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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.

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	FACS Diva 6 (flow cytometry) ZEN Black (microscopy)
Data analysis	GraphPad Prism 6.0 Cytobank 6.3 FCS Express 6 Microsoft Office for Mac 2016

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. 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

The data generated during and analyzed during the current study are available from the corresponding author upon reasonable request.

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	All experiments were designed for two-cohort comparison, those experiments with more than two cohorts all consider the comparison of the (multiple) experimental cohorts to the control cohort as defined for each experiment. Our primary endpoint for each study is the proportion of mice that appear to be cured of disease (therapeutic experiments) or in which disease (tumor growth) is prevented (prophylactic experiments). In our experience with these and related tumor immunotherapy models, we consider the baseline 'cure rate' to be 0% as these tumors are generally progressive and we consider an explicitly beneficial cure rate to be 50%. For these assumptions 10 mice per group are the smallest number of animals that will allow for a statistically significant result, i.e. for 0/10 versus 5/10, two-tailed P value equals 0.0325, whereas for 0/8 versus 4/8 two-tailed P value equals 0.0769 (not significant). (Cohorts of n=9 are not considered as a comparable improved cure rate of exactly 50% in not possible). Therefore, In order to reach statistical significance we aim for groups of 10 mice.
Data exclusions	animal studies: 1 day after the last Flt3L injection mice were assigned to treatment groups according to pre-established criteria. Stratified randomization was used based on tumor size to ensure equal distribution of tumor sizes within each group, and any mice bearing tumors >500mm3 were excluded from the experiment. patients: all patient with indolent NHL and treated with ISV were included in the analysis
Replication	All experiments were repeated in independent experiments at least twice, or more where indicated. Replication attempts were successful.
Randomization	animal studies: 1 day after the last Flt3L injection mice were assigned to treatment groups using stratified randomization based on tumor size to ensure equal distribution of tumor sizes within each group. patients: all patient with indolent NHL and treated with ISV were included in the analysis
Blinding	animal studies: blinding was not possible, since all groups were treated differently and injected repeatedly, so it was necessary for the investigators to know which animals belong to which group, especially since data collection (e.g. tumor measurements) overlapped with treatment patients: investigators were blinded about patient identity during data collection

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
☐	☒ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

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

Antibodies

Antibodies used	anti-mouse: depletion studies: BioXCell: anti-PD1 (RMP1-14, #BE0146), anti-CD8 (2.43, #BE0061), anti-CD4 (GK1.5, #BE0003-1), Wako chemical: anti-asialo-GM1 (polyclonal, #986-10001) flow cytometry: all surface antibodies were used at 1:400, except CD103 (1:100); all intracellular antibodies were used at 1:200 Biolegend: B220-PerCpCy5.5 (RA3-6B2, #103235), CD3 (145-2C11, PE-Cy7 #100319, PerCP-Cy5.5 #100327, APC #100311), CD8-Alexa Fluor 700 (53-6.7, #100729), CD4-BV650 (RM4-5, #100545), CD11b (M1/70, APC-Cy7 #101225, Alexa Fluor 700 #101222), CD11c (N418, PE-Cy7 #117317, Alexa Fluor 700 #117319), CD19-BV711 (6D5, #115555), CD25-BV650 (PC61, #102037), CD80-PE (16-10A1, #104707), CD86-BV510 (GL-1, #105039), CD103 (2e7, PerCP-Cy5.5 #121415, PE #121405), CD137-Biotin (17B5, #106103), F4/80-BV421 (BM8, #123131), Gr1-PerCP-Cy5.5 (RB6-8C5, #108427), LAG3-PE (C9B7W, #125207), Ly6C-Alexa Fluor 700 (HK1.4, #128023), Ly6G PerCP-Cy5.5 (1A8, #127615), I-Ad (39-10-8, FTIC #115005, Alexa Fluor 647 #115009), PD-L1-BV711

(10F.9G2, #124319), TIM3-BV421 (RMT3-23, #119723), XCR1-BV650 (ZET, #148220), TLR3-APC (11F8, #141905), IRF4-Alexa Fluor 647 (IRF4.3E4, #646407), IFNg-PE-Cy7 (XMG1.2, #505825), TNFa-APC (MP6-XT22, #506308),
BD: TLR7-PE (A94B10, #565557), PD1-PE (J43, #551892)
ThermoFisherScientific: IRF8-PE (V3GYWCH, #12-9852-82), Granzyme B-PerCP-eFluor710 (NGZB, #46-8898-82)

anti-human: all antibodies were used at 1ul/test
CD1c (L161, Invitrogen), CD3-PE-CF594 (UCHT1, BD #562280), CD4-PE (RPA-T4, BD #555347), CD5-FITC (UCHT2, BD #555352), CD8-FITC (RPA-T8, Biolegend #301005), CD19 (HIB19, BD, PE#555413, PE-CF594 #562294), CD141-APC (M80, Biolegend #344105), HLA-DR-PE-Cy7 (L243, Biolegend #307615), gamma light chain-PE (MHL-38, Biolegend #316607), kappa light chain-FITC (MHK-49, Biolegend #316506), PD-L1-BV711 (29E.2A3, Biolegend #329721), CD80-BV421 (2D10, Biolegend #305222), CD86-Alexa Fluor 700 (2331, BD #561124),

Validation

all primary anti-mouse and anti-human antibodies were validated for flow cytometry by the manufacturers

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

A20 lymphoma was purchased from ATCC (ATCC TIB-208); 4T1 cells were a gift from the Brown lab; A20-GFP, A20-mCherry, 4T1-GFP and 4T1-mCherry cells were generated in-house using lentiviral transduction; A20-GFP-b2m-/- cells were generated in-house using the Crispr/Cas9 system.

