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: Microsoft Excel

Data analysis:

GraphPad Prism Version 10.1.0 (264)

Digital Pathology: Halo v3.4.
R version 4.2.2 with the following packages:

```
[1] viridis_0.6.2        viridisLite_0.4.1    lubridate_1.9.2
[4] forcats_1.0.0        stringr_1.5.0        purrr_1.0.1
[7] readr_2.1.4          tibble_3.2.1         tidyverse_2.0.0
[10] RColorBrewer_1.1-3   ComplexHeatmap_2.14.0 data.table_1.14.8
[13] EnhancedVolcano_1.16.0 ggrepel_0.9.3      ggsci_3.0.0
[16] SeuratObject_4.1.3   Seurat_4.3.0         tidyr_1.3.0
[19] Matrix_1.5-4         patchwork_1.1.2      CellChat_1.6.1
[22] ggplot2_3.4.2        igraph_1.4.2         dplyr_1.1.1
[25] NMF_0.26             bigmemory_4.6.1      Biobase_2.58.0
[28] BiocGenerics_0.44.0  cluster_2.1.4        rngtools_1.5.2
[31] registry_0.5-1       circlize_0.4.15      premessa_0.3.0
```

omiq
FlowJo (v 10.10.0)
Torrent Suite Software™ v5.8
Ion Reporter™ Analysis Software v5.16 with Oncomine™ Comprehensive Plus Assay w2.0 workflow

All code needed to reproduce the findings in this paper have been uploaded to figshare and is accessible at [https://doi.org/10.6084/m9.figshare.25747089.v3].

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

Source data are provided with this paper. TCR sequencing (Fig. 4, Fig. S3), mutation profiling (Fig. S4) and transcriptional (Fig. S5) raw data generated in this study have been deposited in the European Genome-Phenome Archive (EGA) under accession code EGAD50000001630 [https://ega-archive.org/datasets/EGAD50000001630]. The data are protected and available under restricted access according to the data security guidelines of the University of Basel. Access can be obtained by contacting med-dac@unibas.ch. The processed data are available at figshare [https://doi.org/10.6084/m9.figshare.25747089.v3].
Publicly available data from Lambrechts et al can be obtained from ArrayExpress (accession number E-MTAB-6149 [https://www.ebi.ac.uk/biostudies/ArrayExpress/studies/E-MTAB-6149]) and from the Gene Expression Omnibus database for Song et al (accession code GSE117570 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117570]), Wu et al (accession code GSE148071 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE148071]) and Liu et al (accession code GSE179994 [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE179994]).

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 were recruited irrespective of sex and gender. No specified sex or gender analyses were prespecified or performed. Women in child bearing age as well as non-sterilized men were obliged to use two separate contraceptive measures for the duration of the trial. Self-reported sex was used throughout the study.
Reporting on race, ethnicity, or other socially relevant groupings	No data on race, ethnicity or other socially relevant groupings were collected from participants.
Population characteristics	Characteristics of the study population were previously reported (Rothschild et al Journal of Clinical Oncology 2021, https://doi.org/10.1200/JCO.21.0027). Mean age was 61 years (range: 41-74 years). 52% of participants self-reported as male (35/67 participants), 48% as female (32/67 participants). At registration, 42% of participants were current smokers at registration, 54% former smokers and 5% never-smokers.
Recruitment	This investigator-initiated, open-label, multicenter single-arm, phase II trial was performed at 14 sites within the SAKK network in Switzerland. Patients that presented with stage IIIA(N2) NSCLC at one of these centers or referred from other centers of the SAKK network, and who were potentially eligible for this study were discussed at the local multidisciplinary tumor board. If patients were deemed resectable and operable they were informed about the aims and procedures of the trial as well as potential adverse events and potential hazards. The patients were informed verbally by the local primary investigator or a delegated subinvestigator and in writing by means of the patient informed consent form, which was reviewed and approved by the ethics committee together with the study protocol before the study was initiated. Study participants did not receive financial compensation. There is expected self-selection bias, due to inherent differences in motivation, health literacy and treatment tolernace in patients opting to participate in research studies compared to the general population. This might impact the general application of our findings in assessing response to similar treatment regiments in unselected patients groups. We look forward to studies with larger cohorts to address these questions.
Ethics oversight	The SAKK16/14 clinical trial was carried out in accordance with the most recent version of the Declaration of Helsinki. The study protocol is provided as Supplementary Data. Ethical approval was obtained from Swissmedic and the Ethics Commission for Northwest and Central Switzerland (Ethikkommission Nordwest- und Zentralschweiz, EKNZ).

