

**Accelerated Article Preview**

# COVID-19 vaccine BNT162b1 elicits human antibody and T<sub>H</sub>1 T-cell responses

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Received: 16 July 2020

Accepted: 22 September 2020

Accelerated Article Preview Published  
online 30 September 2020

Cite this article as: Sahin, U. et al. COVID-19  
vaccine BNT162b1 elicits human antibody  
and T<sub>H</sub>1 T-cell responses. *Nature*  
<https://doi.org/10.1038/s41586-020-2814-7>  
(2020).

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## Article

# COVID-19 vaccine BNT162b1 elicits human antibody and T<sub>H</sub>1 T-cell responses

<https://doi.org/10.1038/s41586-020-2814-7>

Received: 16 July 2020

Accepted: 30 September 2020

Published online: 30 September 2020

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An effective vaccine is needed to halt the spread of the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) pandemic. Recently, we reported safety, tolerability and antibody response data from an ongoing placebo-controlled, observer-blinded phase 1/2 coronavirus disease 2019 (COVID-19) vaccine trial with BNT162b1, a lipid nanoparticle (LNP) formulated nucleoside-modified messenger RNA (mRNA) encoding the receptor binding domain (RBD) of the SARS-CoV-2 spike protein<sup>1</sup>. Here we present antibody and T-cell responses after BNT162b1 vaccination from a second, non-randomized open-label phase 1/2 trial in healthy adults, 18–55 years of age. Two doses of 1 to 50 µg of BNT162b1 elicited robust CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses and strong antibody responses, with RBD-binding IgG concentrations clearly above those in a COVID-19 human convalescent sample (HCS) panel. Day 43 SARS-CoV-2 serum neutralising geometric mean titers were 0.7-fold (1 µg) to 3.5-fold (50 µg) those of the HCS panel. Immune sera broadly neutralised pseudoviruses with diverse SARS-CoV-2 spike variants. Most participants had T helper type 1 (T<sub>H</sub>1) skewed T cell immune responses with RBD-specific CD8<sup>+</sup> and CD4<sup>+</sup> T-cell expansion. Interferon (IFN) $\gamma$  was produced by a high fraction of RBD-specific CD8<sup>+</sup> and CD4<sup>+</sup> T cells. The robust RBD-specific antibody, T-cell and favourable cytokine responses induced by the BNT162b1 mRNA vaccine suggest multiple beneficial mechanisms with potential to protect against COVID-19.

In December 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged in China causing coronavirus disease 2019 (COVID-19), a severe, acute respiratory syndrome with a complex, highly variable disease pathology. On 11 March 2020, the World Health Organization (WHO) declared the SARS-CoV-2 outbreak a pandemic. As of 16 September 2020, over 29 million cases have been reported worldwide, with over 930,000 deaths<sup>2</sup>.

The high and worldwide impact on human society calls for the rapid development of safe and effective therapeutics and vaccines<sup>3</sup>.

Lipid nanoparticle (LNP) formulated messenger RNA (mRNA) vaccine technology allows delivery of precise genetic information together with an adjuvant effect to antigen presenting cells<sup>4</sup>. The prophylactic effectiveness of this technology has been proven in preclinical models against multiple viral targets<sup>5–7</sup>. LNP- and liposome-formulated RNA vaccines for prevention of infectious diseases and for treatment of cancer have been shown in clinical trials to be safe and well-tolerated<sup>8</sup>.

mRNA is transiently expressed and does not integrate into the genome. It is molecularly well defined, free of animal-origin materials and synthesized by an efficient, cell-free *in vitro* transcription process from DNA templates<sup>5,9,10</sup>. The fast and highly scalable mRNA manufacturing and LNP formulation processes enable rapid production of many vaccine doses<sup>6,7,11</sup>, making it suitable for rapid vaccine development and pandemic vaccine supply.

