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	DIVA v6.2 (cytometer), Bio-Plex Manager v6.1 (Luminex)
Data analysis	Statistical analysis were performed with SAS (version 9.3 or higher, SAS Institute, Cary, NC, USA), R (version 3.6, The R Foundation for Statistical Computing, Vienna, Austria) and XLSTAT (version 2011.4.04, Addinsoft, Paris, France). Flow cytometry data were analyzed with FlowJo v9 (Treestar)

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

All data supporting the findings of this study are available within the article without any restrictions. The raw data generated in this study are provided in the Source Data file.

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	Sex and gender were not considered in study design Findings of this manuscript do not apply to a specific sex or gender Sex and gender were determined based on self reporting
Reporting on race, ethnicity, or other socially relevant groupings	All the participants in the study are Africans from Conakry, Guinea
Population characteristics	All the participants included in this study are adults healthy participants from Guinea. The median age of the 191 participants varied from 22 to 27 years between groups, with a small proportion of participants aged over 40. Across the different groups, the proportion of males ranged from 44% to 64% .Among the 191 enrolled participants, 31 randomly selected individuals - 11 (35.5%) from the Ad26-MVA, 12 (38.7%) from the rVSV, and 8 (25.8%) from the rVSV-booster groups – were used to assess long term cellular immune responses .
Recruitment	196 adult participants were included in this immunological ancillary study in Guinea from among 4,789 in the whole PREVAC trial. The sub-study was systematically proposed to adult participants included in the main study at Landreah. Participants included in the immunological ancillary study were asked to provide additional written informed consent for additional blood draws. In total, 191 participants were included in the per-protocol population after the exclusion of five participants due to an HIV-positive test (n=4) or discontinuation of the vaccine protocol (n=1) .
Ethics oversight	The study protocol and informed consent, along with participants' informational materials, received approval from the ethics committees of both the sponsor (INSERM IRB 00003888) and the implementing country (Guinea) prior to each version of the protocol being enacted. The study is registered with ClinicalTrials.gov (registration number NCT02876328), EudraCT (2017-001798-18 and 2017-001798-18/3rd), and the Pan African Clinical Trials Registry (PACTR201712002760250)

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	According to methodologist's calculation respecting allocation ratio of randomization 2:1:2:1:1, we plan to enroll up to 230 participants in the substudy under PREVAC versions 2.0, 3.0, and 4.0 (in total).
Data exclusions	All experiments were performed on frozen cells in Paris, France. After thawing, cells with viability <75% were not processed.
Replication	All experiments included a sufficient sample size, taking into account the expected variability when using human PBMC and serum. Representative data were confirmed at least once with an independent experiment.
Randomization	The participants were randomized between arms with the following numbers per group: 79 in the Ad26-MVA arm, 27 in the rVSV arm, 9 in the rVSV-booster arm, and 76 in the pooled placebo arm.
Blinding	PREVAC trial was a double blinded trial. Data collection/generation was blinded to the operator for the different discovery experiments. For this ancillary study, statistician did not perform blind statistical analysis.

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

Multiparametric flow cytometry panel was performed using a battery of antibodies : antibodies: anti-CD4 PE PECF594 # 562281 (1/33), anti-CD8 #560179 (1/20), anti-CD3 Alexa700 #557943 (1/100), anti-IFNγ FITC #557718 (1/20), anti-TNF PE-Cy7 #557647 (1/20), anti-MIP1β PE (1/200) #550078 and anti-IL2 BV421 #564164 (1/33) (all from BD Biosciences)

Validation

All antibodies were commercially available. See the corresponding manufacturer datasheets on webpages for reference and validation

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

PREVAC trial is registered with ClinicalTrials.gov (registration number NCT02876328), EudraCT (2017-001798-18 and 2017-001798-18/3rd), and the Pan African Clinical Trials Registry (PACTR201712002760250).

Study protocol

Regarding the main PREVAC trial, the study protocol and informed consent, along with participants' informational materials, received approval from the ethics committees of both the sponsor (INSERM IRB 00003888) and the implementing country (Guinea) prior to each version of the protocol being enacted. The study protocol has been publishe as pupplementary material of the princeps PREVAC trial publication in New England journal of Medecine (https://www.nejm.org/doi/suppl/10.1056/NEJMoa2200072/suppl_file/nejmoa2200072_protocol.pdf)

Data collection

After it had been established that volunteers met the eligibility criteria and informed consent had been obtained from them or, in the case of children, a parent or guardian background data were collected. Demographics and a short medical history were obtained, blood was drawn as specified by the protocol, and then participants then received their first dose of the vaccine ("prime vaccination"). Randomization occurred at the point of vaccination as described above. For 30min after the vaccination, participants were watched closely, injection site reactions and targeted symptoms were assessed, and possible grade 3 or 4 adverse events were recorded. After the prime vaccination at study entry, initial follow-up visits occurred at: 7 (±3days), 14 (±3days), and 28 (±7days) days. The booster dose of vaccine was administered on day 56 (53 to 66days) with further follow-up visits at 63days (7±3days after the booster vaccination), at 3months (±14days), 6months (±1month), and 12months (±1month). Visits will continue at 24 (±6 month), 36 (±6month), 48 (±6month), and 60 (−6month; +1month) months as part of the PREVAC-UP study.

Outcomes

The primary endpoint of the immunological ancillary study was to assess specific T cell responses induced by the three vaccine strategies and their durability up to five years after the initial vaccination. This endpoint was assessed by intracytokine staining ex vivo or after re-stimulation. The secondary endpoints included measuring serum cytokine and chemokine levels before vaccination (Day 0) and after vaccination (Day 7 and Day 63) using Luminex technology, evaluating the ex vivo gene expression profile in whole blood before and three hours after each vaccination (Day 0 and Day 56), as well as on Day 7 and Day 63 (ongoing study), and performing a phenotypic analysis of B and T cell subsets before and after vaccination (not performed due to insufficient cells remaining after T cell response experiments)

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	Peripheral Mononuclear cells (PBMC), serum and whole blood were frozen in Guinea.Cryopreserved PBMC were thawed and rested, fixed, permeabilized and stained in France according to the demands on each experiment. All details are mentioned in the Methods section
Instrument	LSRII Fortessa 4-laser (488, 640, 561 and 405 nm) cytometer (BD Biosciences)
Software	Data were collected on DIVA v6.2 and analyzed using FlowJo software version 9.9.6 (Tree Star inc.)
Cell population abundance	1-2M of cells were stained and collected on cytometer.
Gating strategy	Gating strategy is presented in Supplementary Figure X

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