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

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Sample size estimation was conducted in the R environment (v 3.2), using the FixDes function of the OptInterim package (v 3.0.1). Using the primary endpoint of EFS at 12 months, the null hypothesis was set to ≤ 48%, the alternative hypothesis to ≥ 65%, type I error to 0.05 and a power to 80%. Minimum sample size to evaluate the primary endpoint was thus calculated to be 64, and target sample size was defined to be 68 (assuming 5% of patients to be non-evaluable).
Data exclusions	Full Analysis Set (FAS): Among the 68 patients enrolled in this study, 67 fulfilled the FAS. One patient did not receive neoadjuvant chemotherapy because of brain metastasis detected in the PET/CT result provided three days after registration. Full Analysis Set - 2 (FAS-2): Of 67 patients fulfilling the FAS, 62 patients were evaluable for the FAS-2. The other five patients did not receive any dose of durvalumab. Safety Analysis: Sixty-seven out of 68 patients enrolled in the trial received at least one dose of neoadjuvant chemotherapy. Therefore, all of them were taken into account for the safety analysis.
Replication	Sample availability was limited. For example for multiple patients there was only a single cryoperserved vial of PBMCs or serum at a certain timepoint, on which the experiment needed to be performed. This as well as considering the large volume of single experiments with hundreds of samples, routine replication could not be achieved. We conducted technical pilot experiments in TCR sequencing applications and for some flow cytometry stainings and compared data from the same samples measured multiple times, finding good overlap.
Randomization	The SAKK 16/14 trial used a single-arm protocol. No patient randomization was conducted.
Blinding	The SAKK 16/14 trial used a single-arm protocol. Therefore, clinical investigators were not blinded. Statistical analysis was conducted centrally by the SAKK, not by staff at individual trial centers and not by staff involved in translational experiments.

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

CyTOF: CD45-106Cd (3106001B), CD45-110Cd (3110001B), CD45-111Cd (3111001B), CD45-112Cd (3112001B), CD45-113Cd (3113001B), CD45-114Cd (3141001B), CD45-116Cd (3116001B), CD19-142Nd (3142001B), CD127-143Nd (3143012B), CD69-144Nd (3144018B), IgD-146Nd (3146005B), CD11c-147Sm (3147008B), CD14-148Nd (3148010B), CD56-149Sm (3149021B), LAG-3-150Nd (3150030B), CD123-151Eu (3151001B), TCRgd-152Sm (3152008B), CD194-153Eu (3149029A), CD3-154Sm (3154003B), PD-1-155Gd (3155009B), CD27-158Gd (3158010B), TIM-3-159Tb (3159037B), CD28-160Gd (3160003B), CTLA4-161Dy (3161004B), FoxP3-162Dy (3162011A), CD294-163Dy (3163003B), CD161-164Dy (3164009B), CD45RO-165Ho (3165011B), CCR7-167Er (3167009A), CD8a-168Er (3168002B), CD25-169Tm (3169003B), CD45RA-170Er (3170010B), CXCR5-171Yb (3171014B), CD38-172Yb (3172007B), HLA-DR-173Yb (3173005B), CD4-174Yb (3174004B), CD57-176Yb (3176019B), CD16-209Bi (3209002B) (all Standard BioTools), Ki67 (350502), CD39 (328202), TCF-1 (655202) (all Biolegend), TOX (14-6502-82, invitrogen).

Aurora: CD56-BUV661 (BD, 750478), CD45-BUV496 (BD, 750179), CD3-BUV395 (BD, 564001), CD19-Spark NIR 685 (Biolegend, 302270), CD14-BUV563 (BD, 741360), HLA-DR-BV570 (Biolegend, 307637), CD4-APC (Biolegend, 300514), CD8-BV510 (Biolegend, 301048), C11c-BUV737 (BD, 741827), CD39-FITC (Biolegend, 328245), CD39-BV421 (Biolegend, 331526), CD57-PE (BD, 560844), PD-1-PE-Cy7 (BD, 561272), CD141-PerCP-Cy5.5 (Biolegend, 344112), CD1c-BV421 (Biolegend, 331526), CD123-PE-CF594 (BD, 562391), Ki-67-BV605 (Biolegend, 350522), Granzyme B-AF700 (BD, 372222), TIM-3-BV650 (Biolegend, 345028), CCR1-FITC (Biolegend, 362909).