Two Phase 1/2 umbrella trials in Germany and the U.S. investigate several LNP-encapsulated RNA vaccine candidates developed in ‘Project Lightspeed’, the joint BioNTech-Pfizer COVID-19 RNA vaccine development program. Recently, we have reported interim data obtained in the U.S. trial (NCT04368728) for the most advanced candidate BNT162b1<sup>1</sup>. BNT162b1 encodes the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, a key target of neutralising antibodies. The RBD antigen expressed by BNT162b1 is fused to a T4 fibritin-derived “foldon” trimerisation domain to

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increase its immunogenicity by multivalent display<sup>12</sup>. The RNA is optimized for high stability and translation efficiency<sup>13,14</sup> and incorporates 1-methylpseudouridine instead of uridine to dampen innate immune sensing and to increase mRNA translation *in vivo*<sup>15</sup>. In the placebo-controlled, observer-blinded U.S. trial, dosages of 10 µg, 30 µg (prime and boost doses 21 days apart for both dose levels) and 100 µg (prime only) were administered. No serious adverse events were reported. Local injection site reactions and systemic events (mostly influenza-like symptoms) were dose-dependent, generally mild to moderate, and transient. RBD-binding immunoglobulin G (IgG) concentrations and SARS-CoV-2 neutralising titers in sera increased with dose level and after a second dose. Fourteen days after the boost, geometric mean neutralising titers reached 1.9- to 4.6-fold of a panel of COVID-19 convalescent human sera.

This study now complements and expands our previous report with available data from the German trial (NCT04380701, EudraCT: 2020-001038-36), providing a detailed characterisation of antibody and T-cell immune responses elicited by BNT162b1 vaccination.

## Study design and analysis set

Between 23 April 2020 and 22 May 2020, 60 participants were vaccinated with BNT162b1 in Germany. Twelve participants per 1 µg, 10 µg, 30 µg, and 50 µg dose level groups received a first dose on Day 1 and were boosted on Day 22 (except for one individual each in the 10 and 50 µg dose-level cohort who discontinued due to reasons not related to the study drug), and 12 participants received a 60 µg prime dose on Day 1 only (Extended Data Fig. 1). The study population consisted of healthy males and non-pregnant females with a mean age of 41 years (range 18 to 55 years) with equal gender distribution. Most participants were Caucasian (96.7%) with one African American and one Asian participant (1.7% each) (Extended Data Tab. 1). Preliminary data analysis focused on immunogenicity (Extended Data Tab. 2).

## Preliminary available safety and tolerability data

Briefly, no serious adverse events (SAE) and no withdrawals due to related adverse events (AEs) were observed for any dose. Similar to the U.S. trial, most reported solicited systemic events in the 10 µg and 30 µg groups were reactogenicity, with a typical onset within the first 24 hours after immunisation (Extended Data Fig. 2). Injection site reactions within 7 days of the prime or boost were mainly pain and tenderness. Reactogenicity was dose level dependent, and was more pronounced after the boost dose. The associated symptomatology such as fever, chills, headache, muscle pain, joint pain, injection site pain, and tenderness, was mostly mild or moderate, with occasional severe manifestations (Grade 3). In the 30 µg dose level cohort, 2 out of 12 (16.7%) subjects experienced severe local reactogenicity, 6 out of 12 (50%) subjects reported severe systemic reactogenicity, which were primarily headache, chills, fatigue or muscle pain, and in 1 subject out of 12 (8.3%) fever. These AEs were transient, resolved spontaneously or were manageable with simple measures (*e.g.* paracetamol). Based on the reactogenicity reported after the 50 µg boost dose, participants who had received an initial 60 µg dose did not receive a boost.

Whereas no relevant change in routine clinical laboratory values occurred after BNT162b1 vaccination, a transient increase in C-reactive protein (CRP) and temporary reduction of blood lymphocyte counts were observed in a dose-dependent manner in vaccinated participants (Extended Data Fig. 3). CRP is a well-known inflammatory serum protein previously described as biomarker for various infectious disease vaccines and an indicator of vaccine adjuvant activity<sup>16–19</sup>. Based on our previous clinical experience with RNA vaccines the transient decrease in lymphocytes is likely attributable to innate immune stimulation-related redistribution of lymphocytes into lymphoid tissues<sup>20</sup>. Concomitant neutropenia was not observed. Both CRP levels and lymphocyte counts

are considered pharmacodynamics markers for the mode-of-action of RNA vaccines.

