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☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

The current study is a secondary investigation using patient samples collected from existing clinical trials. Therefore, the sample sizes in this report were determined by the original clinical trial designs and sample availability

2. Data exclusions

Describe any data exclusions.

Please see the above response. No additional inclusion/exclusion criteria were applied.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All in vitro experiments were repeated with primary cells from different subjects in each separate experimental run. All attempts at replication of these experiments were successful.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

The clinical trials were single-treatment studies; the comparison groups of patients in the current study were defined by the observed clinical responses.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were blinded to clinical responses as correlative assays were conducted using de-identified subject samples.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒	A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	A statement indicating how many times each experiment was replicated
☐	☒	The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☒	☐	A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒	The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒	A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒	Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Flow cytometry data were analyzed using FlowJo software. For ATAC Seq. data, BedTools, HOMER and Metascape were used. Analysis of TCR VB and BCR IgH sequencing was done using the ImmunoSeq Analyzer.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	B. Jena and L. Cooper (MD Anderson Cancer Center) provided the CAR anti-idiotype detection reagent.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Information about flow cytometry antibodies can be found in the methods section. All clinical and research samples were analyzed in the Product Development and Correlative Sciences laboratories at the University of Pennsylvania and antibodies were validated in these laboratories. Western blot antibodies against FLAG and Hsp90 were validated using serial dilutions of HEK293T cell lysates as shown in Supplementary Figure 1c.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	NALM-6, K562 and HEK293T cell lines were originally obtained from the American Type Culture Collection (ATCC). The OSC-CLL line was derived and provided by investigators at Ohio State University.
b. Describe the method of cell line authentication used.	Cell line authentication was performed by the University of Arizona (USA) Genetics Core based on criteria established by the International Cell Line Authentication Committee. Short tandem repeat (STR) profiling revealed that these cell lines were above the 80% match threshold.
c. Report whether the cell lines were tested for mycoplasma contamination.	Low passage working banks of cells were tested for mycoplasma using the MycoAlert detection kit according to the manufacturer's (Lonza) instructions. Mycoplasma testing and authentication are routinely performed before and after molecular engineering.
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	NALM-6, OSU-CLL, K562 and HEK293T cell lines are not listed in the latest version of the database of commonly misidentified cell lines maintained by ICLAC (Version 8.0, released 1 December 2016).

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	N/A

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	We did not perform covariate analyses in this secondary correlative investigation.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☒ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. 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).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Primary human PBMC or T-cells were isolated from the peripheral blood of CLL patients and healthy donors. Immunophenotyping was performed by surface staining with flow cytometry antibodies immediately following pre-incubation with Aqua Blue dead cell exclusion dye (Invitrogen). For intracellular staining, cells were fixed and permeabilized using the Foxp3 Fixation/Permeabilization Kit (eBioscience) or the Cytofix/Cytoperm Kit (BD Biosciences). The GolgiStop protein transport inhibitor containing monensin and GolgiPlug protein transport inhibitor containing brefeldin A (BD Biosciences) were used when staining for intracellular cytokine production.
6. Identify the instrument used for data collection.	Samples were acquired on a custom 17-color, 19-parameter special order LSRFortessa (BD Biosciences). Routinemeasurements of the expansion and persistence of CAR T-cells, as well as peripheral B-CLL burden were conducted with a six-parameter Accuri C6 flow cytometer (BD Biosciences).
7. Describe the software used to collect and analyze the flow cytometry data.	Data were analyzed using FlowJo software (TreeStar).
8. Describe the abundance of the relevant cell populations within post-sort fractions.	Cell frequencies/abundances are listed in the flow plot (insets).
9. Describe the gating strategy used.	Gating strategies are depicted in Extended Data Figure 2.

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