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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	No code was used for data collection.
Data analysis	Somatic mutations in WES data were detected by an implementation of the Cancer Genome Analysis WES Characterization Pipeline (as implemented in Braun et al, Nature Med, 2020; hereafter, referred to as the CGA pipeline) in a cloud-based analysis platform, Terra (https://terra.bio/). We utilized version 0.2 of the CGA pipeline. As QC steps, the CGA pipeline ran deTiN (v1.8.5) and ContEst for estimating tumor-in-normal and cross-patient contaminations, respectively. The pipeline applies multiple artifact filters such as the read-realignment filter by BLAT and the read orientation bias filters followed by SNV and indel calling with MuTect and Strelka. All somatic alterations were annotated using Oncotator. Tumor ploidy and purity was determined using ABSOLUTE, and this was used to assign clonality (cancer cell fraction; CCF) for each somatic mutation. RNA-seq data were aligned to the human reference genome (hg19) and transcriptome (GENCODE v19) using STAR (v2.6.1), and expression was quantified (transcripts per million; TPM) using RSEM (v1.3.1). All coding mutations were visualized using the Integrated Genomics Viewer (IGV v2). Antigen prediction was performed using HLAthena and NetMHCpan version 4 (eluted ligand rank). Flow cytometry data were acquired on a BD LSR Fortessa with FACSDiva and analyzed with Flow Jo (version 10.8.1). For scRNA-seq, the 10x Cell Ranger pipeline (version 6.1.2) was utilized for data demultiplexing, data alignment and count quantification, and TCR clonotype assembly. Single-cell RNA sequencing data were imported and read using a custom function built upon the Seurat 4.3.0 pipeline. doubletFinder (version 2.0.3), scDblFinder (version 1.12.0) were used for doublet detection. Harmony 0.1.1 was used for batch correction. We use clustree 0.5.0 for optimal clustering resolution. Heatmapps were generated using pheatmap v1.0.12. For TCR diversity and clonotype analysis, we utilized the R package VDJdive version 1.3.5 and scRepertoire (v1.8.0). For bulk TCR processing from PBMCs, code is available on GitHub at: https://github.com/Wu-Lab-DFCI-Harvard/bulkrhTCR_Script. Disease-free survival is reported as the time (in months) of disease recurrence or death from the start of treatment. Analysis and

visualizations were generated using the R packages "survival" (v.3.5.5) and "survminer" (v0.4.9). The Kaplan-Meier curves for KEYNOTE-564 and IMmotion-010 were reconstructed using DigitizeIt software (v2.5.9) together with the "IPDfromKM" package in R (v0.1.10).

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 list of somatic mutations for all patients (mutation annotation file) is available in Table S1. The IFNγ ELISpot responses for ex vivo peptide pool stimulation, in vitro individual peptide stimulation, and autologous tumor testing are available in Table S2. The median normalized protein expression (NPX) values of circulating soluble proteins is available in Table S3. The cell numbers and T cell clonotype metrics from scRNA-seq and TCR-seq of skin samples is available in Table S4. The T cell clonotype metrics for tumor-infiltrating T cells and for peripheral T cells are available in Table S5. The individual T cell clonotypes and their corresponding phenotypes are available in Table S6. The reference genome (hg19) and transcriptome (GENCODE v19) are publicly available (gs://firecloud-tcga-open-access/tutorial/reference/annotation.db.ucsc.hg19.tar and gs://firecloud-tcga-open-access/tutorial/reference/rna-seq/gencode.v19.genes.v7_model.patched_contigs.gtf, respectively). All raw DNA are RNA sequencing files for the tumor samples are deposited in dbGaP (phs003710.v1.p1).

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	Self-reported sex was described for all study participants. Of the 9 patients in the current study, 7 were self-reported as males and 2 as females. This roughly reflects the increased prevalence of renal cell carcinoma among men.
Reporting on race, ethnicity, or other socially relevant groupings	Self-reported race and ethnicity were collected as part of the medical record.
Population characteristics	Age, demographic information, and tumor characteristics were collected from the medical records. Age, performance status, tumor histology, stage, grade, and size were reported. Age: median 65.5 years, range 50.4 - 75.7 years. Sex: 22% female, 78% male. ECOG performance status: 78% 0, 22% 1 Tumor histology: 100% clear cell Stage: 78% stage III, 22% stage IV ISUP grade: 11% grade 2, 89% grade 3 Primary tumor size: 44% 4-7cm, 33% 7-10cm, 22% > 10cm Prior treatment: none
Recruitment	All potentially eligible subjects (renal mass, clinical T3 or T4 with plan for resection, no obvious exclusions) seen at the Dana-Farber Cancer Institute medical oncology clinic by participating oncologists, or at the Brigham and Women's Hospital urology clinic by participating urologists, were identified and provided an opportunity to consent and screen for the trial. Study eligibility included age >= 18, ECOG performance status <= 1, suspected stage III or IV clear cell renal cell carcinoma (at initial registration; confirmed stage III or IV clear cell renal cell carcinoma at secondary registration), undergoing curative intent surgery (nephrectomy and/or metastasectomy to no evidence of disease), no active autoimmune disease, and no prior therapy with immunomodulatory agents. A potential source of bias is that patients who were not seen by participating medical oncologists and urologists would not participate in the trial.
Ethics oversight	Dana-Farber / Harvard Cancer Center (DF/HCC) Institutional Review Board.

