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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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

ICS flow cytometry data was collected using the FACS VERSE instrument (BD Biosciences) and FACSSuite software version 1.0.6 .
Multimer flow cytometry phenotyping data was collected using a Symphony A3 flow cytometer (BD) and DIVA Version 9.1. Flow cytometry data was analysed with FlowJo software version 10.6.2 (FlowJo LLC, BD Biosciences)
ELISpot plates were scanned using an AID Classic Robot ELISPOT Reader and analysed by AID ELISPOT 7.0 software (AID Autoimmun Diagnostika).

S1- and RBD-binding IgG data were captured as median fluorescent intensities (MFIs) using a Luminex reader.
For SARS-CoV-2 neutralisation assay, total cell counts per well were enumerated by nuclear stain (Hoechst 33342) and fluorescent virally infected foci were detected with a Cytation 7 Cell Imaging Multi-Mode Reader (Biotek) with Gen5 Image Prime version 3.09.

For VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay (for single amino acid exchange S glycoproteins), fluorescent foci were quantified using the SpectraMax i3 plate reader with MiniMax imaging cytometer (Molecular Devices).
For VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay (for multiple site mutations), luminescence was quantified using the Infinite F200 pro multiplate Reader (Tecan).

No custom software codes have been developed.

Data analysis

Flow cytometry data was analysed using FlowJo software version 10.6.2 (FlowJo LLC, BD Biosciences).

ELISpot plate scan and QC was performed using AID ELISPOT 7.0 software (AID Autoimmun Diagnostika). T-cell responses stimulated by peptides were compared to T-cell responses stimluated with cell culture medium only as a negative control using an in-house ELISpot data analysis tool (EDA), based on two statistical tests (distribution-free resampling) according to Moodie et al. (refer to Material&Methods section in the manuscript for references), to provide sensitivity while maintaining control over false positives.

S1- and RBD-binding IgG data captured as median fluorescent intensities (MFIs) were converted to U/mL antibody concentrations using a

reference standard curve (reference standard composed of a pool of five convalescent serum samples obtained >14 days post-COVID-19 PCR diagnosis and diluted sequentially in antibody-depleted human serum) with arbitrarily assigned concentrations of 100 U/mL and accounting for the serum dilution factor.

For SARS-CoV-2 and VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay (for single amino acid exchange S glycoproteins), titers were calculated in GraphPad Prism version 8.4.2 by generating a 4-parameter (4PL) logistical fit of the percent neutralisation at each serial serum dilution. The 50% neutralisation titre (VNT50) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.
For VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay (for multiple site mutations), titers were calculated in GraphPad Prism version 9.0.0 by generating a 4-parameter (4PL) logistical fit of the percent neutralisation at each serial serum dilution. The 50% pseudovirus neutralisation titre (pVNT50) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in luminescence signal.

All statistical analyses were performed using GraphPad Prism software versions 8.4.2 and 9.0.0.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request. SARS-CoV-2 complete genome sequences were downloaded from GISAID nucleotide database (https://www.gisaid.org) on March 20th, 2020 as referred in Baum et al., 2020. Upon completion of this clinical trial, summary-level results will be made public and shared in line with data sharing guidelines.