## Vaccine-induced antibody response

RBD-binding IgG concentrations and SARS-CoV-2 neutralising titers were assessed at baseline, 7 and 21 days after the BNT162b1 priming dose (Days 8 and 22), and 7 and 21 days after the boosting dose (Days 29 and 43), except for the 60 µg cohort, which received a priming dose only (Fig. 1, Extended Date Tab. 3).

Immunised participants showed a strong, dose-dependent vaccine-induced antibody response. Twenty-one days after the priming dose (for the four dose levels ranging from 1–50 µg), geometric mean concentrations (GMCs) of RBD-binding IgG had increased in a dose level dependent manner, with GMCs ranging from 265–1,672 U/mL (Fig. 1). Seven days after the boosting dose (Day 29), RBD-binding IgG GMCs in participants vaccinated with 1–50 µg BNT162b1 showed a strong, dose-level dependent booster response ranging from 2,015–25,006 U/mL. At Day 43 (21 days after boost), RBD-binding antibody GMCs were in the range of 3,920–18,289 U/mL in BNT162b1 vaccinated individuals as compared to a GMC of 602 U/mL measured in a panel of convalescent sera from 38 SARS-CoV-2 infection patients. The patients were 18–83 years of age, and sera were drawn at least 14 days after polymerase chain reaction (PCR)-confirmed diagnosis. In the 60 µg dose-level cohort, which received a priming dose only, RBD-binding IgG GMCs were 755 U/mL by Day 43, indicating that a boosting dose to increase antibody concentrations is necessary.

SARS-CoV-2 neutralising antibody geometric mean titers (GMTs) increased modestly in a dose-dependent manner 21 days after the priming dose (Fig. 2a, Extended Date Tab. 4). Substantially higher serum-neutralising GMTs were achieved 7 days after the booster dose, reaching 36 (1 µg dose level), 158 (10 µg dose level), 308 (30 µg dose level), and 578 (50 µg dose level), compared to 94 for the convalescent serum panel. On Day 43 (21 days after the boost), the neutralising GMTs and RBD-binding GMCs decreased (with exception of the 1 µg dose level). Serum virus neutralising GMTs were strongly correlated with RBD-binding IgG GMCs (Fig. 2b) with lower ratios of serum neutralising GMT to RBD-binding IgG GMC elicited by the vaccine than by SARS-CoV-2 infection. In summary, antibody responses elicited by BNT162b1 in study BNT162-01 largely mirrored those observed in the U.S. study<sup>1</sup>.

To demonstrate the breadth of the neutralising response, a panel of 16 SARS-CoV-2 RBD variants identified through publicly available information<sup>21</sup> and the dominant (non-RBD) spike variant D614G<sup>22</sup> was evaluated in pseudovirion neutralisation assays. Sera collected 7 days after the second dose of BNT162b1 showed high neutralising titers to each of the SARS-CoV-2 spike variants (Fig. 2c, Extended Date Tab. 5).

## Vaccine-induced T-cell responses

CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses in BNT162b1 immunised participants were characterised prior to priming vaccination (Day 1) and Day 29 (7 days after boost vaccination for cohorts 1 µg–50 µg) using direct *ex vivo* IFN $\gamma$  enzyme-linked immunosorbent spot (ELISpot) assay with peripheral blood mononuclear cells (PBMCs) from 51 participants across the 1 µg to 60 µg dose-level cohorts (Fig. 3). In this assay, CD4<sup>+</sup> or CD8<sup>+</sup> T cell effectors were stimulated overnight with overlapping peptides representing the full-length sequence of the vaccine-encoded RBD.

Of 42 participants receiving prime-boost vaccination (cohorts 1 µg to 50 µg), 40 (95.2%, including all participants treated with ≥10 µg BNT162b1) mounted RBD-specific CD4<sup>+</sup> T cell responses. While the magnitude varied between individuals, participants with the strongest CD4<sup>+</sup> T cell responses to RBD had more than 10-fold of the memory responses observed in the same participants when stimulated with cytomegalovirus (CMV), Epstein Barr virus (EBV), influenza virus and tetanus