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	This was a phase I study of safety and tolerability of this vaccination strategy in renal cell carcinoma. The initial cohort size of patients treated with vaccine + ipilimumab was designed to identify dose-limiting toxicity (DLT). If the true but unknown rate of dose-limiting toxicity is low, i.e., 10%, the probability of observing either no DLTs or a single DLT in 5 patients is 0.92. The sample. With the study design, observing more than a single DLT was unacceptable. Additionally, sample size was designed to identify toxicities of out interest. For 5 patients treated with vaccine plus ipilimumab, if the true but known probability of toxicity is 0.1, 0.2, 0.3, 0.4, or 0.5, the probability of observing it in at least 1 of 5 patients would be 0.41, 0.67, 0.83, 0.92, or 0.97, respectively. For the secondary endpoint of induction of IFNg T cell response, we estimated that there would be approximately 100 peptides among patients treated with vaccine plus ipilimumab, and at least 40 patients treated with vaccine alone. Then, using a Fischer exact test, and assuming a low rate of 5% CD8+ response in the vaccine alone group, we would have 90% power to detect a 25% increase in CD8+ T cell response. All sample size calculations for the original clinical trial are provided in the protocol, as part of Supplementary Information.
Data exclusions	The current studies reports on the first 9 patients treated on study with sufficient follow-up (n = 5 treated with vaccination plus ipilimumab, and n = 4 with vaccination alone). As noted in the methods, a third cohort (vaccination plus a higher dose of ipilimumab) was accrued until December 2022 with follow-up ongoing, and results of that immature cohort are not reported here.
Replication	This study used exclusively primary human material from participants in the trial, and so replication was not typically performed. For flow cytometry, Olink analysis, and all sequencing studies (single-cell RNA-sequencing, TCR sequencing), no replication was performed. For the IFNg ELISpot, we performed technical replicates (triplicate). Results from each replicate is provided in supplementary table S2.
Randomization	There was no randomization in this phase I clinical study. Specifically, randomization is not applicable to this individual cohort, phase I clinical trial. Of note, there is formal comparison between arms in the main figures of this study (and therefore, no adjustment for covariates).
Blinding	There was no blinding in this phase I clinical study, as there was no randomization. Investigators performing the flow cytometry, IFNg ELISpots, and single-cell analysis directly were blinded to clinical outcomes.

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	All flow cytometry antibodies were purchased from BD Biosciences or BioLegend: CD3 (clone UCHT1, APC-Cy7, Biolegend cat#300426) - dilution 1:20 per million PBMC/100uL. CD4 (clone L200, PerCP-Cy5.5, BD Biosciences cat#552838) - dilution 1:5 per million PBMC/100uL. CD8 (clone RPA-T8, AlexaFluor-488, BD Biosciences cat#557696) - dilution 1:20 per million PBMC/100uL. CD45RO (clone UCHL1, PE, BD Biosciences cat#555493) - dilution 1:5 per million PBMC/100uL. PD-1 (clone EH12.2H7, BrilliantViolet 785, Biolegend cat#329930) - dilution 1:40 per million PBMC/100uL. IFN-gamma (clone B27, APC-R700, BD Biosciences cat#564981) - dilution 1:20 per million PBMC/100uL. TNF (clone MAb11, PE-Cy7, BD Biosciences cat#560923) - dilution 1:20 per million PBMC/100uL. IL-2 (clone MQ1-17H12, BrilliantViolet 605, BD Biosciences cat#564165) - dilution 1:20 per million PBMC/100uL. For ELISpot, antibodies were purchased from Mabtech: For capture (primary antibody), plates were coated with IFN-gamma antibody (Mabtech, clone 1-D1K, cat#3420-3-250) - dilution 1:500. For detection (secondary antibody), anti-human IFN-gamma antibody was used (Mabtech, clone 7-BG-1 biotin, cat#3420-6-250) - dilution 1:1000
Validation	All antibodies used in the manuscript were purchased from reputable commercial vendors, and all validation studies were performed by the commercial vendors themselves. Additional information on validation can be found on the manufacturers' websites, listed below.

