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	TENALEA clinical trial data management system
Data analysis	R version 4.2.2 SAS version 9.4 Whole-Transcriptome Sequencing - Salmon (v1.10.0) – for transcript abundance estimation - hg38.p13 and Gencode v42 – reference genome annotations - DESeq2 – for count normalization and log2 transformation - Singscore – for gene set scoring - MSigDB – for hallmark gene sets - Ayers et al. – for interferon-γ signature reference - Custom marker genes – for immune cell subtype abundance estimation Whole-Exome Sequencing - seqpurge – for adapter trimming - bwa (v0.7.17) – for read alignment to GRCh38 reference genome - rumidup (https://github.com/NKI-GCF/rumidup) – for UMI-aware duplicate marking - Genome Analysis Toolkit (GATK, v4.3) – for somatic variant calling using Mutect2

- FACETS – for tumor purity and ploidy estimation
- Freebayes (v1.3.6) – for BRCA1/2 germline variant calling
- CNVkit (v0.9.10) – for copy number variation analysis
- Popova et al. method – for homologous recombination deficiency classification

ctDNA Analysis
- AVENIO ctDNA Expanded Kit V2 (Roche) – for plasma sequencing
- Bio-Rad PrimePCR Mutation Detection Design tool – for ddPCR assay design
- QX600 Droplet Digital PCR System (Bio-Rad) – for ddPCR analysis
- CLSI EP17 protocol – for limit of blank determination
- GraphPad Prism 9.3.0

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 study protocol and statistical analysis plan are available in Supplementary Note 1 and 2. The RNA and DNA data generated in this study have been deposited in the European Genome-Phenome Archive under accession code EGAS50000000781 [https://ega-archive.org/studies/EGAS50000000781]. Both genomic data and deidentified clinical data are available under restricted access for academic use, within the limitations of the provided informed consent and under General Data Protection Regulation law. Requests for access to genomic data can be submitted via https://ega.nki.nl/, while requests for clinical data should be sent to repository@nki.nl. All data requests will be reviewed by the NKI Institutional Review Board (IRB) and must be supported by the Principal Investigator of the study. The researcher will need to sign a data access agreement with the NKI after approval. Estimated time to response is 2–4 weeks. All remaining data can be found in the Article, Supplementary and Source data files.

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	The study population consists of female with ovarian cancer. Patients were included regardless of gender. The study protocol did not include pre-specified gender-based analysis.
Reporting on race, ethnicity, or other socially relevant groupings	Race, ethnicity, or other socially relevant groupings were not reported in this manuscript.
Population characteristics	Patients aged ≥ 18 years were eligible for the study if they had histologically confirmed FIGO stage IV epithelial ovarian, fallopian tube, or peritoneal cancer and were willing and able to provide three pre-treatment tumor biopsies from a metastatic site. Other key inclusion criteria included a WHO performance status score of 0–1, normal blood counts, and adequate organ function. Patients with immunosuppressive treatment, immunodeficiency, human immunodeficiency virus, hepatitis B or C infection, active autoimmune disease, or active other malignancy were ineligible. All patients provided written informed consent before enrolment.
Recruitment	Patients that presented with initial diagnosis of advanced ovarian cancer, either at the Netherlands Cancer Intitute or referred from other centers, and who were potentially eligible for this study were informed of the study. Patients who were deemed eli
Ethics oversight	The protocol was approved by the local ethics committee at the Netherlands Cancer Institute. All patients provided written informed consent prior to enrolment. This study was performed in accordance with the Declaration of Helsinki.

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	For this exploratory phase II study, no formal sample size calculation was performed. The study aimed to recruit 30 patients to guarantee enough and timely accrual as well as evaluation of the immune-activating capacity of the treatment.
Data exclusions	One patient received one cycle of chemotherapy and discontinued treatment (and the study) before receiving pembrolizumab. This patient was excluded in the analysis, which is described in the results section of the manuscript.
Replication	Replication is not applicable for clinical data. Translational experiments on human samples were not replicated due to limited material.
Randomization	Not applicable, no randomization was performed.
Blinding	No blinding was performed as this was a single-arm study. Radiologists and pathologists were blinded for survival outcomes when assessing imaging and pathologic response,

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:
PD-1, CAL20 clone, 1/250 dilution, catalogno: ab237728, Lotno: GR3361014, Abcam
CD8, C8/144B clone, 1/200 dilution, catalogno: M710301-2, Lotno: 41527763, Agilent (DAKO)
PD-L1, 22C3 clone, 1/40 dilution, catalogno:M365329-2, Lotno: 11508061, Agilent (DAKO)

Multiplexed immunofluorescence:
CD20, L26 clone,1/500 dilution, catalogno: M0755, Lotno: 20083766, Agilent (DAKO)
CD3, SP7 clone, 1/400 dilution, catalogno: RM-9107-5, Lotno: 9107S2006A, Thermo Scientific
CD8, C8/144B clone, 1/100 dilution, catalogno: M7103, Lotbo: 41311427, Agilent (DAKO)
CD68, KP1 clone, 1/300 dilution, catalogno: M0814, Lotno:41345911, Agilent (DAKO)
FOXP3, 236A/E7 clone, 1/50 dilution, catalogno: ab20034, Lotno: GR3373016-1, Abcam
PanCK, AE1AE3 clone, 1/100 dilution, catalogno: ab27988, Lotno: GR3334419-5, Abcam

Validation

Each IHC protocol has been developed and validated under standard operating procedures in a pathology lab. Each new antibody lot is validated by testing multiple dilutions and evaluating them with the pathologist in a standardized method, using positive control tissues suitable for the antibody (images and protocol details available upon request).

