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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	FACs data were acquired with the use of FACS DIVA V8.0.1 (BD Biosciences), bioluminescence data were acquired and analysed with the use of Living Image v4.3.1 (Xenogen) software, clinical data were collected with the use of MACRO v4.9.1. xPONENT® software (v4.0, Millipore) was used for plexed cytokine acquisition
Data analysis	STATA 15.1 and Graphpad prism version 7 were used for statistical analyses, BIAevaluation software Version 2.0 (GE Healthcare) was used for affinity analyses as stated in the methods, Milliplex Analyst v5.1 and FCAP Array Software v3.0 were used to analyse cytokine analysis

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

The whole exome sequencing data files from the CARPALL study are available in controlled-access format from the European Genome-Phenome Archive (http://www.ebi.ac.uk/ega; accession number EGAS00001003733). Sequencing data requests will be reviewed by the Independent Data Monitoring Committee and Trial Management Group of the CARPALL study and may be subject to patient confidentiality. After approval, a data access agreement with UCL will be required. All requests for raw and analyzed data and materials will be reviewed by UCL Business (UCLB) to verify if the request is subject to any intellectual property or confidentiality obligations.

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	The response rate with CD19CAR T cells is expected to be at least 50% (data from the 3 major US studies found 60-80%), when it is normally 20% or less with conventional chemotherapy in this high risk cohort. A trial of 15 patients has 80% power to detect a difference of 20 vs 50%, with one-sided 7% statistical significance (A'Hern phase II sample size; 'Sample size tables for clinical studies' software, by Machin et al). If at least 6 patients have a response, this would be evidence of efficacy for CD19CAR T-cells.
Data exclusions	There were no data exclusions
Replication	All data were representative of 2 experiments or the data were pooled from at least 2 experiments. All attempts at replication were successful
Randomization	Randomisation was not incorporated into the design of this study as this was a Phase 1/2 first in Man study focusing on toxicity and biological endpoints. Further, since the majority of patients had relapsed post transplant, there are no effective curative approaches available with which to randomize against.
Blinding	Investigators were blinded in the assessment of tumour burden for in vivo tumour model experiments and the determination of tumour burden by bioluminescence. There was no blinding carried out in the clinical study as it was a single cohort study.

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	The following antibodies were used for phenotypic analysis of CAR T cells in accordance with manufacturer's instructions or as indicated: Anti-CAT CAR Idiotype antibody (bespoke product, Evitria 1/40) CD2 APC (Mnaufacturer Miltenyi, cat no. 130-098-579, clone LT2, 1/100), CD3 PerCPCy5.5 (Biolegend 300430 UCHT1, 1/200), CD4 PE-Vio770 (Miltenyi, 130-100-454, M-T466, 1/50) , CD8 PE or FITC (Biolegend 300908 and 300906 respectively, HIT8A, 1/100), CD19 BV605 (Biolegend, 115539, HIB19 1/100), CD19 PE (Biolegend, 392506, 4G7, 1/100), CD45RA BV605 (Biolegend, 304134, HI100, 1/100), CCR7 APC (Biolegend, 353214, G043H7, 1/100), CD107a FITC (BD), CD223 APC-eFluor 780 (LAG-3, eBioscience), CD279 BV421 (PD1 Biolegend 1/100), CD366 or TIM3 BV711(Biolegend 345024 F38-2E2, 1/100), CD95 BV711 (BD 563132, DX2, 1/50), CD127 BV421 (Biolegend 351310, A019D5, 1/10), CD107A FITC (BD, 555800, H4A3) IFN-gamma APC (502512, 4S.B3), TNF-alpha BV421 (502932, MAb11), IL-2 BV605 (500332, MQ1-17H12, Biolegend), Anti-Rabbit Goat F(ab')2 FITC (Jackson Immunoresearch, 1/100), Anti-Rabbit IgG BV421 (Biolegend, 1/100), Anti rat IgG PE (Biolegend 405406 Poly4054, 1/200). For clinical assays e.g. CAR T cell persistence in patient samples, manufacturer's guidelines were followed. anti-His monoclonal antibody (GE Life sciences, 27-4710-01) was used in accordance with manufacturer's guidelines for competitive scFv binding assays
Validation	Primary antibodies were validated and titrated with appropriate positive and negative controls in order to determine the optimal stain concentration for each test. Serum cytokine measurements are validated to UK ISO standards, CAR T cell detection with the CAT CAR anti-idiotype antibody were validated against healthy donor controls and pure populations of CAR T cells in accordance with standard operating protocols in UK NEQAS and ISO accredited laboratories