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	In the part of the clinical study reported here five dose levels (1 µg, 10 µg, 20 µg, 30 µg) of the BNT162b2 vaccine candidate were assessed at one site in Germany with 12 healthy volunteers per dose level in a dose escalation and de-escalation design. Sentinel dosing was performed in each dose-escalation cohort. The inclusion of 12 subjects per group is considered to be adequate for a safety assessment of each vaccine per dose level. The probability to observe a particular TEAE with incidence of 15% at least once in 12 subjects per group is 85.8%.
Data exclusions	Clinical safety and serology data available until data extraction date of 23 October 2020 were included. Cut-off date for intracellular cytokine staining data included in the manuscript was 17 February 2021. Cut-off date for ELISPOT data included in the manuscript was 28 January 2021. For serology/cell-mediated immunity correlation analyses (Ext. Data Fig. 6), data were only plotted for prime/boost vaccinated participants with detectable T-cell response. All participants with sufficient PBMC material available at day 1 and day 29 were included in the ICS analyses. In Fig. 3a, CD4 non-responders (<0.03% total cytokine producing T cells; 1 µg, n=2 [S pool 1] and n=1 [S pool 2]; 10 µg, n=1) were excluded. In Ext. Data Fig. 7c, one participant from the 20 µg dose level cohort with a strong pre-existing CD4+ T cell response to S pool 2 was excluded. All participants with sufficient PBMC material available were included in the ELISPOT analyses. Fig. 2a and Ext. Data Fig. 5a and b spot count data from two participants from the 20 µg dose level cohort could not be normalized and have been excluded. In Fig. 2b, participants without a T-cell response were excluded. In Ext. Data Fig. 6b, only data from participants with both CD4+ and CD8+ T-cell responses were included. Data shown are preliminary and not fully source-data verified.
Replication	A parallel clinical study of very similar design has been conducted in the USA involving the same populations, vaccine candidates and doses. The results for safety and immunogenicity align closely. The US study is randomized placebo controlled. Serology: Participant sera were tested in duplicate and geometric mean concentration (S1- or RBD-specific IgG dLIA) or titer (virus neutralisation and pseudovirus neutralisation assay) were plotted. T cell immunity: Participant PBMCs were tested as single instance in ICS and multimer analyses. Participant PBMCs were tested in duplicates in ELISpot analyses. Spot counts were summarized as mean values of each duplicate. Data shown are preliminary and not fully source-data verified.

Randomization	Randomization was not performed in order to facilitate operational efficiencies with the sentinel design, also knowing that a parallel randomized, placebo-controlled study was being conducted in the same vaccine constructs in the USA.
Blinding	This is a non-randomized open-label phase I/II trial. Investigators were not blinded in order to facilitate operational efficiencies with the sentinel design, also knowing that a parallel randomized, placebo-controlled study was being conducted in the same vaccine constructs in the USA.

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Flow cytometry (specificity/host+reactivity/fluorochrome/clone/manufacturer/catalogue number/lot number/dilution/extra- or intracellular):
CD3/mouse anti-human/BV421/UCHT1/BD Biosciences/562426/9113553/1:250/extracellular+intracellular
CD4/mouse anti-human/BV480/RPA-T4/BD Biosciences/746541/0171955/1:50/extracellular+intracellular
CD8/mouse anti-human/BB515/RPA-T8/BD Biosciences/564526/0037189/1:100/extracellular+intracellular
IFNγ /mouse anti-human/BB700/B27/BD Biosciences/566394/XX/1:250/intracellular
IFNγ /mouse anti-human/PE-Cy7/B27/BD Biosciences/557643/9332967/1:50/intracellular
IL-2/rat anti-human/PE/MQ1-17H12/BD Biosciences/554566/9337013/1:10/intracellular
IL-4/rat anti-human/APC/MP4-25D2/BD Biosciences/554486/9185677/1:500/intracellular