Each Multiplex protocol has been developed and validated under standard operating procedures in a pathology lab. Each new antibody lot is validated by testing multiple dilutions using the correct fluorescence dye and evaluating them with the pathologist in a standardized method, using positive control tissues suitable for the antibody (images and protocol details available upon request). The antibodies have also been tested and evaluated in the full multiplex panel.

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: NCT03126812
Study protocol	The study protocol will be made available upon request and will be uploaded together with initial submission of the manuscript.
Data collection	All patients with stage IV HGSOC were screened. Patients were consulted in the outpatient clinic of the Netherlands Cancer Institute. Long-term follow-up was either performed at the outpatient clinic, by telephone, or through telemedicine. Clinical data, including data regarding adverse events, were collected from time of informed consent until 90 days after the last administration of the study drug. Long-term survival data will be collected until five years after initial diagnosis. The first patient was enrolled on December 21, 2017 and the last patient on May 2, 2022. Data cutoff for the current analysis was April 1, 2024.
Outcomes	Primary endpoint of the present study is: • expression and changes in expression of CD3+, CD8+, FOXP3+ regulatory T cells, CD68+ macrophages, CD20+ B cells and CD8/ FOXP3 ratio in pre-treatment and on-treatment tumor samples by multiplex immunofluorescence (MIF) and comparison between minor and major pathologic responders; • expression and changes in inflammation signatures, immune checkpoints, immune suppressive pathways and IFN-induced gene expression in pre-treatment and on-treatment tumor samples by whole transcriptome sequencing and comparison between minor and major pathologic responders. Secondary endpoints are: • AEs, as characterized by type, frequency, severity (as graded using CTCAE version, v4.03), seriousness, and relation to study therapy AEs will be graded by the investigator according to the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. Treatment related AEs (TRAEs) are defined as those with initial onset or increasing in severity after the first dose of study medication and are related to study medication and will be categorized into immune-related AEs, chemotherapy-related AEs and surgery-related AEs. Endpoints include maximum toxicity and relation to treatment. Details on safety endpoints are provided in Section 7.5. The Safety Set will be used for the analysis of AEs. • Best overall response based on RECIST 1.1 The best overall response is the best response recorded from the start of the study treatment until the end of neoadjuvant treatment according to RECIST v1.18. A CT scan is done after two cycles of chemotherapy in all patients. In case of prolongation of neoadjuvant chemotherapy to > 3 cycles, a CT scan is repeated after 4 or 6 cycles. For a patient to be classified as a complete responder according to RECIST, CA-125 tumor marker levels must be within the normal range. Analysis of radiological response will be performed on the FAS. • The ability to perform a complete cytoreduction as well as the pathologic major response rate (no viable invasive tumor left in the resection specimen) The proportion of patients in whom a complete cytoreduction was achieved (CC0, no residual disease), as well as a summary of the other surgical results (CC1, <2.5mm residual disease; CC2a, 2.5-10mm; CC2b >10mm, no resection, no surgery) Pathological tumor response based on the Chemotherapy Response Score (CRS), as identified on routine hematoxylin and eosin (H&E) staining. Major pathologic responders were defined as CRS 3. Since the study by Böhm et al.9 did not show a clear prognostic separation of CRS 1 and CRS 2, minor pathologic responders were defined as either CRS 1 or CRS 2. Analysis of this endpoint will be performed on the FAS. • Feasibility The percentage of patients who completed the standard treatment according to protocol (6 cycles of chemotherapy and interval cytoreductive surgery) and the percentage of patients who completed the experimental treatment according to protocol (neo-adjuvant, adjuvant, and maintenance pembrolizumab). In addition, the relative dose intensity (RDI) of the chemotherapy and pembrolizumab will be assessed, calculated as follows: RDI=DDI/SDI With DDI = delivered dose intensity = delivered dose/actual number of days to complete dose, and SDI = standard dose intensity = standard total dose/standard number of days to complete dose. For DDI, the duration of a missing cycle will be imputed by the standard duration as per protocol (21 days). For SDI, the standard dose for a missing cycle will be imputed from available cycles using a last-observation-carried-forward approach. Analysis of this endpoint will be performed on the FAS. • Progression free survival (PFS) and Overall survival (OS) Definition of PFS: Time from start chemotherapy to tumor progression or death from any cause, whichever occurs first. Disease recurrence or progression was confirmed by imaging (according to RECIST version 1.1) or elevated CA-125 concentrations, according to the Gynecologic Cancer InterGroup (GCIG) criteria. If none of these events are observed, PFS will be censored at date of last contact. The date of last contact will be obtained from the medical records of the Netherlands Cancer Institute and will be either physical or telephone consultations.

Definition of OS: Time from start chemotherapy to death from any cause. If no death is observed, OS will be censored at the date when the patient was last known to be alive. Survival status will be obtained from medical records or requested from the general practitioner or the National Death Registry if not available in the medical record.
A survival sweep will be performed at the time of data analysis.

Analysis of this endpoint will be performed on the FAS.

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.