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Raji, K562 and 293T cell lines were obtained from ATCC. SupT1 cells were purchased from ECACC and transduced with an SFG vector to express human CD19 (SupT1-CD19) and single cell selected by flow cytometry to generate a cell line, NALM6 expressing GFP and firefly luciferase were provided by Dr. Hilde Almasbak
Authentication	Cell line authentication was not carried out
Mycoplasma contamination	All cell lines tested negative for mycoplasma
Commonly misidentified lines (See ICLAC register)	RAJI cells directly obtained from ATCC were utilized as target cells for a discrete number of in vitro experiments, alongside other CD19-expressing target cell lines e.g. NALM6 and engineered SupT1 cells which are not on the misidentified register and gave results as expected.

Animals and other organisms

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

Laboratory animals	NOD-SCID-γ–(NSG,female, aged 6-10 weeks) were obtained from Charles River Laboratory (Wilmington, MA)
Wild animals	These were not utilized in this study
Field-collected samples	These were not utilized in this study
Ethics oversight	All animal studies were approved by the University College London Biological Services Ethical Review Committee and licensed under the Animals (Scientific Procedures) Act 1986 (ASPA)

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	1. Children and young adults (age 24 years or younger) with high risk/relapsed CD19+ haematological malignancy 2. Within the cohort of 14 treated patients, 13 were male (ALL being more common in boys than girls) 3. Agreement to have a pregnancy test, use adequate contraception (if applicable) 4. Written informed consent
Recruitment	Participants were recruited via a national referral pathway involving a national multi-disciplinary team contributed to by representatives from all UK treating centers. As a result, there was multi-party oversight of the referrals process, reducing bias from e.g. self referrals or referral from any single center
Ethics oversight	Research Ethics Committee (REC)

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

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT02443831
Study protocol	http://www.ctc.ucl.ac.uk/TrialDetails.aspx?Trial=116&term=carpall
Data collection	3 hospitals, registration from 03may2016 to 12jun2018; The end of the trial will be 10 years after the last patient has received the CD19CAR T-cells (or the last patient last visit if this occurs earlier).
Outcomes	1) Toxicity evaluation following CD19CAR T-cell infusion; 2) Proportion of patients achieving molecular remission at 1 month post-CD19CAR T-cell infusion; 3) Proportion of patients in molecular remission without further therapy at 2 years; 4) Persistence and frequency of circulating CD19CAR transduced T-cells in the peripheral blood; 5)Incidence and duration of hypogammaglobulinaemia; 6) Relapse rate, Disease-Free Survival and Overall Survival at 1 and 2 years after immunotherapy with CD19CAR transduced T-cells

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	Samples were either derived from healthy donors or study patients. Red cells were lysed using Ammonium-Chloride-Potassium (ACK) Lysing Buffer, water lysis method or removed by ficoll density gradient centrifugation. Up to 1x10^6 cells were stained in 100ul of staining buffer containing PBS or PBS/FCS 2%. Cells were washed 1-2 times prior to analysis. For samples stained in BD Trucount tubes, to obtain absolute cell numbers, cells were stained according to manufacturer's guidelines.
Instrument	Samples were run on BD Fortessa, BD LSRII or BD FACSCanto II instruments
Software	BD FACSDiva Software Version 8.0.1, Flowjo, Treestar Version X
Cell population abundance	N/A
Gating strategy	For experiments defining proportions or characteristics of CAR T cells, samples were pre-gated on viable CAR+ CD45+ CD3+ cells falling within a lymphocyte morphological gate after doublet exclusion. Where necessary, fluorescence minus one (FMO) or negative / healthy donor controls were used to set threshold gates for expression

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