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toxoid-derived immuno-dominant peptide panels (Fig. 3a–c). In the 60 µg cohort, treated with the priming dose only, both immunogenicity rate (5/9, equals 55.6%) and response strength was lower compared to the other cohorts, indicating the importance of booster vaccination. No CD4<sup>+</sup> T cell responses were detectable at baseline, except for one participant with a low number of preexisting RBD-reactive CD4<sup>+</sup> T cells, which increased significantly after vaccination (normalised mean spot count from 63 to 1,519, in the 50 µg dose cohort). For two participants from the 1 µg cohort the baseline data was not evaluable. The strength of RBD-specific CD4<sup>+</sup> T cell responses correlated positively with both RBD-binding IgG and with SARS-CoV-2 neutralising antibody titers (Extended Data Fig. 4a, b), being in line with the concept of intramolecular help<sup>23</sup>. The two participants immunised with 1 µg BNT162b1 lacking CD4<sup>+</sup> response had no detectable virus neutralising titers (VNT<sub>50</sub>) (Extended Data Fig. 4b).

Among vaccine-induced CD8<sup>+</sup> T cell responses (32/42 participants receiving the prime-boost dosing, 76.2%), strong responses were mounted by the majority of participants (Fig. 3a) and were quite comparable with memory responses against CMV, EBV and influenza virus in the same participants (Fig. 3b, c). Individuals immunised with a single dose of 60 µg had a lower response rate (4/9, equals 44%) and weaker CD8<sup>+</sup> T-cell responses to RBD. The strength of RBD-specific CD8<sup>+</sup> T cell responses correlated positively with vaccine-induced CD4<sup>+</sup> T cell responses but did not significantly correlate with SARS-CoV-2 neutralising antibody titers (Extended Data Fig. 4c, d).

Of note, while at 1 µg BNT162b1 the CD4<sup>+</sup> and CD8<sup>+</sup> rate of response was lower (9 and 8 out of 11 participants, respectively), the magnitude of vaccine-induced T cells in some participants was almost as high as with 50 µg BNT162b1 (Fig. 3a).

To assess functionality and polarization of RBD-specific T cells, cytokines secreted in response to stimulation with overlapping peptides representing the full-length sequence of the vaccine encoded RBD were determined by intracellular staining (ICS) for IFN $\gamma$ , IL-2 and IL-4 specific responses in pre- and post-vaccination PBMCs of 52 BNT162b1 immunised participants. RBD-specific CD4<sup>+</sup> T cells secreted IFN $\gamma$ , IL-2, or both, but in the vast majority of subjects did not secrete IL-4 (Fig. 4a–c, Extended Data Tab. 6). Similarly, fractions of RBD-specific CD8<sup>+</sup> T cells secreted IFN $\gamma$  and IL-2.

The mean fraction of RBD-specific T cells within total circulating T cells obtained by BNT162b1 vaccination was substantially higher than that observed in fifteen participants who recovered from COVID-19. Fractions of RBD-specific IFN $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells reached up to several percent of total peripheral blood CD8<sup>+</sup> T cells (Fig. 4c). Analysis of supernatants of PBMCs stimulated *ex vivo* with overlapping RBD peptides from a subgroup of five vaccinated participants detected proinflammatory cytokines TNF, IL-1 $\beta$  and IL-12p70, but neither IL-4 nor IL-5 (Fig. 4d).

In summary, these findings indicate that BNT162b1 induces functional and proinflammatory CD4<sup>+</sup>/CD8<sup>+</sup> T cell responses in almost all participants, with T<sub>H</sub>1 polarisation of the helper response.

## Discussion

We observed concurrent production of neutralising antibodies, activation of virus-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and robust release of immune-modulatory cytokines such as IFN $\gamma$ , which represents a coordinated immune response to counter a viral intrusion (for review<sup>24</sup>). IFN $\gamma$  is a key cytokine for several antiviral responses. It acts in synergy with type I interferons to inhibit replication of SARS-CoV<sup>25</sup>. Patients with IFN $\gamma$  gene polymorphism related to impaired IFN $\gamma$  activity have been shown to display 5-fold increased susceptibility to SARS<sup>26</sup>. The robust elicitation of IFN $\gamma$ -producing CD8<sup>+</sup> T cells indicates that a favourable cellular immune response with anti-viral and immune-augmenting properties complements the strong neutralising antibody response.

