased leukopaks at the MGH blood bank which were obtained from volunteers and are considered non-human subjects research. Demographic information such as age, sex, etc are not available for these volunteers.
Recruitment	Patients with aggressive B-cell lymphoma who were treated with axicabtagene ciloleucel or tisagenlecleucel were selected for inclusion based on having the appropriate diagnosis, sample and cell number availability for the required time points, and attempting to balance the number of responders and non-responders included. Patients were not strictly randomly or prospectively chosen and the number of non-responders in our cohort is artificially inflated for the purposes of our study compared to what is expected based on the performance of the two CAR-T cell products. No compensation was provided for participation. Healthy donors were volunteers from MGH blood bank; considered non-human subjects research.
Ethics oversight	Samples were obtained after written informed consent under IRB-approved protocols at the Dana-Farber/Harvard Center Center (DFHCC #16-206 and 17-561).

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

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	Tumor cells were cultured as recommended by ATCC. CAR T cells were generated from healthy donors with lentiviral transduction.
Instrument	BD Fortessa-X20
Software	Flow cytometry was collected using BD FACSDiva software v8.0.1 and analyzed using Flowjo v10.8.1
Cell population abundance	Sorted samples were evaluated via scRNAseq as discussed in the study design resulting in additional confirmation of population identities (e.g CAR+ and CD4/CD8 populations)
Gating strategy	Sorted cells were assessed with viability using 7-AAD and gated on single cells. Gate boundaries were established from simultaneous staining of healthy donor PBMC fractions with each sorting batch.

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