IHC: CD4 (790-4423), CD3 (790-4341), CD8 (790-4460), CD20 (790-2531) (all Ventana/Roche Diagnostics), FoxP3 (abcam, ab99963).

See Supplementary Data - Antibody Panels for detailed information (clone, dilution).

Validation

CyTOF: Purchased Metal isotope pre-conjugated antibodies were all tested in multiple dilutions using samples from multiple healthy donors. Each new in-house preparation of metal-conjugated antibodies was tested in multiple dilutions and counterstained. To validate specificity of inhibitory receptor staining (PD-1, TIM-3, LAG-3, CD39), healthy donor PBMCs were pre-activated with agonistic anti-CD3/CD28 antibodies (pre-coating with 2 µg/ml anti-CD3 (clone: OKT3) and 2 µg/ml anti-CD28 (clone: CD28.2) for 2 h at 37°C, activation for 24 hours). To validate specificity of self-conjugated anti-transcription factor (TOX, TCF1) antibodies, we used counterstains against well established surface markers expressed by the same PBMC subsets (PD-1, TIM-3 for TOX, CD27, CD45RA for TCF1). Finally, we treated THP-1 monocytes with 1 µg/ml IFNγ for 24 hours to test staining with self-conjugated anti-PD-L2, and we stained an aliquot of these cells using an established flow antibody and acquired them with flow cytometry.

Aurora: All antibodies were purchased pre-conjugated from common vendors. All antibody clones were titrated over multiple concentrations before used on the same instrument the final sample was acquired on.

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, NCT02572843

Study protocol

The study protocol is made available as Supplementary Data.

Data collection

Details are provided in the study protocol. Biospecimen collection is outlined in Fig. 1. Follow-up visits were slotted one month after surgery, then every 3 months for 2 years, then every 6 months for years 3-5, and then every 12 months life-long. Tumor assessments were performed locally at the partner sites.

Outcomes

he primary endpoint was event-free survival at 12 months. Secondary endpoints were EFS, OS, objective response rate, pCR, MPR, rate of nodal downstaging, R0 resection, pattern of recurrence, adverse events and postoperative 30-day mortality.

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.

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

Sample preparation is described in the Methods section. Briefly, PBMCs were isolated with density centrifugation and cryopreserved. Samples were then thawed, Fc blocked and stained with a fixable live/dead dye in PBS. Following washing, surface stain was performed in FACS buffer (PBS with 2% FBS, 2 mM EDTA and 0.5% sodium azide). After washing, cells were permeabilized with FoxP3 Fix/Perm kit, followed by intranuclear staining in permeabilization buffer.

Instrument	All samples were acquired on a Cytex Aurora instrument in a five laser configuration. Unstained and live/dead cell reference controls were prepared, alongside CompBeads (BD) conjugated to the same antibodies used in the staining.
Software	Data was acquired with Cytek Spectroflow software. Data was exported as FCS files and analyzed using FlowJo (v 10.10.0).
Cell population abundance	In graphs indicating cell population abundance as measured by flow cytometry, the parent gate is indicated above the graph. The y axis shows the the abundance of the indicated cell population within the parent gate (for example, parent gate: CD39+ PD-1+ CD8+ T cells, y axis: %Ki67+).
Gating strategy	Shown in Supplementary Fig. 8a. FSC/SSC gating was used to gate on lymphocytes and on myeloid cells. FSC-A/FSC-H and SSC-A/SSC-H were used to exclude doublets, followed by exclusion of dead cells, and exclusion of CD45 negative cells. Only CD45+, single live cells were taken forward. For this cell pool, CD3 and CD19 were used to identify T and B cells, respectively. Within T cells, CD4 and CD8 were gated before any further analysis. Cut-offs for PD-1, CD39, CD57 and Ki67 are shown in the Figure. From CD3 CD19 double negative cells, CD14 and CD56 were used to identify monocytes and NK cells, respectively. Cells also negative for these two markers were considerec lineage (Lin) negative. From Lin negative cells, CD123 was used to exclude pDC, followed by identification of cDC by HLA-DR and CD11c staining. Subsets of cDC were identified by CD141 and CD1c were identified within that gate. TIM-3 levels were only assessed with geometric mean fluorescent intensity.

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