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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	NA
Data analysis	NA

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

The flow cytometry raw data will shared upon request under a collaborative agreement.

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	Patients of any sex and gender were eligible for this study.
Reporting on race, ethnicity, or other socially relevant groupings	NA
Population characteristics	Patients with CD19 and/or CD20 and/or CD22 expressing hematologic malignancies were enrolled to this study.
Recruitment	Patients were recruited at the OSU James Hospital or Nationwide Children's Hospital.
Ethics oversight	The clinical study was approved by the OSU Institutional Review Board (IRB). IRB approved protocol OSU 21170 (2022C0021).

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	Statistical design is described on page 95 of the protocol, included in the supplemental materials. This study used the 3+3 design.
Data exclusions	No data were excluded.
Replication	NA
Randomization	NA
Blinding	NA

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	human CD19PE, clone HIB19 (catalogue# 555413 BD), human CD20 PE clone H1 (catalogue#561174 BD), human CD22 PE clone HIB22 (catalogue# 562859BD); mouse IgG1K (catalogue# 555749 BD) was used as an isotype control; live/dead fixable Near-IR from Thermo Fisher (catalogue# L34981). was used to distinguish between live and dead cells. Quantitative flow cytometry was done using BD Quantibrite™Beads, PE Fluorescence Quantitation Kit purchased from BD Bioscience. His-tagged CD22 protein

(Acrobiosystems, catalog # CD2-H52H8) and biotin-tagged CD19 CAR detection reagent (Miltenyi Biotec, catalog # 130-129-550). secondary antibodies (His Antibody, PE, Miltenyi Biotec, catalog # 130-120-718 and Biotin Antibody, APC, Miltenyi Biotec, catalog # 130-110-952) along with immunophenotyping antibodies (Miltenyi Biotec, REAfinity™) to CD45 (clone REA747/5B1, catalog # 130-110-637), CD3 (clone REA613/SK7, catalog # 130-113-138), CD4 (clone REA623/SK3, catalog # 130-113-230), CD8 (clone REA34/HIT8a, catalog # 130-110-680) and the viability dye 7-AAD (BD Biosciences, catalog # 559925). Controls included a fluorescence minus two (FMT; lacking CD22 protein, His tag and CAR19 detection reagent) along with fluorescence minus one (FMO) controls for each CAR detection reagent, creating a four tube panel for CAR detection. Antibodies used for spectral flow are listed in Supplementary Table 2.

Validation

All antibodies used in this study were purchased from recognized commercial vendors with publicly available validation data.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	No cell lines were used in this manuscript
Authentication	NA
Mycoplasma contamination	NA
Commonly misidentified lines (See ICLAC register)	NA

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	5–7-week-old female NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) were purchased from the Jackson Laboratory
Wild animals	NA
Reporting on sex	Only female mice were chosen for these experiments given their proven less aggressive behavior compared to male mice. Both the MCL and DLBCL PDXs were generated lymphoma cells from male patients.
Field-collected samples	NA
Ethics oversight	All animal studies were approved by the OSU Institutional Animal Care and Use Committee (IACUC). IACUC approved protocol: 2020A00000010-R2. Expiration date: 12/17/2028

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	NCT05418088
Study protocol	The clinical study protocol was provided as supplemental material and submitted in a separate PDF document.
Data collection	Clinical trial was conducted from 2022-06-30 to present.
Outcomes	The primary outcome was to determine the recommended phase II dose of CAR T cells (range 0.5 to 2 million /kg) and describe dose limiting toxicities. Study design was a classic 3+3 and the recommended dose is 2 million /kg. There were no dose limiting toxicities.

Plants

Seed stocks	NA
Novel plant genotypes	NA
Authentication	NA

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	See the Methods section of the manuscript for detailed sample preparation information. Briefly, cryopreserved peripheral blood samples obtained from patients with B-ALL, CLL, nodal MCL and DLBCL were cultured in standard RPMI1640 with 10% FBS and 5% CO2 and used immediately upon thawing. Cryopreserved normal human T cells isolated from peripheral blood mononuclear cells (PBMCs) using EasySep Human T Cell Isolation Kit (STEMCELL Technologies, Vancouver, Canada) were used as negative control. Cryopreserved NTTs and trispecific CAR T infusion product samples cryopreserved at the time of harvest were utilized for correlative studies. In addition, cryopreserved PBMCs derived from Ficoll-Paque separation of blood samples drawn at timepoints pre- and post-CAR T infusion (Day -6, Day 0, Day +2, 4, 6, 14, 21, 30, 90, 6 months and at 12 months) were used for correlative analysis. NTTs derived from apheresis products, trispecific CAR T infusion product, and PBMCs at Days 7, 14, and 30 post-CAR T infusion along with healthy donor PBMCs were analyzed.
Instrument	Quantitative flow cytometry for clinical product validation was calibrated using BD Quantibrite™Beads and run on BD LSRFortessa Flow Cytometer. Beckman Coulter CytoFLEX flow cytometer and data analyzed with Beckman Coulter Kaluza Analysis Software for product validation. Cytek Aurora 5 laser for spectral flow cytometry.
Software	See the Methods section of the manuscript for detailed sample preparation information. Briefly, flow cytometry data was uploaded to web-based software OMIQ for analysis (https://app.omiq.ai/) and analyzed as previously described (reference #47 from the manuscript). Bar plots and statistical analysis were performed suing Prism 10; edgeR analysis via OMIQ software was used for statistical analysis. For further validation, flow cytometry data were reevaluated using FlowJo (BD) by creating 2-dimensional (2D) plots and using concomitant statistical approaches.
Cell population abundance	See the Methods section of the manuscript for detailed sample preparation information. Briefly, cryopreserved de-identified lymphoma and leukemia patient samples were thawed and washed with PBS. A cell count for total cell number and viability was done using hemocytometer. 1 million cells were taken for quantitative flow for each patient sample. No sorting was performed.
Gating strategy	Beads were run on BD LSRFortessa Flow Cytometer, setting the threshold for FSC or SSC, and 10,000 events were collected. The gate was adjusted around bead singles on FSC-H vs SSC-H plot. Singlet bead population was analyzed using histogram plot of FL2-H. After instrument adjustment, patient samples were acquired. Manual analysis was done using the geometric means of each histogram for the four bead peaks and Linear regression of Log10 per bead against Log10 fluorescence was plotted. Calibrated beads contain a specific number of fluorophore molecules bound per bead and are used to standardize and convert the mean fluorescence intensity (MFI) in flow cytometry into a count of fluorophores. These allow for the calculation of the number of target antigens per cell when using antibodies under saturating conditions, considering the Fluorophore to Protein Ratio (F:P) of each antibody. A calibration curve correlating instrument detection channel values and standardized fluorescence intensity units on constructed with R2 of 0.9995. A sequential gating strategy (singlets, debris exclusion, CD45+ then viability) is utilized prior to determining the %CD3 and % CAR. The FMT control, FMO controls, and positive population clusters were used to determine the transduction efficiency of the product. Further gating strategies are included in supplementary figure 9.

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