All flow cytometry antibodies were purchased from BD Biosciences or BioLegend:
CD3 (clone UCHT1, APC-Cy7, Biolegend cat#300426) - https://www.biolegend.com/en-gb/clone-search/apc-cyanine7-anti-human-cd3-antibody-3929?GroupID=BLG5900
CD4 (clone L200, PerCP-Cy5.5, BD Biosciences cat#552838) - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/percp-cy-5-5-mouse-anti-human-cd4.552838
CD8 (clone RPA-T8, AlexaFluor-488, BD Biosciences cat#557696) - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/alexa-fluor-488-mouse-anti-human-cd8.557696
CD45RO (clone UCHL1, PE, BD Biosciences cat#555493) - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-mouse-anti-human-cd45ro.555493
PD-1 (clone EH12.2H7, BrilliantViolet 785, Biolegend cat#329930) - https://www.biolegend.com/nl-nl/products/brilliant-violet-785-anti-human-cd279-pd-1-antibody-7980
IFN-gamma (clone B27, APC-R700, BD Biosciences cat#564981) - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/apc-r700-mouse-anti-human-ifn.564981
TNF (clone MAb11, PE-Cy7, BD Biosciences cat#560923) - https://www.bdbiosciences.com/en-eu/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/pe-cy-7-mouse-anti-human-tnf.560923
IL-2 (clone MQ1-17H12, BrilliantViolet 605, BD Biosciences cat#564165) - https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/research-reagents/single-color-antibodies-ruo/bv605-rat-anti-human-il-2.564165

For ELISpot, antibodies were purchased from Mabtech:
For capture (primary antibody), plates were coated with IFN-gamma antibody (Mabtech, clone 1-D1K, cat#3420-3-250) - https://www.mabtech.com/products/anti-human-ifn-g-mab-1-d1k-unconjugated-3420-3
For detection (secondary antibody), anti-human IFN-gamma antibody was used (Mabtech, clone 7-BG-1 biotin, cat#3420-6-250) - https://www.mabtech.com/products/3420-6

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	NCT02950766
Study protocol	The study protocol is included in the supplementary appendix.
Data collection	Data was collected as described i the Methods section. Patients were enrolled in the first cohort (vaccine plus 2.5mg of subcutaneous ipilimumab at each vaccine site) and the second cohort (vaccine alone) between March 2019 and September 2021. Clinical outcome and safety data are reported until the clinical data cutoff of July 7, 2023. Data was collected at Dana-Farber Cancer Institute and Brigham and Women's Hospital during and beyond the enrollment period.
Outcomes	Primary Objectives: To evaluate safety and tolerability of administering NeoVax and locally delivered Ipilimumab in patients with fully resected, high-risk clear cell renal cell carcinoma (ccRCC). To determine the maximum tolerated dose (MTD) of locally administered Ipilimumab when delivered with NeoVax. Secondary Objectives To assess the induction of neoantigen-specific cellular immune responses following administration of NeoVax with Ipilimumab. To determine the proportion of patients alive without recurrence at two years after surgery following administration of NeoVax with Ipilimumab.

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	PBMCs were thawed and rested overnight at 37oC in RPMI (Life Technologies) containing 10% human serum (Sigma-Aldrich), 1% penicillin and streptomycin (Life Technologies), and 20ng/mL of IL-7 (Peprotech). The following day PBMCs were stimulated with vaccine peptide pools (2µg/mL per individual peptide) in completed R10 media (RPMI, 10% FBS, 1% Pen/Strep). After one hour of co-culture at 37oC, transport inhibitors GolgiStop and GolgiPlug were added. Following 5 hours of incubation at 37◦C, samples were washed with 1X PBS and stained with live/dead dye in 1X PBS for 20 min at 4oC. Samples were subsequently washed with Staining buffer (1X PBS, 10% FBS, 1% penicillin and streptomycin). Fc receptors were blocked for 15 min at 4oC with Human TruStain FcX in staining buffer. Surface staining antibodies were then added: CD4 (clone L200), CD8 (clone RPA-T8), CD45RO (clone UCHL1), and PD-1 (clone EH12.2H7). Following 30 minutes of incubation at 4oC, samples were washed twice before fixation and permeabilized with CytoFix/CytoPerm for 15 minutes at 4oC. Samples were then washed twice and resuspended in 1X Perm/Wash buffer to conduct intracellular staining with: IFN-γ (clone B27), TNF (clone Mab11), IL-2 (clone MQ1-17H12), and CD3 (clone UCHT1). Following 30 minutes of incubation at 4oC, samples were washed twice with Perm/Wash, once with staining buffer, and then resuspended in CytoFix fixation buffer until acquisition. All flow reagents were purchased from BD Biosciences or Biolegend.
Instrument	Data were acquired on a BD LSR Fortessa.
Software	Data was acquired on the BD LSR Fortess with FACSDiva, and then all analysis was performed with Flow Jo (version 10.8.1)
Cell population abundance	There was no post-sort population analyzed in this study. Gating was performed as described in Extended Data Figure 4.
Gating strategy	Gating was performed as described in Extended Data Figure 4. Brief, after gating on live cells (BioLegend Zombie Yellow negative), a lymphocyte gate was drawn using FSC/SSC. Doublets/mulitplets were excluded by gating in FSC-A vs FSC-H and SSC-A vs SSC-H. CD3+ T cells were gated, and then CD4 vs CD8 cells wete gated and analyzed separately. For CD4 T cells, gates were drawn on CD4 vs each individual cytokine (IFNgamma, TNF, or IL-2). This process was repeated for CD8 T cells. For PD-1 and CD45RO, median fluorescence intensity (MFI) was reported.

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