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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.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	LivingImage software v. 4.3.1 (Caliper LifeSciencies), FCS Dive (BD), Illumina HiSeq 2500 platform, Accuri C6 Sampler Software (BD)
Data analysis	GraphPad Prism 6.05 (GraphPad Software, Inc.), FlowJo v.10 (FlowJo LLC), BD Accuri 6 software (BD), Fiji software (ImageJ), LivingImage software v. 4.3.1 (Caliper LifeSciencies), Pindel, Genome Analysis ToolKit, Agilent TapeStation (RIN), Burrows-Wheeler Aligner (BWA-MEM), ImmunoSeq Analyzer (Adaptive Biotechnologies), Excel (Microsoft), INSPIIRED, BLAT

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 upon request. 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 requests for raw and analyzed data and materials are promptly reviewed by the University of Pennsylvania Center for Innovation to see if the request is subject

to any
intellectual property or confidentiality obligations. Patient-related data not included in the paper were generated as part of clinical trials and may be subject to patient
confidentiality. Any data and materials that can be shared will be released via a Material Transfer Agreement. All raw and analyzed sequencing data can be found at the
NCBI Sequence Read Archive (accession number: SRP155722; analyses of lentiviral integration sites and mutations associated with CD19 negative relapse), and Adaptive
Biotechnologies' immuneACCESS database (doi:10.21417/B7V06M; http://clients.adaptivebiotech.com/pub/Ruella-2018-naturemedicine).

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size was calculated by estimating the confidence interval and margin of error.
Data exclusions	No data were excluded. A priori criteria for exclusion were developed.
Replication	All experiments include biological replicates. When available and possible we used CART cells derived from multiple normal donors and different cell lines. All attempts at replication were successful.
Randomization	Randomization was performed using softwares (e.g. randomizer.org).
Blinding	Blinding was not relevant to this study as the measurements could not affected by the operator

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Unique biological materials were obtained from patients in clinical trials and may be available via material transfer agreement if allowed by protocol/informed consent. Data access requests will be evaluated by the University of Pennsylvania Center for Innovation who manages the intellectual property of this project.

Antibodies

Antibodies used	FIn the terminology used in this manuscript, CART19 refers to all CAR T-cell products directed against CD19, CTL019 refers to the Penn/Novartis-developed murine CART19 product, and CAR19 refers to the chimeric antigen receptor itself. CD19 protein expression was studied by flow cytometry and confocal microscopy using the following CD19-specific monoclonal antibodies (mAbs): OTI3B10, HIB19, SJ25-C1, MHCD1928, BU12, CB19, J3-119 and EPR5906. An Alexa Fluor-647-conjugated anti-idiotype mAb (kindly provided by Drs. B. Jena and L. Cooper)19 and unconjugated anti-idiotype mAb (kindly provided by Novartis) followed by Alexa Fluor-647-conjugated goat-anti-human IgG Fcγ (Jackson ImmunoResearch) were used in flow cytometry assays. Detection of CAR22 was performed using CD22-Fc/His (Sino Biologicals) and anti-His-APC (R&D) or PE (AbCam) or directly

PE-conjugated CD22 protein. HA22 CARs were detected by staining cells with biotinylated α-murine F(ab)2 (Jackson ImmunoResearch) followed by staining with Streptavidin-PE (BD Pharmingen). The following mAbs were used for antigen detection: α-CD3-APC-H7 (SK7, BD Pharmingen), α-CD4-BV785 (OKT4, BioLegend), α-CD8a-PE-Cy5.5 (RPA-T8, eBioscience), α-CD10-PE-Cy7 (eBioCB-CALLA, eBioscience), α-CD14-V500 (M5E2, BD Horizon), α-CD19-BV510 (HIB19, BioLegend), α-CD19-Pac Blue (SJ25-C1, Thermo Fisher Scientific), α-CD19-FITC (BU12, LifeSpan Biosciences), α-CD19-PE (CB19, Abcam), α-CD19-PerCP-eF710 (J3-129, eBioscience), α-CD19-APC (J3-119, Beckman Coulter), α-CD19-BV785 (HIB19, BioLegend), α-CD22-PE-Cy7 (HIB22, BioLegend) α-CD22-PE (HIB22, BioLegend), α-CD22-PE (SHL-1, BioLegend), α-CD22-PE (RFD-4), α-CD45-BV421 (HI30 BioLegend), α-CD56-PE (CMSSB, eBioscience), and α-NRP1-BV421 (12C2, BioLegend). Alexa 488-conjugated goat-α–rabbit IgG (Abcam) was used as secondary antibody. In all analyses, the population of interest was gated based on forward vs. side scatter characteristics followed by singlet gating, and live cells were gated using Live Dead Fixable Aqua (Invitrogen) and time gating was included for quality control.

Validation

Antibodies were titrated using positive and negative cells. All antibodies were validated using human primary peripheral blood mononuclear cells or the human leukemia cell line NALM6. All antibodies used in this study were titrated prior to use, and fluorescence minus one (FMO) controls were created for each antibody panel to set gates for positive events.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

The NALM6 cell line was originally obtained from DSMZ (Braunschweig, Germany).
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Authentication

Authentication was performed by STR profiling

Mycoplasma contamination

The cell line was tested for the presence of mycoplasma contamination (MycoAlert™ Mycoplasma Detection Kit, LT07-318, Lonza, Basel, Switzerland). All the cell line were negative for mycoplsma.

Commonly misidentified lines
(See ICLAC register)

Nalm6, however authentication was performed by STR profiling

Animals and other organisms

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

Laboratory animals

NOD-SCID-Gamma chain deficient mice, male/female randomized, 6-8 week old

Wild animals

No wild animals were used in this study

Field-collected samples

No field-collected samples were used in this study

Human research participants

Policy information about studies involving human research participants

Population characteristics

This paper does not describe the results of a clinical trial, rather a case report of a patient in trial NCT01626495. Here we report a 20-year-old male B-ALL patient (Patient 107) in his third relapse after chemotherapy and a cord blood transplant who enrolled in our phase 1 trial (NCT01626495) to evaluate the safety, feasibility, and engraftment of CTL019 in pediatric and young adult B-ALL.

Recruitment

NCT01626495, please refer to Maude SL, NEJM, 2014.

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

Flow cytometry was performed as previously described: Kenderian, S.S., et al. CD33 Specific Chimeric Antigen Receptor T Cells Exhibit Potent Preclinical Activity against Human Acute Myeloid Leukemia. Leukemia (2015).

Instrument

Flow cytometry was performed on an LSRII or a four-laser Fortessa-LSR II cytometer (Becton-Dickinson) or a 4-color Becton Dickinson (BD) Accuri flow cytometer or a 4-color FACSCalibur (BD).

Software	FCS files were analyzed with FlowJo X 10.0.7r2 (Tree Star) or the Accuri C6 software (BD).
Cell population abundance	Cell lines were sorted on a BD Influx Cell Sorter (BD Biosciences). Purity was assessed by flow cytometry on the sorting product using validated antibodies and with relevant controls. Cell frequencies/abundances are listed on the flow plot (insets).
Gating strategy	Relevant populations were gated as follows: time gate--> SSC/FSC --> single cells --> live cells --> gating of interests. Negative (FMO or biological) and positive controls were included. Please refer to Suppl. Fig 11 for a gating strategy example.

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