nature portfolio

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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	Raw sequencing reads were processed using CellRanger pipeline using default settings (10x Genomics, software version 3.1.0).
Data analysis	Filtered gene expression matrices generated by CellRanger pipeline were used for downstream analysis using Seurat package (version 3). Loupe Browser was used to resolve Hashtag identities of cellular barcodes (10x Genomics version 3). Pathway analysis and biological processes analysis was performed using IPA software (Qiagen). TCR clonotype analysis was performed using immunarch R package (version 1.0.0). Shannon index was calculated on TCR clonotypes using abdiv package (https://github.com/kylebittinger/abdiv). CellChat package was used to infer cell–cell communications between the following cell types via interaction-network analysis (https:// github.com/sqjin/CellChat. NetAnalysis_signalingRole_heatmap). Cluster identities were analysed using SingleR package. Code for data processing and analysis is available at https://github.com/SzymonSzymura/LPLvaccine and Zenodo (10.5281/zenodo.12761537)

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

Processed and raw sequencing data files have been deposited to Gene Expression Omnibus (GEO) database under accession code GSE243545 https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE243545. Dataset "A single cell immune cell atlas of human hematopoietic system" is available from Human Cell Atlas Project https://explore.data.humancellatlas.org/projects/cc95ff89-2e68-4a08-a234-480eca21ce79 and was downloaded on April 24th 2020.

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	Sex and gender were not considered in the study design. Sex/ gender are described in Table 1.
Reporting on race, ethnicity, or other socially relevant groupings	This study consenting from didn't require race, ethnicity or other socially relevant grouping.
Population characteristics	A total of nine patients were enrolled and treated on trial, three in the 500µg cohort and six in the 2500µg cohort. The baseline characteristics of patients are described in Table 1. The median age of all patients was 67, and the majority were male (78%).All patients with asymptomatic phase, previously untreated LPL with surface IgG, IgA or IgM phenotype tissue diagnosis and a monoclonal heavy and light chain as determined by flow cytometry. All primary diagnostic lymph node and/ or bone marrow biopsies were reviewed at the University of Texas M.D. Anderson Cancer Center (UTMDACC).
Recruitment	These patients were recruited by UTMDACC. Recruitment was based on first-come/first-serve basis if meeting eligibility. Patient recruitment occurred from 3/4/2015 to 3/4/2019. All patients consented to donated bone marrow and peripheral blood samples after discussion and review of the consent from their treating physician.
Ethics oversight	The trial protocol and all its amendments were approved by the UTMDACC institutional review Board (UTMDACC IRB, protocol number 2009-0465). All patients provided written informed consent before study enrollment. All correlative tissues analyses were conducted with UTMDACC IRB. For samples transfer from UTMDACC to City of Hope (MTA#19267) and analyzed at City of Hope(COH), review and approval was COH institutional review Board (Protocol Number: 17421).

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	The 3+3 design was employed in this study. This is a statistical approach utilized in phase 1 oncology trials to identify recommended doses for phase 2 trials. The experiments conducted in this study were determined by a sample of nine patients(patient. The 2 pre-defined dose levels – Cohort 1 (500 µg DNA vaccine; 3 patients) and Cohort 2 (2500 µg DNA vaccine; 6 patients) – were chosen based on the pre-clinical experience. Total of 9 patients were enrolled. Eight patients participated in providing both pre- and post-treatment samples, with one patient opting out of providing a post-treatment sample. Therefore, a total of 17 patients bone marrow samples were included in the study. For all of the analysis comparing pre- and post-vaccine conditions, 8 paired pre- and post- vaccine samples were used.
Data exclusions	From the protocol: 1. HIV, Hepatitis B and/or Hepatitis C infection. 2. Pregnant or lactating females. 3. Patients with previous history of malignancy within the last 5 years except curatively treated squamous or basal cell carcinoma of the skin or curatively treated carcinoma in-situ of other organs. 4. Any medical or psychiatric condition that in the opinion of the principal investigator would compromise the patient's ability to tolerate this treatment. 5. Patients with New York Heart Association Class 3 or 4 disease. 6. Patients with a history of autoimmune diseases except for Hashimoto's thyroiditis. 7. Patients with positive ANA and/or anti-dsDNA antibodies.