CD3/mouse anti-human/BUV396/UCHT1/BD Biosciences/563546/903095/1:50/extracellular
CD69/mouse anti-human/BUV496/FN50/BD Biosciences/750214/0234125/1:150/extracellular
CD45RA/mouse anti-human/BUV563/HI100/BD Biosciences/612926/0108723/1:200/extracellular
CD27/mouse anti-human/BUV737/L128/BD Biosciences/612829/0048020/1:200/extracellular
CD103/mouse anti-human/BUV805/Ber-ACT8/BD Biosciences/748501/0234121/1:150/extracellular
CD8/mouse anti-human/BV480/RPA-T8/BD Biosciences/566121/8298740/1:200/extracellular
CD49a/mouse anti-human/BV605/SR84/BD Biosciences/742359/0234071/1:100/extracellular
CD279 (PD-1)/mouse anti-human/BV650/EH12.1/BD Biosciences/564104/0064420/1:20/extracellular
CD197 (CCR7)/rat anti-human/BV786/3D12/BD Biosciences/563710/0163845/1:15/extracellular
CD4/mouse anti-human/BB515/SK3/BD Biosciences/565996/9343113/1:50/extracellular
CD28/mouse anti-human/BB700/L293/BD Biosciences/745905/0259534/1:100/extracellular
CD38/mouse anti-human/PE-CF594/HIT2/BD Biosciences/562288/0036633/1:600/extracellular
HLA-DR/mouse anti-human/APC-R700/G46-6/BD Biosciences/565127/9204365/1:150/extracellular
CD16/mouse anti-human/APC-eFluor780/CB16/Thermo/47-0168-42/2152036/1:100/extracellular
CD14/mouse anti-human/APC-eFluor780/61D3/Thermo/47-0149-42/2126831/1:100/extracellular
CD19/mouse anti-human/APC-eFluor780/HIB19/Thermo/47-0199-42/2145095/1:100/extracellular

Tetramers:
HLA-A*02:01 - YLQPRTFLL - BV711; HLA-A*02:01 - YLQPRTFLL - PE-Cy7; 1:24
HLA-A*02:01 - RLQSLQTYV - BV711; HLA-A*02:01 - RLQSLQTYV - PE; 1:24
HLA-B*35:01 - LPFNDGVYF - BV421; HLA-B*35:01 - LPFNDGVYF - PE; 1:24
HLA-B*35:01 - QPTESIVRF - APC; HLA-B*35:01 - QPTESIVRF - PE; 1:24
HLA-B*35:01 - IPFAMQMAY - BV711; HLA-B*35:01 - IPFAMQMAY - PE-Cy7; 1:24
HLA-A*24:02 - NYNYLYRLF - BV711; HLA-A*24:02 - NYNYLYRLF - PE; 1:24
HLA-A*24:02 - QYIKWPWYI - BV421; HLA-A*24:02 - QYIKWPWYI - PE; 1:24
HLA-A*24:02 - KWPWYIWLGF - BV421; HLA-A*24:02 - KWPWYIWLGF - BV711; 1:24

Reagents for in-house Monomer and Tetramer production:
HLA-A*02:01 easymer; Immunaware; 1001-01; E190040.3; 1:6
HLA-B*35:01 easymer; Immunaware; 1072-01; E200058.1; 1:6
HLA-A*24:02 easymer; Immunaware; 1020-01; E190042.1; 1:6
Streptavidin – PE; Biosciences; 554061; 9049627; 1: 560
Streptavidin – APC; Biosciences; 554067; 8326901; 1:220
Streptavidin – BV421; Biosciences; 563259; 8291845; 1: 110

Streptavidin – BV711; Biosciences; 563262; 9283452; 1:110
Streptavidin – PE-Cy7; Biosciences; 557598; 9336544; 1:220
β2M; mouse; anti human; Biolegend; 316305; B286120; 1:250

Fixable Viability Dye/eF780/eBioscience/65-0865-14/2185428/1:1,666

ELISpotPro kit/cat. no. 3420-2APT-10/lot no. 370/Mabtech:
Primary anti-IFNg antibody/clone c1-D1K/pre-coated plates
Secondary anti-IFNg antibody/clone 7-B6-1 (ALP conjugate)/1:250
CD3/clone CD3-2/1:1,000

S1- and RBD-binding IgG assay:
goat anti-human IgG/R-PE/polyclonal/Jackson Labs/109-115-098/147186/1:500

Validation

Commercially available antibodies were selected based on their antigen specificity and suggested application as described on the manufacturer`s website and data sheets. The antibody concentrations for staining were optimized by titrating down each reagent starting at the manufacturer`s recommendation. The optimal amounts of the reagents were defined by (i) minimal unspecific shift of the negative population and (ii) a maximal separation of the negative and positive population. Individual antibody validation reports are not evident from the BD Biosciences website.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Vero cells (CCL-81), Vero E6 cells (CRL-1586) and HEK293T (CRL-3216) were obtained from ATCC.

