nature portfolio

Corresponding author(s): Leo D. Wang

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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	Siemens MAGNETOM Verio 3.0 Tesla scanner; GE Discovery DST HP60 PET-CT scanner; NanoZoomer 2.0-HT digital slide scanner; NanoZoomer S360 Digital Slide Scanner; Olympus BX46 transmitted light microscope with an SC-180 Olympus camera; MACSQuant Analyzer 10; FLEXMAP 3D® (Luminex);
Data analysis	Vital Images Vitrea v6.7.2; ITK-SNAP v3.8.0; FlowJo v10.1; GraphPad Prism v9; 10x Genomics Cell Ranger v5.0 and Ensemble 98; Seurat v4; R v4.0.2. Scripts to conduct the RNAseq analyses will be made publicly available on GitHub at https://github.com/Banovich-Lab/19130_Pediatric_CART

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

All requests for raw and analyzed data and materials will be promptly reviewed by the intellectual property office of City of Hope to verify whether the request is subject to any intellectual property or confidentiality obligations. Raw preclinical and clinical data are stored at City of Hope with indefinite appropriate backup. Patient-related data not included in the paper were generated as part of the clinical trial and might be subject to patient confidentiality restrictions. Any data and materials that can be shared will be released via a material transfer agreement. Sequencing data will be made publicly available on appropriate no-cost repositories (e.g., GEO).
Scripts to conduct the RNAseq analyses will be made publicly available on GitHub at https://github.com/Banovich-Lab/19130_Pediatric_CART

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	The sex of each patient is reported in the main text results section and in Table S4.
Reporting on race, ethnicity, or other socially relevant groupings	Patient characteristics are included in the main text results section.
Population characteristics	See above.
Recruitment	Research participants were identified at City of Hope through the clinical practices of the PI, co-Is and participating clinicians and through direct referrals from outside hospitals and physicians.
Ethics oversight	City of Hope Institutional Review Board

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	We studied 3 participants on treatment plan 1 and plan for 12 participants on treatment plan 2, allowing 3 additional participants on treatment plan 1 and 6 for replacement of unevaluable research participants (minimum=6, maximum=24). A sample size of 12 will provide us with i) maximum margin of error of 0.25 for a 90% confidence interval (90% CI) for the DLT rate, and ii) to detect a toxicity with a true rate of 0.20 in 93% of trials.
Data exclusions	Study participants who did not receive the full schedule of 4 T cell doses were excluded from disease response analysis.
Replication	Statistical tests were employed to ensure significance of results within our study. However the study is a preliminary report limited to a single site, and investigation if true replicability is beyond the scope of the study. Single cell replicate experiments were included in the reported statistics.
Randomization	This is a a Phase I, open-label, non-randomized feasibility/safety study.
Blinding	This is a Phase I, open-label, non-randomized feasibility/safety 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 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

CCR7-PE (R&D Systems Cat. # FAB197P)
CD3-APC (BD Biosciences Cat. # 340440)
CD3-VioGreen (BD Biosciences Cat. # 563109)
CD4-FITC (BD Biosciences Cat. # 340133)
CD4-PerCP (BD Biosciences Cat. # 347324)
CD8-APC-Cy7 (BD Biosciences Cat. # 348793)
CD19-PE-Cy7 (BD Biosciences Cat. # 557835)
CD27-APC-Cy7 (BD Biosciences Cat. #302816)
CD27-PE (BD Pharmingen Cat. # 555441)
CD45RA-FITC (BD Bioscience Cat. # 555488)
CD57-FITC (BD Biosciences Cat. # 555619)
CD62L-FITC (BD Biosciences Cat. # 347443)
CD62L-PE (BD Biosciences Cat. # 341012)
LAG-3-PE (eBiosciences Cat # 12-2239-42)
LAG-3-FITC (Lifespan Biosciences Cat. # LS-C344745)
PD-1-FITC (eBiosciences Cat. # 11-9969-42)
PD-1-PE (eBiosciences Cat. # 12-9969-42)

Validation

All antibodies are validated by specificity to their respective target on human cells as provided by the manufacturer's information.

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

NCT04510051

Study protocol

Full trial protocol is available as Supplementary Information

Data collection

Data were collected at City of Hope, Duarte, CA, between December 2020 and May 2023.

Outcomes

The Primary Objectives are:
• to assess the feasibility and safety of lymphodepleting chemotherapy followed by cellular immunotherapy utilizing IL13(EQ)BBζ/CD19t+ Tn/mem cells implementing intraventricular delivery for participants with IL13Rα2+ recurrent/refractory pediatric brain tumors. A single dose schedule of 4-weekly IL13(EQ)BBζ/CD19t+ Tn/mem cell infusions alone (Treatment plan 1) is being examined first as a safety precaution.
The Secondary Objectives are:
• To describe persistence and expansion of CAR T cells in peripheral blood (PB) and CSF.
• To describe cytokine levels (PB and CSF) over the study period.
• In research participants who receive the full schedule of 4 CAR T cell cycles:
o to estimate 6-month PFS rate per disease,
o to estimate disease response rates per disease,
o to estimate 1-year overall survival rate per disease.
• To evaluate the use of circulating tumor DNA (ctDNA) to evaluate tumor burden
• For study participants who undergo an additional biopsy/resection or autopsy:
o to evaluate CAR T cell persistence in the tumor tissue and the location of the CAR T cells with respect to the infusion site, and
o to evaluate IL13Rα2 antigen expression levels on tumor tissue pre and post CAR T cell therapy.

Primary Endpoints: Grade 3 toxicities, DLTs, and all other toxicities;
Secondary Endpoints:
• CAR T cells detected in peripheral blood and CSF.
• Peripheral blood and CSF cytokine levels.

• Peripheral blood and CSF immune cell characterization.
• Progression free survival: Defined as from time of lymphodepletion to the event date (progression or death).
• Overall survival: from time of lymphodepletion to date of death.
• Disease response by RANO criteria.
• CAR T cells detected in tumor tissue, if available.
• IL13Rα2 antigen expression levels in tumor tissue, if available.
• Circulating tumor DNA assessments.

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	Cell suspensions were washed and resuspended in PBS before staining with appropriate antibodies.
Instrument	MACSQuant Analyzer 10 (Miltenyi Biotec)
Software	FlowJo software (v10.9.0), FCSExpress 7 (Version 7.16.0047) and GraphPad Prism Software (v9.5.1).
Cell population abundance	Refer to percentages on figures.
Gating strategy	gating strategies varied by sample, but in general single-cell suspensions were gated by FSC/SSC, then SSC-A v. SSC-W for doublet discrimination, then viability (unless permeablized), then nested biaxial plots depending on populations of interest.

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

Magnetic resonance imaging

Experimental design

Design type	N/A
Design specifications	N/A
Behavioral performance measures	N/A

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	3 Tesla
Sequence & imaging parameters	standard clinical imaging sequencing and parameters: pre- and post-gadolinium sequences of brain and spine were acquired on a Siemens Viro 3 Tesla scanner.
Area of acquisition	whole brain and spine

Diffusion MRI ☐ Used ☒ Not used

Preprocessing

Preprocessing software	Regions of contrast-enhancing tumor were outlined by a radiologist for measurement and volumes were calculated on the Medtronic Stealthstation with Stealth3DTM software (v 2.2.0).
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*

Noise and artifact removal

Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).

Volume censoring

Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

Statistical modeling & inference

Model type and settings

Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).

Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

(See Eklund et al. 2016)

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis