nature portfolio

Corresponding author(s): Prof. dr. Hans W. Nijman

Last updated by author(s): Jun 14, 2024

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	We utilized Research Electronic Data Capture (REDCap) software, version 13.7.19, for data collection in our study. REDCap is a secure, web-based application designed to support data capture for research studies.
Data analysis	We performed data analysis using a combination of software tools, including GraphPad Prism (version 9.1.0), R (version 4.3.2), the immunoSEQ analyzer of Adaptive Biotechnologies (version 3.0), and the GeoMx Spatial Data Analysis Service. GraphPad Prism was used for statistical analysis and visualization of experimental results, while R was used for advanced single-cell analysis and data visualization. The ImmunoSEQ analyzer and GeoMX Spatial Data Analysis Service were used to pre-analyze the TCR- and spatial transcriptomic data respectively before moving it into R for a more in-depth analysis.

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 data generated in this study may contain personally identifying information are thus not openly available under current (GDPR) guidelines. Data are available from the corresponding author upon reasonable request. Data are located in controlled access data storage at University Medical Center Groningen, The Netherlands.

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 trial reported in this work focussed on the treatment of uterine cancer, which predominantly affects individuals assigned female at birth. Due to the relative rareness of uterine cancer in individuals assigned male at birth, these were not represented in our current pilot trial and we strive to increase inclusivity and equitable representation across diverse gender identities within follow-up trials. Nevertheless, our study's findings contribute valuable insights into the efficacy and safety of Pembrolizumab in individuals with uterine cancer, while underscoring the need for broader considerations of sex and gender in future studies.
Reporting on race, ethnicity, or other socially relevant groupings	We collected data on ethnicity as reported by the patients themselves. This decision was supported by the ethical board due to the recognized potential for differences in treatment response based on ethnic background.
Population characteristics	Population characteristics can be found in Table 1 of the manuscript
Recruitment	Recruitment procedures were designed to ensure thorough patient information and minimize biases. The recruitment process comprised the following steps: Initial Screening by Gynaecologic Oncologist: Patients diagnosed with uterine cancer were initially screened by their gynecologic oncologist. Patients who met the initial eligibility criteria and expressed interest in learning more about the clinical trial were considered for further evaluation. Patient Information Sheet Distribution: Eligible patients were provided with a patient information sheet detailing the study's purpose, procedures, potential risks and benefits, and their rights as participants. This information sheet served as a comprehensive resource for patients to understand the study requirements and implications. The information sheet was available in Dutch and English, and available for translation upon request. Informational Consultation with Research Physician and Medical Oncologist: Patients who expressed continued interest in participating in the trial underwent an informational consultation with our research physician and medical oncologist. During this consultation, patients received additional information about the study protocol, treatment procedures, and potential outcomes. The presence of both a research physician and a medical oncologist ensured that patients received comprehensive information from specialists representing different disciplines. Informed Decision Making: Following the informational consultation, patients were encouraged to take sufficient time to consider all aspects of the study before deciding to participate. Participation was entirely voluntary, and patients were assured that they could decline participation or withdraw from the study at any time without repercussions. Patients were also provided with contact information for the study team to address any further questions or concerns. Minimizing Bias: To minimize the potential for self-selection bias or other biases influencing participation, our recruitment process involved independent specialists from gynecologic oncology and medical oncology. This multidisciplinary approach aimed to provide patients with comprehensive and unbiased information to facilitate informed decision-making regarding study participation.
Ethics oversight	The trial was discussed and approved by the medical ethical board of the University Medical Center Groningen and subsequently, a no-objection statement was provided by the Dutch Central Committee on Research Involving Human Subjects in the role of Competent Authority.

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

Field-specific reporting

Life sciences study design

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

Sample size	The feasibility study aimed to recruit a total of 10 dMMR and 10 POLE-EDM patients. With a sample size of 10 for each group, we estimated that the hypothesized objective rate of 50% pathologic responses could be established to within a 95% confidence interval of +/- 31%, sufficient for further exploring pembrolizumab in follow-up trials. Due to a failure to identify and recruit patients, the POLE-EDM arm was closed prematurely and the study was terminated after the inclusion of 10 dMMR patients.
Data exclusions	For some patients, not all study data could be collected (tumour material, MRI scans) due to either human error or COVID restrictions during the period of the trial. Therefore, these tissues / time points are not incorporated in the analysis. Where applicable, this is reported in a fully transparent manner within the manuscript.
Replication	To ensure the reproducibility of our experimental findings, we implemented the following measures: We meticulously documented the methodology in our manuscript, providing comprehensive details on the various experiments conducted. When necessary, we supplemented our descriptions with references to pertinent literature or external manuals. All experiments were conducted following standardized operating procedures (SOPs), which were established beforehand to minimize variations between experiments. Prior to use in patient material, all reagents and assays underwent rigorous validation procedures to ensure their reliability and accuracy. We have made our raw data, analyses, and experimental protocols openly accessible where possible. Interested parties can access these materials through open access channels, or by contacting the corresponding author for reasonable requests.
Randomization	Randomization was not implemented in our clinical trial due to its design as an open-label, phase-I study.
Blinding	Blinding was not utilized in our clinical trial owing to its nature as an open-label, phase-I study.

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	We provide the antibodies in the following order: Fluorochrome; Antigen; Clone; Catalog no; Lot no; Company; Dilution. BV605; CD45; HI30; 304042; B339820; Biolegend; 3 µl / 10^6 cells APC-CY7; CD8; SK1; 344714; B341619; Biolegend; 3 µl / 10^6 cells PE; CD4; OKT4; 12-0048-42; 2054907; Invitrogen; 3 µl / 10^6 cells BV421; CD19; HIB19; 302234; B342418; Biolegend; 3 µl / 10^6 cells APC; PD-1; EH12.2H7; 329908; B334074; Biolegend; 3 µl / 10^6 cells PE-CY7; CD3; HIT3a; 300316; B317797; Biolegend; 3 µl / 10^6 cells FITC; CD14; HCD14; 325604; B302821; Biolegend; 3 µl / 10^6 cells FITC; CD56; HCD56; 318304; B268830; Biolegend; 3 µl / 10^6 cells FITC; Epcam; 9C4; 324204; B327432; Biolegend; 3 µl / 10^6 cells PerCP-Cy5.5; CXCR5; J252D4; 356910; B345921; Biolegend; 3 µl / 10^6 cells
Validation	All antibody clones have been extensively validated for target specificity in previous work (see also manufacturors sites). Additionally, all antibodies underwent testing in our own laboratory on previously identified positive samples to confirm specificity.

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	NCT04262089
Study protocol	The study protocol can be accessed on reasonable request by contacting the corresponding author.
Data collection	The investigator was responsible for maintaining adequate records to enable the conduct of the study to be fully documented. This included: • The IMPD, signed protocol, and amendments • Signed and dated informed consents per institutional policy • Signed, dated, and completed CRFs, and documentation of CRF corrections • Notification of SAEs and related reports • All study drug dispensing and accountability logs • Shipping records of investigational product and trial-related materials • Dated and documented IRB/IEC protocol approvals, and all correspondence between the investigator and the IRB/IEC • Normal laboratory values and laboratory certifications • Curricula vitae and current medical licenses for principal investigator and all sub-investigators • Case report forms (CRFs) • Source documents Recruitment of study participants took place between 16-12-2020 and 01-09-2022. After inclusion of the 12th PAM-patient recruitment stopped. Data collection took place between the inclusion of the first patient (16-12-2020) up until the cut-off point for follow-up (01-11-2023). The data were collected at the University Medical Center Groningen (UMCG), located in Groningen, The Netherlands. If necessary, additional data were gathered from collaborating community health centers in the surrounding region. Specifically, information was obtained from the pathology laboratory, clinical chemistry, oncology laboratory, medical imaging center, surgery room, and the outpatient clinics of gynecological oncology and medical oncology all available in the Electronic Patient Dossier of the UMCG. Data was coded and handled confidentially. Data in the database were coded with a subject identification code to handle the data anonymously and to protect the subject's privacy. The codes were not related to an individual and the key to the code was safeguarded by the coordinating investigator and the principal investigator. The originals of all source documents are stored for a period of 15 years after the study has been finalized and all study-related documents are archived on a UMCG server for 15 years. The handling of data in this study was in agreement with the Dutch Personal Data Protection Act (in Dutch:wet Algemene verordening gegevensbescherming (AVG).) The anonymized patient data collected during this study was entered into a Clinical Trial Database Management System (CTDMS). For this study, REDCap was used. The database was a secure web platform for building and managing online databases and surveys. Access to the database was secured via a username/password combination. Users were only given access to the database at the request of the principal investigator, or a delegate. REDcap was fully GCP and GDPR-compliant. After the study ended, the database was locked. For each patient, a Patient Data Report was created including notes, discrepancies, and the audit trail, and was sent to the principal investigator. Also, a copy of the collected data was made available to the investigator. The database was kept available for as long as the statutory period determined. Just before the statutory period ended, the principal investigator or his/her department was notified that the data would be deleted., a copy of the collected data will be made available to the investigator. The database will be kept available for as long as the statutory period determines. Just before the statutory period ends, the principal investigator or his/her department will be notified that the data will be deleted.
Outcomes	The primary objective was to obtain an efficacy estimate of 2 cycles of neo-adjuvant pembrolizumab in primary dMMR uterine cancer. This estimate would have been used to inform follow-up multicenter trials involving postponement of standard-of-care. Hereto, objective response rates were determined using pathology to assess the percentage of residual viable tumor from the surgical specimen. The secondary endpoint was to assess the objective response rate of the tumor by MRI using RECIST1.1 in uterine cancer patients treated with 2 cycles of neo-adjuvant pembrolizumab. Exploratory parameters were: 1. To assess safety of neo-adjuvant pembrolizumab in the treatment of dMMR UC 2. To assess whether a pipelle biopsy could be used as a predictor for tumor response to neo-adjuvant pembrolizumab. Pathologic response assessment was predefined as: Hematoxylin and eosin staining on endometrium tissue collected during standard-of-care hysterectomy was assessed by an experienced pathologist for • Evidence of necrosis and/or viable tumor cells. • Degree of immune infiltration, including T-cell density and proliferation markers. • Degrees of inflammation, fibrosis, and mucin, consistent with an ongoing immune response.