Authentication

none of the cell lines used were authenticated

Mycoplasma contamination

all cell lines tested negative for micoplasma

Commonly misidentified lines
(See ICLAC register)

none used

Animals and other organisms

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

Laboratory animals

mice: Balb/c wt, Balb/c-Batf3-/-, Balb/c-JEDI, Balb/c-RAG1-/-, C57/Bl6-RAG1-/-, all male and female, all 8-12 weeks

Wild animals

study did not involve wild animals

Field-collected samples

study did not involve samples collected from the field

Ethics oversight

All experiments were reviewed and approved by the Institutional Animal Care and Use Committee of the Icahn School of Medicine at Mount Sinai.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics

patients (64% male and 36% female) with indolent non-Hodgkin's B cell lymphoma (low grade) were enrolled into our ongoing in situ vaccine trial (NCT01976585, see also below). Median age 54 years (range 32-68), 9 diagnosed with follicular lymphoma (FL), 1 diagnosed with marginal zone lymphoma and 1 diagnosed with small lymphocytic lymphoma. For FL 6 patients presented with grade 1/2, 1 patient presented with grade 2, 2 patients presented with grade 3a. 6 patients presented with stage 4a, 3 patients presented with stage 3a. Most patients were previously untreated unless otherwise indicated in the manuscript (3 patients received R-CHOP and idelalisib prior to this study). All patients were treated with the same in situ vaccine regimen.

Recruitment

participants were recruited by the Icahn School of Medicine at Mount Sinai as part of our clinical trial NCT01976585 after biopsy-confirmed diagnosis of low-grade B-cell lymphoma. To be included patients had to have at least on site of disease that is accessible for intratumoral injection percutaneously and measurable disease other than the injection site. ECOG performance status of 1 or better and adequate bone marrow, renal and hepatic function. Additional detail can be found www.clinicaltrials.gov

Ethics oversight

This protocol was approved by the Mount Sinai Institutional Review Board and is being performed in accordance with Federal Law

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	NCT01976585
Study protocol	The study protocol is available from the corresponding author while the study is still ongoing
Data collection	data are collected at the Icahn School of Medicine at Mount Sinai in New York, NY. Recruitment started in Dec 2013 and is still ongoing. Patients included in this manuscript were recruited between Dec 2013 and April 2018. Data/sample collection for each patient started on the day of the first Flt3L injection; blood samples were collected on day 0 (pre-treatment), day 10 and day 25 of the trial; biopsies were obtained before treatment and, where possible, at 3 months and/or 6 months after the start of the treatment.
Outcomes	primary and secondary outcome measures can also be found on the trial page on www.clincialtrials.gov - Primary Outcome Measures : response rate [Time Frame: week 12]: Overall objective response rate at time of best response as defined by International Harmonization (Cheson) Criteria. - Secondary Outcome Measures : 1)safety profile [Time Frame: week 1]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 2) safety profile [Time Frame: week 2]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 3) safety profile [Time Frame: week 4]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 4) safety profile [Time Frame: week 6]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 5) safety profile [Time Frame: week 8]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 6) safety profile [Time Frame: week 12]: Patient reported and clinical observation of adverse events, including changes in physical examination, peripheral blood hematology and serum chemistry 7) tumor-specific immune response [Time Frame: baseline]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response. 8) tumor-specific immune response [Time Frame: week 2]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response. 9) tumor-specific immune response [Time Frame: week 4]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response. 10) tumor-specific immune response [Time Frame: week 6]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response. 11) tumor-specific immune response [Time Frame: week 8]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response. 12) tumor-specific immune response [Time Frame: week 12]: Pre- to post-treatment induction of systemic (peripheral blood) tumor-specific immune response.

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	Tumors, spleens and lymph nodes were homogenized by forcing the tissue through a 70µm nylon mesh. Bone marrow cells were isolated by crushing femur and tibia in HBSS using a glass pestle and filtering through a 70um nylon mesh. Cell suspensions were pelleted at 500xg for 5 minutes at 4°C and resuspended in Pharm Lyse lysing buffer (BD) to remove red blood cells for 5 minutes. Tumor cell suspensions were then depleted of tumor cells by magnetic separation using CD19-nanobeads according to the manufacturer's protocol (Mojosort, Biolegend). For DC isolation lymph nodes were digested using 400U/ml Collagenase D (Worthington) for 20 minutes at 37C. Cells were stored at 4°C until further usage.
Instrument	BD LSR Fortessa
Software	collection: FACS DIva 6 analysis: Cytobank 6.3
Cell population abundance	purity of post-sort samples was 95% or higher as determined by flow cytometry
Gating strategy	cells were first gated in intact cells using FSC/SCC, doublets were excluded using FSC-W vs SSC-A, cells were then gated on live cells using a viability dye, followed by cell type specific gating using fluorescently labeled antibodies

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