The detection of IFN $\gamma$ , IL-2 and IL-12p70 but not IL-4 and IL-5 indicates a favorable T<sub>H</sub>1 profile and the absence of a potentially deleterious

T<sub>H</sub>2 immune response. CD4<sup>+</sup> and CD8<sup>+</sup> T cells may confer long-lasting immune memory against coronaviruses as indicated in SARS-CoV-1 survivors, in whom CD8<sup>+</sup> T-cells persisted for 6–11 years<sup>24,27</sup>. Some cases of asymptomatic virus exposure have been associated with cellular immune response without seroconversion indicating that SARS-CoV-2 specific T cells could be relevant in disease control even in the absence of neutralising antibodies<sup>28</sup>. In our study, almost all vaccinated volunteers mounted RBD-specific T cell responses detected with an *ex vivo* ELISpot assay, which was performed without prior expansion of T cells and captures only high-magnitude T cell responses. While the strength of the T-cell responses varied considerably between participants, we observed no clear dose dependency of the T-cell response strength within the tested dose range (1 µg to 50 µg). Of note, even with a dose as little as 1 µg mRNA-encoded immunogen stimulation and robust expansion of T cells was accomplished in most of the subjects.

The study confirms the dose-dependency of RBD-binding IgG and neutralisation responses and reproduces our previous findings for the 10 and 30 µg dose levels of BNT162b1 in the U.S. trial<sup>1</sup>. The observed strong boost response for BNT162b1 is in line with an absence of a limiting anti-vector immunity, which is a characteristic advantage of the RNA-based vaccine platform.

The ratio of serum virus neutralisation GMT to recombinant RBD-binding IgG GMC is lower after immunisation with BNT162b1 than after SARS-CoV-2 infection. As noted previously, this difference may be attributed, in part, to BNT162b1 eliciting antibodies that bind epitopes that are exposed on the RNA-encoded RBD immunogen but buried and inaccessible in the spikes of SARS-CoV-2 virions, differentially increasing RBD-binding IgG GMCs after immunisation. In addition, SARS-CoV-2 infection may elicit neutralising antibodies that recognise epitopes that are exposed on virions and located outside the RBD, differentially increasing the serum neutralising GMT after infection<sup>29,30</sup>.

As reported for other types of vaccines, mRNA vaccine induced B cell responses typically peak two weeks after boost and thereafter drop over time until reaching a sustained memory phase with only gradual decline<sup>31</sup>. The RBD-binding antibody concentrations and SARS-CoV-2 neutralising titers elicited by two doses of BNT162b1 appear to follow this pattern showing a decline on day 43. A long-term trend based on the contraction phase cannot be extrapolated. A description of the durability of the antibody response to BNT162b1 will emerge over the planned six months of serological follow up in this study and two years of follow up in the corresponding U.S. study. A distinguishing observation for this RNA-based vaccine candidate is that two injections of BNT162b1 at a dose level as low as 1 µg are capable of inducing RBD-binding IgG levels higher than those observed in convalescent sera, and serum neutralising antibody titers that were still increasing up to Day 43.

As was also observed in the U.S. trial of this vaccine candidate<sup>1</sup>, reactogenicity to BNT162b1 is dose level dependent, and a higher proportion of participants had severe reactogenicity after the second dose, leading to a decision not to administer a boost at the 60 µg dose level. The number of subjects who reported severe AEs was more pronounced in the German trial as compared to the placebo controlled U.S. trial. BNT162b1 demonstrated in principle a manageable tolerability at dose levels that elicited robust immune responses. Meanwhile, BNT162b2, derived from the same nucleoside-modified vaccine platform but encoding the full spike protein has been assessed in two clinical trials and has been found to have a milder reactogenicity profile<sup>32</sup>. Based on the more favourable systemic tolerability, BNT162b2 was selected for advancing into a phase 2/3 trial.

A purely RBD-directed immunity might be considered prone to escape of the virus by single amino acid changes in this small domain. To address this concern, neutralisation assays were conducted with 17 pseudotyped viruses, 16 of which enter cells using a spike with a different RBD variant found in circulating strains and one of which uses the dominant spike variant D614G. All 17 variants were efficiently neutralised by the five tested BNT162b1 immune sera. At present, there

is probably insufficient immunity to SARS-CoV-2 in the human population to drive antigenic drift. If escape from RBD-elicited immunity were to emerge in the future, the versatility of the RNA platform could facilitate fast adaptation to the newly emerging viral strains.

Limitations of our clinical study include the small sample size and its restriction to participants below 55 years of age. Another constraint is that we did not perform further T cell analysis e.g. deconvolution of epitope diversity, characterisation of HLA restriction, T cell phenotyping and TCR repertoire analysis before and after vaccination, due to the limited blood volumes that were available for biomarker analyses. Similarly, we did not assess the induction of tissue-resident memory CD8<sup>+</sup> T cells. Further, as vaccine-induced immunity can wane over time, it is important to study persistence of potentially protective immune responses. Samples to assess persistence are not yet available but are planned per study protocol and will be reported elsewhere. The results reported here were obtained from immunisation with one of four vaccine candidates in the study. Upcoming reports of the Project Lightspeed will present data obtained for other COVID-19 vaccine candidates, including BNT162b2, the RNA-based vaccine candidate that encodes the full-length SARS-CoV-2 spike glycoprotein and is being tested in a phase 3 efficacy trial<sup>32</sup>.

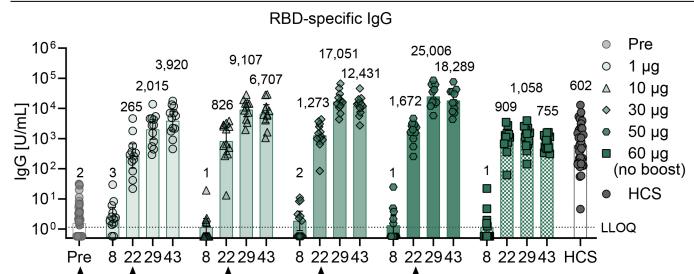
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Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-020-2814-7>.