In order to assess the correlation of pathological response seen in the biopsy and the surgical specimen. A pipelle biopsy was obtained during surgery and assessed as the tumor specimen from hysterectomy. At the time of assessment by the pathologist, he or she was blinded for any study-related information.

Radiologic responses were predefined as:
In order to assess responses, MRI was performed at baseline (week 5) and was compared to MRI performed after the second cycle of pembrolizumab (week 10).

Tumor response was classified according to a RECIST 1.1.
• Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) had a reduction in the short axis to <10 mm.
• Partial Response (PR): At least a 30% decrease in the sum of diameters of target lesions, taking as reference the baseline sum diameters.
• Progressive Disease (PD): At least a 20% increase in the sum of diameters of target lesions, taking as reference the smallest-sum-on-study (this included the baseline sum if that was the smallest on study). In addition to the relative increase of 20%, the sum also demonstrated an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions was also considered progression).
• Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.
In order to assess systemic immune responses induced by pembrolizumab: a vena puncture to obtain 60 mL blood for PBMC preparation was performed prior to administration of pembrolizumab (visits 2), during pembrolizumab treatment (visit 3), after pembrolizumab treatment (visit 4) and twice during follow up at week 20 and 32.

Safety and toxicity of pembrolizumab were monitored using GCP guidelines.

Recording of concurrent illness/therapy and adverse events (AE):
All AEs were followed until they abated, or until a stable situation was reached.
All AEs from the first study treatment to 30 days after the planned surgery. AEs occurring later than this were only documented if a relationship to the study drug was suspected.

For the proposed trial and in accordance with NCI CTCAE5, an adverse event was defined as any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which did not necessarily have a causal relationship with this treatment. An abnormal objective finding was reported as an AE when one of the following criteria was met:
• The finding was associated with clinically significant symptoms.
• The finding led to a change in the study dosing or discontinuation from the clinical trial and/or significant additional concomitant drug treatment or other therapy.
• The finding lead to any of the outcomes included in the definition of an SAE
• The finding was considered to be clinically relevant by the investigator

Treatment-related surgical postponements
The number of treatment-related surgical postponements were counted to assess feasibility. A treatment-related delay was defined as all delays over 30 days caused by treatment-related adverse events which occurred during the trial.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A

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	Tumour digests were thawed in FCS and resuspended in RPMI + 10% FCS. After 1.5h incubation at 37°C/5% CO2, samples were stained and incubated with a CXCR5 PerCP-Cy5.5 antibody for an additional 0.5h at 37°C. After a total of 2h recovery at 37°C/5% CO2, samples were centrifuged and resuspended in 100 µl PBS + 2% FCS. Samples were incubated for 30-45 minutes on ice (in the dark) with the following antibodies: CD45 BV605, CD8 APC Cy7, CD4 PE, CD19 BV421, PD1 APC, CD3 PE-Cy7, and the dump channel antibodies CD14 FITC, CD56 FITC and EpCAM FITC. 3 µl of each antibody was used per 1x10e6 cells. Samples were washed twice with PBS + 2% FCS and filtered using a 35 µm strainer (Falcon) before sorting on a Beckman Coulter MoFlo Astrios cytometer.
Instrument	Sorting was performed on a Beckman Coulter MoFlo Astrios cytometer. UltraComp eBeads (Thermo Fisher Scientific) were used as compensation controls.
Software	FlowJo (Version 10.8.1) [Computer Software]. Ashland, Oregon: FlowJo, LLC; 2023 was used to visualize the flow and sorting data
Cell population abundance	CD4: +/- 23% of CD3+-CD45+ cells; CD8: 35% CD3+-CD45+ cells
Gating strategy	CD45+ CD3+ CD19- CD8+ and CD4+ cells were sorted.

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