nature research

Corresponding author(s): Todd M. Allen, James L. Riley

Last updated by author(s): Jul 10, 2020

Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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	BD FACSDiva Software version 8.0.1 (Flow cytometry) QuantaSoft version 1.7.4.0917 (ddPCR) LightCycler 480 Software release 1.5.1.62 SP3 (real-time PCR) Viia 7 QuantStudio Real-time PCR Software v1.3 (real-time PCR) Magellan version 7.2 (Tecan plate reader)
Data analysis	Prism 8 for macOS version 8.2.1 Prism 7 for PC version 7.05 JMP Pro version 14.2.0 Flowjo software version 10

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All of the construct sequences described in this article are published (see Leibman et al. PMID 29023549). All materials described in this manuscript are available via a material transfer agreement with the University of Pennsylvania or the Ragon Institute. All data are available from the corresponding authors upon request.

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	In general, study arms were designed with sample sizes of 8 mice per group in order to provide an adequate power (80% or more) to detect an effect size of 1.60. These power calculations were based on simulation studies using 10,000 Monte Carlo samples and two-sided Wilcoxon rank sum test with type 1 error rate of 5%. However, due to the nature of the model system used (BLT humanized mice), batch sizes are variable as they are determined by the quality and size of the human donor tissue. As such, sample sizes were chosen to balance statistical power with the need to test treatment arms within the same donor batch of BLT mice. For example, when sample sizes were small due to batch size restrictions, non-parametric statistical tests were used. In general, sample sizes of no less than 6 mice were used in a given study. Animal studies in which experimental arms fell below the target of 8 mice were repeated in independent experiments to confirm findings.
Data exclusions	No data points were excluded from the analyses detailed in this manuscript.
Replication	In essence, all major findings were replicated in multiple batches of BLT humanized mice, and all attempts at replication were successful. For example: 1) Non-inferiority of BLT-derived CAR T cells. In vitro experiments comparing CAR T cell functions in BLT T cell-derived and human PB-derived CAR T cells were performed using 3 distinct BLT mouse samples representing 3 genetically distinct donor sources as well as 3 distinct adult human PMBC donors (Fig. 1 and Fig. S1). 2) Costimulatory domain effects. Data shown in Fig. 2 regarding effects of 4-1BB vs CD28 costimulation integrated into the CAR, were observed again in Fig. 4 and Fig. 5, representing 4 distinct batches of BLT mice generated from 4 genetically distinct donor tissues. 3) Dual CARs demonstrate superior effector functions in vivo. Controlled studies in which purified populations of 41BBz/CD28z Dual CARs were shown to demonstrate enhanced in vivo expansion and protection from CD4+ T cell loss (Fig. 5c,d, Fig. S16) were repeated in a completely distinct BLT cohort (Fig. 5f,g, Fig. S18), and further controlling for CAR expression levels by purifying doubly transduced 41BBz/41BBz CARs and CD28z/CD28z CARs alongside Duals CARs and by the same manufacturing and selection process (Fig. S17). 4) HIV-susceptible CAR T cells increase acute viremia and/or HIV rebound. Data demonstrating this finding and depicted in Fig. 6a are derived from 6 independent studies. 5) Acceleration of viral control when CAR T cells given at the time of ART initiation. This result was repeated in two distinct batches of BLT mice, generated at two different facilities and with two different HIV isolates, depicted in Fig. 6e,f and Fig. S21d,e.
Randomization	Due to variability in reconstitution levels and phenotypes among humanized mice, groups were normalized rather than randomized in order to limit bias in experimental groups. In general, groups were normalized using data from pre-study blood draws and flow cytometry-based phenotyping on the following criteria: - # of CD4+ T cells/uL of blood - # of CCR5+ CD4+ T cells/uL of blood - % of memory CD4+ T cells - Weight of the mice at study start (health status) - Plasma viral load (where applicable)
Blinding	Technicians responsible for blood and tissue collection were blinded to the study groups. Blood processing and downstream flow cytometery and qRT-PCR for viral loads were performed without strict identification of study groups, only mouse identifiers. Mouse IDs and data were formally assigned to study groups only at the time of data analysis.