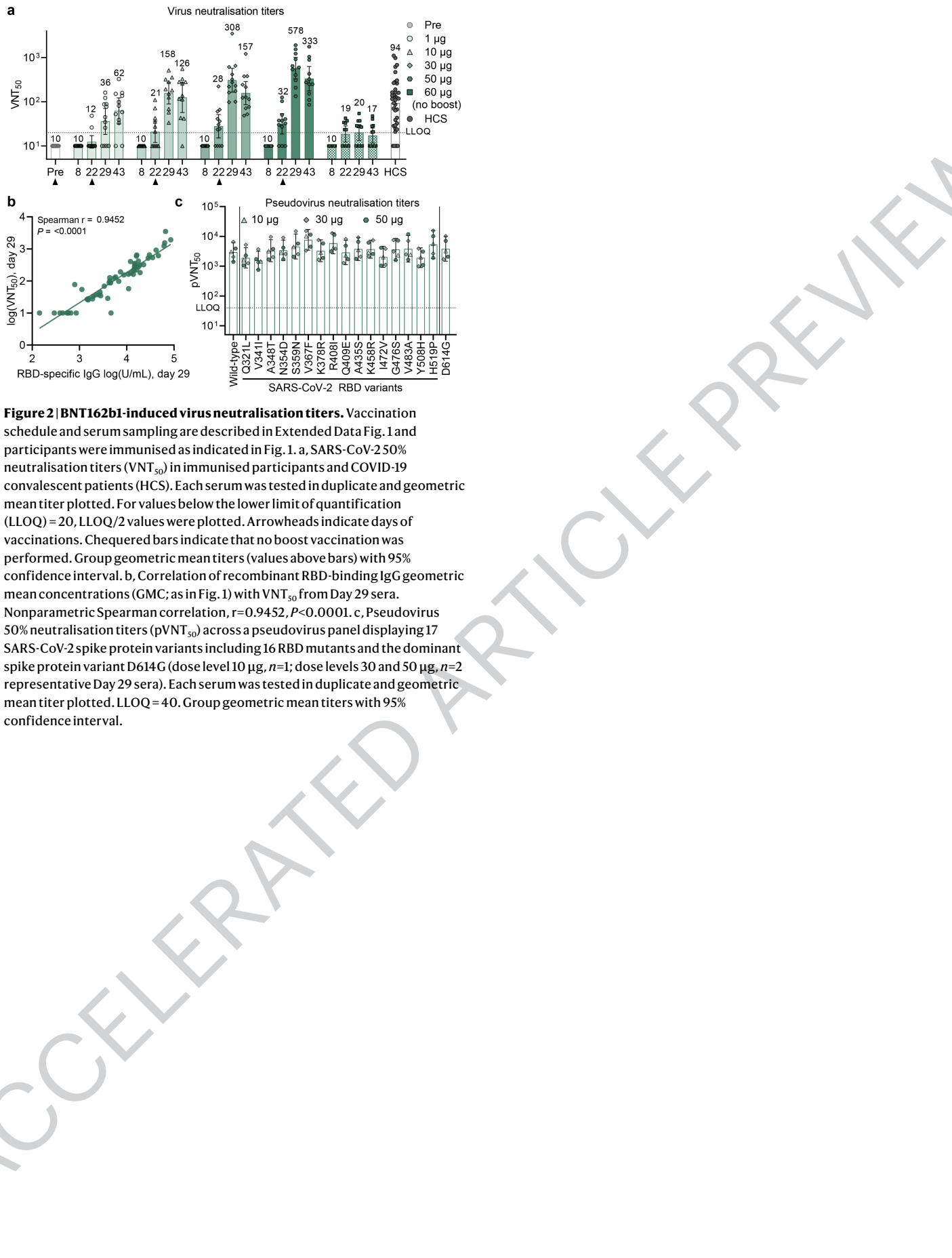
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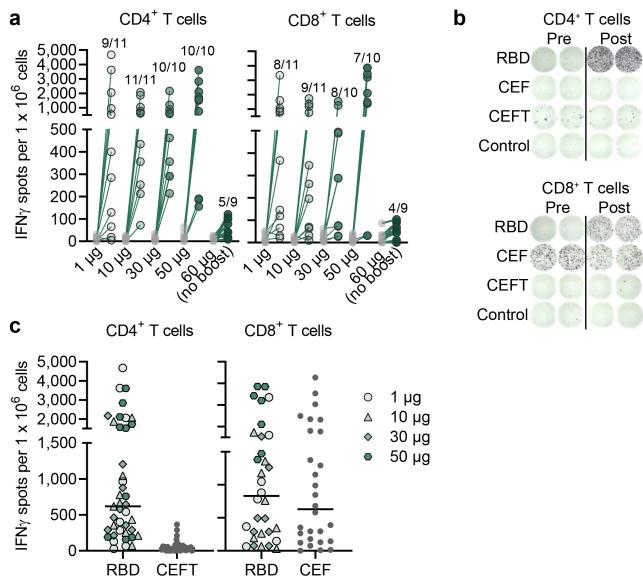
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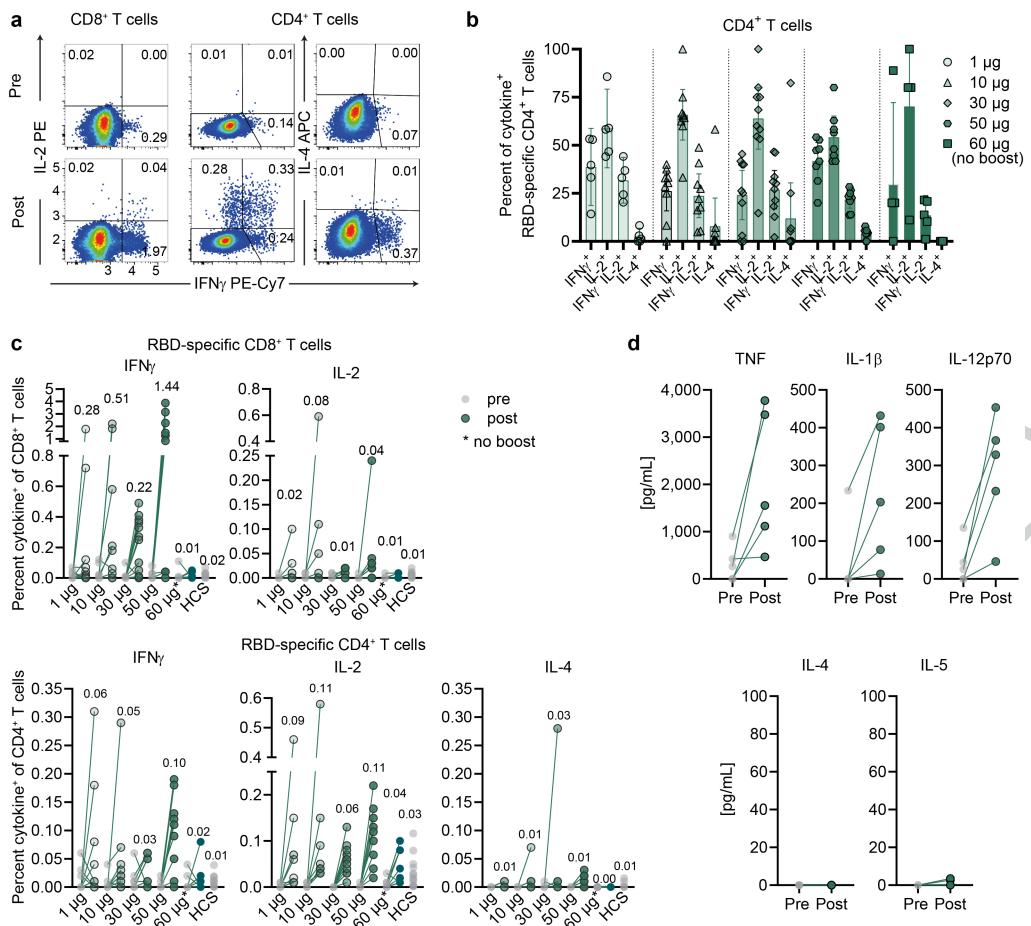
**Fig. 1 | BNT162b1-induced IgG concentrations.** Vaccination schedule and serum sampling are described in Extended Data Fig. 1. Participants were immunised with BNT162b1 on Days 1 (all dose levels) and 22 (all dose levels except 60 µg) ( $n=12$  per group, from Day 22 on  $n=11$  for the 10 µg and 50 µg cohort). Pre-dose responses across all dose levels were combined. COVID-19 convalescent samples (HCS,  $n=38$ ) were obtained at least 14 days after PCR-confirmed diagnosis and at a time when the donors were no longer symptomatic. Each serum was tested in duplicate and geometric mean concentration plotted. For values below the lower limit of quantification (LLOQ) = 1.15, LLOQ/2 values were plotted. Arrowheads indicate days of vaccination. Chequered bars indicate that no boost vaccination was performed. Group geometric mean concentrations (GMC; values above bars) with 95% confidence interval.