Authentication

Vero and Vero E6 cells were sourced from ATCC, which maintains a quality management system commensurate to ISO 9001:2015, ISO 13485:2016, ISO 17025:2017, and ISO 17034:2016. Cells were certified by the vendor and propagated according to the manufacturer's instructions.

Mycoplasma contamination

All used cell lines were tested negative for mycoplasma contamination after receipt and before expansion and cryopreservation.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Healthy men and non-pregnant women 18 to 55 years of age with equal gender distribution. Most participants were Caucasian (96.7%) with one African American and one Asian subject (1.7% each). Key exclusion criteria included previous clinical or microbiological diagnosis of COVID-19; receipt of medications to prevent COVID-19; previous vaccination with any coronavirus vaccine; a positive serological test for SARS-CoV-2 IgM and/or IgG at the screening visit; and a SARS-CoV-2 NAAT-positive nasal swab within 24 hours before study vaccination; those with increased risk for severe COVID-19; immunocompromised individuals, those with known infection with HIV, hepatitis C virus, or hepatitis B virus and those with a history of autoimmune disease.

Recruitment

Recruitment was performed by teaching investigators according to inclusion and exclusion criteria without any bias. No protocol-specified methods. The sites are experienced phase 1 units with established rostas of potential subjects who they can invite for screening for inclusion. Also the sites advertise through their own web-site. Some subjects self-referred via the sponsor.

Ethics oversight

The trial was carried out in Germany in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines and with approval by an independent ethics committee (Ethik-Kommission of the Landesärztekammer Baden-Württemberg, Stuttgart, Germany) and the competent regulatory authority (Paul-Ehrlich Institute, Langen, Germany). All subjects provided written informed consent.

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

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

ClinicalTrials.gov Identifier: NCT04380701, see also manuscript

Study protocol

The full clinical study protocol will be submitted before acceptance, and a comprehensive description of the clinical trial design, eligibility criteria and endpoints is available at https://clinicaltrials.gov/ct2/show/study/NCT04380701.

Data collection

Serum for antibody assays was obtained on day 1 (pre-prime), 8±1 (post-prime), 22±2 (pre-boost), 29±3, 43±4, 50±4, and 85±7 (post-boost). PBMCs for T cell studies were obtained on day 1 (pre-prime), 29±3, 43±4, and 85±7 (post-boost). Tolerability was assessed by patient diary.
All formal protocol-determined visits were conducted on-site at the investigators premises (in each case a dedicated phase 1 unit). All study procedures such as blood sample, physical examinations, screening checks were conducted at the study sites. The

only exceptions were the completion of the subject diaries, which was done by the subjects at home. Diaries were collected by the sites at the subjects' next scheduled visits and the data entered on site. There was also dedicated telephone follow-up, 48 hrs following dosing, to ensure subject well-being, which was documented on site by the investigator conducting the call.

Outcomes

Primary objective: To describe the safety and tolerability profiles of prophylactic BNT162 vaccines in healthy adults after single dose (SD; prime only) or prime/boost (P/B) immunization.
Endpoints: Solicited local reactions & solicited systemic reactions (listed in subject diaries, to be graded by subjects) and unsolicited treatment-emergent adverse events.

Secondary objectives: To describe the immune response in healthy adults after SD or P/B immunization measured by a functional antibody titer, e.g., virus neutralization test or an equivalent assay available by the time of trial conduct.
Endpoints: Functional antibody responses; fold increasese in functional antibody titers; number of subjects with seroconversion