Replication	The individual patients serve as biological replicates. The functional tumor idiotype-specific-T cell responses assay was measured in technical triplicates from eight post-vaccination patients BM samples (Figure 2I). Experiments were performed in once (LPL-1, 2, 3, 4, 8, 9) due to limited patient sample availability or twice (LPL-5, 7) with similar results. Covariates were not controlled for in this study due to small sample size.
Randomization	There was no randomization in the phase 1 clinical study.
Blinding	This study is a clinical trial to test the safety of a new vaccine. It is open-label, Phase 1, and designed to test the safety and tolerability of the vaccine in patients with LPL. Therefore, blinding is not applicable.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[ ]	[x] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used	Cell hashtag antibodies: TotalSeq-C0251 (Biolegend, Cat #394661, clone LNH94, 2M2, lot B282243, 1ug per sample), TotalSeq-C0252 (Biolegend, Cat #393663, clone LNH94, 2M2, lot B293608, 1ug per sample). Flow cytometry antibodies: anti-HLA-DR AlexaFluor 647 (BD Biosciences, Cat# 563591, clone Tu39, lot 8087988, 5ul per sample), anti-CD38 PE-Cy7 (BD Biosciences, Cat# 560677, clone HIT2, lot 2129025, 5ul per sample), anti-CD19 V450 (BD Biosciences, Cat# 560354, clone HIB19, lot 4015718, 5ul per sample) IHC antibodies: CD79a (Cell Signaling Technology, Cat# 13333, clone D1X5C, dilution 1:100), CD138 (Ventana, Cat# 760-4248, clone B-A38, ready-to-use format), HLAII (Dako Cat#M0775, clone CR3/42, dilution 1:800)
Validation	Antibody validation is provided on manufacturer's website: https://www.biolegend.com/en-us/explore-new-products/totalseq-c0251-anti-human-hashtag-1-antibody-17162 https://www.biolegend.com/fr-ch/search-results/totalseq-c0252-anti-human-hashtag-2-antibody-17163 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-647-mouse-anti-human-hla-dr-dp-dq.563591 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-mouse-anti-human-cd38.560677 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/v450-mouse-anti-human-cd19.560353 https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/v450-mouse-anti-human-cd19.560353 hhttps://www.cellsignal.com/products/primary-antibodies/cd79a-d1x5c-xp-174-rabbit-mab/13333 https://diagnostics.roche.com/us/en/products/lab/cd138-syndecan-1-b-a38-rtd000717.html

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	BJ cells were obtained from ATCC (CRL-2522, male), MWCL-1 (male) cell line was a kind gift of Asnell lab, Mayo Clinic. BCWM.1 (female) cell line was a kind gift of Treon Lab, Dana Farber Cancer Institute
Authentication	BJ cell line was purchased directly from ATCC (CRL-2522). MWCL-1 and BCWM.1 cells were not authenticated. No commonly misidentified cell lines were used in this study.
Mycoplasma contamination	All cell lines are regularly tested in our lab for the mycoplasma contamination and were confirmed mycoplasma negative.
Commonly misidentified lines (See ICLAC register)	NA

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJEguidelines for publication of clinical research and a completedCONSORT checklist must be included with all submissions.

Clinical trial registration	NCT01209871
Study protocol	The study protocol was added as supplementary data.
Data collection	This was an open-label phase I trial conducted at the University of Texas M.D. Anderson Cancer Center (NCT01209871). Patients were enrolled between March 2015 and August 2017. All patients were enrolled and data collected at M.D. Anderson Cancer Center
Outcomes	PRIMARY OBJECTIVES: 1. To evaluate the safety and feasibility of using a novel lymphoma deoxyribonucleic acid (DNA) vaccine encoding macrophage inflammatory protein 3 alpha (MIP3a)-fused lymphoma idiotype in single chain format. 2. To determine the maximum tolerated dose (MTD) of the vaccine. (primary endpoint) SECONDARY OBJECTIVES: 1. To assess the immunogenicity of the vaccine to generate tumor-specific cellular and humoral immune responses. EXPLORATORY OBJECTIVES: To characterize tumor microenvironment of LPL bone marrow specimens pre- and post-vaccine (outlined in the section "Correlative studies") of the study protocol. OUTLINE: This is a dose-escalation study. Patients receive autologous lymphoma immunoglobulin-derived single-chain variable fragment (scFV)-chemokine DNA vaccine intradermally (ID) at 0, 4, and 8 weeks. After completion of study treatment, patients are followed up at 4 weeks, and then every 6 months for 1 year. Detailed information is provided in Table 2.

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	Briefly, cells were washed in Stain Buffer (BD Biosciences Cat# 554657), blocked with Fc blocking reagent (Human TruStain FcX, Biolegend Cat# 422302) followed by staining with fluorophore conjugated antibodies (anti-HLA-DR AlexaFluor 647, BD Biosciences Cat# 563591, anti-CD38 PE-Cy7, BD Biosciences Cat# 560677, anti-CD19 V450, DB Biosciences Cat# 560354).
Instrument	Cells were analyzed on BD LSRFortessa
Software	Data analysis and visualization was performed in FlowJo version 10.8.1.
Cell population abundance	MWCL-1: Mature B cells 83.1%, Plasma cells: 6.31%. BCWM.1: Mature B cells 80.7%, Plasma cells: 4.13%
Gating strategy	Gating based on CD19/CD38 staining into Mature B cells: CD19highCD38low, Plasma cells: CD19lowCD38high

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