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used

Format: antigen specificity (clone; cat#)
BioLegend: CD45 (HI30 and 2D1; 304023 and 368513), CD19 (HIB19; 302251), CD3 (OKT3; 317335), CD4 (OKT4 and RPA-T4; 317415 and 300553), CD8 (RPA-T8 and SK1; 301065 and 344745), CD45RA (HI100; 304125 and 304110), CD27 (LG.3A10; 124229), CCR7 (G043H7; 353211), CCR5 (J418F1; 359107), CD271 (ME20.4; 345107 and 345109), PD-1 (EH12.2H7; 329919), TIGIT (VSTM3; 372715), 2B4 (C1.7; 329521), CD107a (H4A3; 328637), IL-2 (MQH-17H12; 500331), Perforin (B-D48; 353303 and 353307), anti-mouse CD45 (30-F11; 103115), CD20 (2H7; 302313), CD14 (HCD14; 325619), CD56 (HCD56; 318331)
BD Biosciences: CD45 (HI30; 564106), CD3 (UCHT1; 612941), CD8 (SK1; 612889), CD45RA (HI100; 612846), CCR7 (3D12; 560922), TNF (Mab11; 563996), IFN-γ (4S.B3; 612845), Granzyme B (GB11; 563389), MIP-1β (D21-1351; 560687), GM-CSF (BVD2-21C11; 562857), Active Caspase-3 (C92605; 564096)
R&D: Human EGFR (Cetuximab Biosimilar, Hu1; FAB9577G-100)
Beckman Coulter: HIV-1 Core Antigen (KC57; 6604667)
eBioscience: T-bet (4B10; 25-5825-80), EOMES (WD1928; 46-4877-42), TOX (TXRX10; 12-6502-82)

Validation

Antibody validation was performed by the manufacturer for all antibodies listed.
Before use, all antibodies were validated and titrated on human peripheral blood mononuclear cells and the optimal dilution was found to be 1:100.
Antibodies used to detect production of effector cytokines (IL-2, TNF, GM-CSF, INF-gamma, and MIP-1B) were validated and titrated on PMA/Ionomycin-stimulated human PBMCs and optimal staining dilutions were found to be between 1:50 to 1:400 for all antibodies tested.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

HEK 293T/17 cells - ATCC
K562 (wild type) – ATCC
K562.Env - C. Ellebrecht and A. Payne, University of Pennsylvania

Authentication

ATCC uses morphology, karyotyping, and PCR based approaches to confirm the identity of human cell lines and to rule out both intra- and interspecies contamination. These include an assay to detect species specific variants of the cytochrome C oxidase I gene (COI analysis) to rule out inter-species contamination and short tandem repeat (STR) profiling to distinguish between individual human cell lines and rule out intra-species contamination.

Mycoplasma contamination

All cell lines were tested for mycoplasma contamination using the Lonza MycoAlert assay and were found to be negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this study.

Animals and other organisms

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

Laboratory animals

Mus musculus; NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG); female or male; 6-8 weeks of age at time of humanization; 18-24 weeks of age at time of study start

Wild animals

No wild animals were used in this study.

Field-collected samples

No field collected samples were used in this study.

Ethics oversight

Humanized mouse experiments performed at the Ragon Institute of MGH, MIT and Harvard were approved by the Massachusetts General Hospital Institutional Animal Care and Use Committee (IACUC) under the approved protocol 2016N000483. Humanized mouse experiments performed at the University of Pennsylvania were approved by the University of Pennsylvania IACUC under the approved protocol 805606. All animal studies were carried out in accordance with recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

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

Human fetal tissue was obtained from a third party commercial supplier. The population comprised pregnant females located in California. No other demographic information is available.

Recruitment

Participants had previously decided to have an abortion and had previously completed an informed consent for the abortion prior to being recruited by a third party commercial supplier to donate aborted material for research purposes. No other information about recruitment is available.

Ethics oversight	The use of anonymized fetal tissue purchased from the third party commercial supplier was deemed Not Human Subjects Research by the Partners Healthcare Institutional Review Board.

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	Cells were derived from either whole blood or single cell suspensions from tissues collected from BLT-humanized mice. For analysis of peripheral blood, plasma was removed by low speed centrifugation, cells were resuspended in PBS, and whole blood was stained with antibodies prior to fixation of cells and lysis of RBCs. Single cell suspensions were isolated from tissues by layering dissociated tissue cell suspensions over a density gradient and removing the mononuclear cell layer after centrifugation.
Instrument	BD FACSymphony High Parameter Flow Cytometer
Software	BD FACSDiva Software version 8.0.1 (data collection) FlowJo version 10.5.3 (data analysis)
Cell population abundance	For NGFR selections to purify C34-CXCR4+ cells (Figure 6), post-selection flow was used to determine purities that were >98%.
Gating strategy	General gating strategies were as follows: lymphocyte gate, human CD45+ and mouse CD45-, CD3+ and other lineage negative, CAR T cell marker positive (i.e. GFP+, iRFP670+, NGFR+), CD4+ for CD8-CAR and CD4-bright for CD4 CAR Supplementary Figures added to depict specific gating strategies: - Gating of target cells in in vitro elimination assays, Supplementary Fig. 1f - Gating of endogenous memory CD4+ T cells, Supplementary Fig. 3a - Sorting gates for C34-CXCR4+/- CAR T cells, Supplementary Fig. 22a - Sorting gates for endogenous central memory CD4+ T cells, Supplementary Fig. 22b

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