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

Cytokine-producing T cells were identified by intracellular cytokine staining. PBMCs thawed and rested for 4 hours in OpTmizer medium supplemented with 2 µg/mL DNAseI (Roche), were restimulated with different portions of the wild-type sequence of SARS-CoV-2 S protein (N-terminal pools S pool 1 [aa 1-643] and RBD [aa1-16 fused to aa 327-528 of the S protein], and the C-terminal S pool 2 [aa 633-1273]) (2 µg/mL/peptide; JPT Peptide Technologies) in the presence of GolgiPlug (BD) for 18 hours at 37 °C. Controls were treated with DMSO-containing medium. Cells were stained for viability and surface markers (in flow buffer comprising D-PBS [Gibco] supplemented with 2% FBS [Sigma], 2 mM EDTA [Sigma-Aldrich]), and Brilliant Stain Buffer Plus [BD Horizon™, according to the manufacturer's instructions] or in Brilliant Stain Buffer [BD Horizon™]) for 20 minutes at 4 °C. Afterwards, samples were fixed and permeabilized using the Cytofix/Cytoperm kit according to manufacturer's instructions (BD Biosciences). Intracellular staining was performed in Perm/Wash buffer supplemented with Brilliant Stain Buffer Plus (according to the manufacturer's instructions) for 30 minutes at 4 °C.

Antigen-specific CD8+ T cells were identified and characterized in Multimer staining experiments. Frozen aliquots of PBMCs were thawed and 2x10^6 cells were stained for 20 minutes at room temperature with each pMHC multimer cocktail at a final concentration of 4 nM in Brilliant Staining Buffer Plus (BSB Plus [BD Horizon™]). Surface and viability staining was carried out in flow buffer (DPBS [Gibco] with 2% FBS [Biochrom], 2 mM EDTA [Sigma-Aldrich]) supplemented with BSB Plus for 30 minutes at 4° C. Finally, the cells were fixed for 15 minutes at 4°C in 1x Stabilization Fixative (BD Biosciences).

Instrument

Samples were acquired on a FACS VERSE instrument (BD Biosciences) for identification of cytokine-producing T cells. For multimer analysis, samples were acquired on a Symphony A3 instrument (BD Biosciences)

Software

For data analysis FlowJo software version 10.6.2 (FlowJo LLC, BD Biosciences) was used.

Cell population abundance

Bulk PBMCs were used. No cell sorting was performed.

Gating strategy

The gating strategies are detailed in the respective figure or in the supplementary information. Briefly, singlets were gated based on their location in the FSC-A/FSC-H plot. Debris was excluded in the subsequent FSC-A/viability dye plot. Viable cells were gated from non-debris in the FSC-A/viability dye plot. From viable cells, lymphocytes were gated based on their size and granularity in the FSC-A/SSC-A plot. From lymphocytes, CD3+ T cells were gated in the CD3/SSC-A plot. From CD3+ T cells, CD4+ and CD8+ T cells were gated in the CD4/CD8 plot. From CD4+ T cells, IFNg+, IL-2+, IL-4+ or IFNg+ IL-2+ T cells were gated by plotting CD4/IFNg, CD4/IL-2, CD4/IL-4, or IFNg/IL-2. From CD8+ T cells, IFNg+, IL-2+ or IFNg+ IL-2+ T cells were gated by plotting CD8/IFNg, CD8/IL-2, or IFNg/IL-2.

The gating strategy for the identification and characterization of ag-specific CD8+ T is shown in detail in the supplementary Fig. 2. Singlets were gated in a FSC-A/FSC-H plot. Viable lymphocytes were addressed by successive gating in a SSC-A/FCS-A plot followed by excluding DUMP positive cells (dead cells, CD14, CD19, CD16) in a FSC-A/DUMP plot. From lymphocytes, CD3+ T cells were gated in a CD3/FSC-A plot. From CD3+ T cells, CD4+ and CD8+ T cells were gated in the CD4/CD8 plot. Ag-specific CD8+ T cells were gated as multimer double positive cells in a 2D dot plot with a combination of two fluorochromes labeling a defined MHC-epitope (10 combinations using 5 fluorochromes). From CD8+ or CD8+ multimer+ T cells, further T cell subsets were determined in a CCR7/CD45RA plot (memory phenotype) or CD38/HLA-DR plot or CD3/PD-1(activation status) or CD28/CD27 (stages of differentiation).

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