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	The sequencing data were demultiplexed and raw single-cell RNAseq reads were aligned to GRCh38-3.0.0 using Cell Ranger version 3.1.0 pipeline (10× Genomics). The count matrix for each sample was read using Seurat V3.04 and made into a Seurat object. All of the Seurat objects were merged and batch corrected with Harmony for CD3+ cells and CD3- cells, respectively. The Seurat objects were processed by the standard Seurat workflow. The count matrix was log normalized and the top 2000 most variable genes were calculated by the vst method in Seurat. The UMAP was generated using the first 50 dimensions of the Harmony coordinates. To find clusters, the shared nearest neighbor (SNN) graph was built on the first 50 dimensions of the Harmony coordinates followed by the Louvain community detection algorithm with a resolution of 1.5. Image acquisition was performed using the PhenoImager HT multispectral imaging platform (Akoya Biosciences)
Data analysis	scRNA-seq and scTCR-seq data were processed and analyzed by custom Python and R scripts. The codes are available at https://github.com/crazyhottommy/hodgkin_lymphoma_publication_scRNAseq_analysis. Code used for image analyses is available at https://github.com/dfci/pythologist.

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 scRNA-seq and scTCR-seq data are publicly available through the European Genome-phenome Archive (EGA) study ID EGAS00001007569 and dataset ID EGAD00001011360. External, previously published data sets included in this manuscript as a validation cohorts are publicly available: Yuen K.C. et al., Nature Medicine 27, 560 (2021): EGAD00001005481, EGAS00001004008 and GSE145281; Mulder K et al. Immunity 54,1883-1900 (2021): GEO:GSE178209 and https://gustaveroussy.github.io/FG-Lab/.

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' sex and gender are included in Supplementary Data 1.
Reporting on race, ethnicity, or other socially relevant groupings	Patients' race and ethnicity information were not available.
Population characteristics	Full description of the human research participants and the covariate-relevant population characteristics is detailed in the following publications: Armand P, et al. J Clin Oncol. 36, 1428–1439 (2018) Ansell, S.M., et al. Blood Adv. (2023)
Recruitment	The recruitment of participants of the clinical trial was based on predetermined in- and exclusion criteria. Full description of the recruitment of participants of the clinical trials is detailed in the https://clinicaltrials.gov/ website and the corresponding publications: Armand P, et al. J Clin Oncol. 36, 1428–1439 (2018) Ansell, S.M., et al. Blood Adv. (2023)
Ethics oversight	The study was approved by institutional review boards (IRB) at the Dana-Farber Cancer Institute and Brigham & Women's Hospital.

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 obtained baseline and on-treatment cryopreserved peripheral blood mononuclear cells (PBMCs) from 20 patients who received anti-PD-1 therapy (nivolumab) on the Checkmate 205 clinical trial. This multi-center, multi-cohort phase II study included patients with relapsed/refractory cHL following autologous stem cell transplantation (ASCT) alone or ASCT and brentuximab vedotin. PBMCs were collected immediately before the initiation of therapy (cycle 1 day 1 [C1D1]) and during treatment at cycle 4 day 1 (C4D1). In addition, cryopreserved PBMCs were obtained from 11 patients with newly diagnosed, previously untreated cHL and 13 healthy donors for comparison.
Data exclusions	No data were excluded from the analysis.
Replication	No replication was possible as the study used clinical trial samples. We utilized two independent, publicly available scRNA-seq studies (Yuen K.C. et al. Nature Medicine 27, 560 (2021); Mulder K. et al. Immunity 54,1883-1900 (2021)) to extend certain findings to solid tumors.
Randomization	Patients samples came from a clinical trial and analysis was done based on best overall response.
Blinding	ScRNA-seq analyses of trial patient samples were performed blinded to clinical parameters.

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	Immunohistochemistry antibodies: PD-L1 1:100 Clone 405.9A11, #G.J.F.19 PAX5 1:100 Clone 24/Pax-5 (RUO), #610862 BD Biosciences Multiplex Immunofluorescence antibodies: PAX5 1:100 Clone 24/Pax-5 (RUO), #610862 BD Biosciences CTLA-4 1:100 E2V1Z, #53560S Cell Signaling Technology CD4 1:200 4B12, #M731001-2 Agilent Dako Ki67 1:100 MIB-1, #GA62661-2 Agilent Dako FOXP3 1:100 D2W8E, #98377S Cell Signaling Technology
Validation	PAX5 and CD4 IF antibody validation described in Patel, S.S., et al. The microenvironmental niche in classic Hodgkin lymphoma is enriched for CTLA-4-positive T cells that are PD-1-negative. Blood 134, 2059-2069 (2019). PD-L1 antibody was validated and described in Ansell SM etal. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. NEJM 2015. CTLA-4, Ki67 and FOXP3 IF antibodies were validated by manufacturer. CTLA-4: https://www.cellsignal.com/products/primary-antibodies/ctla-4-e2v1z-rabbit-mab/53560; Ki67: https://www.agilent.com/en/product/immunohistochemistry/antibodies-controls/primary-antibodies/ki-67-antigen-(dako-omnis)-76239; FOXP3: https://www.cellsignal.com/products/primary-antibodies/foxp3-d2w8e-rabbit-mab/98377.

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	ClinicalTrials.gov identifiers: Study of Nivolumab in Patients With Classical Hodgkin's Lymphoma (Registrational) (CheckMate 205) (NCT02181738); Yuen et al. (Nature Medicine 26, 693–698 (2020)): IMvigor210: NCT02108652.
Study protocol	The studies have reported and the full protocols are available on clinicaltrials.gov.
Data collection	The full description of the data collection is detailed in the https://clinicaltrials.gov/ website and the corresponding publications. - CheckMate 205 (NCT02181738): Armand P, et al. J Clin Oncol. 36, 1428–1439 (2018) Ansell, S.M., et al. Blood Adv. (2023) - IMvigor210 trial cohorts (NCT02951767, NCT02108652): Rosenberg JE, et al. Lancet, 2016;387(10031):1909-1920. Balar AV, et al. Lancet, 2017;389(10064):67-76.
Outcomes	The full description of the data collection is detailed in the https://clinicaltrials.gov/ website. CheckMate 205 (NCT02181738): https://clinicaltrials.gov/study/NCT02181738 and IMvigor210 trial cohorts (NCT02951767, NCT02108652): https://clinicaltrials.gov/study/NCT02951767 and https://clinicaltrials.gov/study/NCT02108652.

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.