**Figure 2 | BNT162b1-induced virus neutralisation titers.** Vaccination schedule and serum sampling are described in Extended Data Fig. 1 and participants were immunised as indicated in Fig. 1. a, SARS-CoV-2 50% neutralisation titers ( $VNT_{50}$ ) in immunised participants and COVID-19 convalescent patients (HCS). Each serum was tested in duplicate and geometric mean titer plotted. For values below the lower limit of quantification (LLOQ) = 20, LLOQ/2 values were plotted. Arrowheads indicate days of vaccinations. Chequered bars indicate that no boost vaccination was performed. Group geometric mean titers (values above bars) with 95% confidence interval. b, Correlation of recombinant RBD-binding IgG geometric mean concentrations (GMC; as in Fig. 1) with  $VNT_{50}$  from Day 29 sera. Nonparametric Spearman correlation,  $r=0.9452$ ,  $P<0.0001$ . c, Pseudovirus 50% neutralisation titers ( $pVNT_{50}$ ) across a pseudovirus panel displaying 17 SARS-CoV-2 spike protein variants including 16 RBD mutants and the dominant spike protein variant D614G (dose level 10 µg,  $n=1$ ; dose levels 30 and 50 µg,  $n=2$  representative Day 29 sera). Each serum was tested in duplicate and geometric mean titer plotted. LLOQ = 40. Group geometric mean titers with 95% confidence interval.



**Figure 3 | Frequency and magnitude of BNT162b1-induced CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses.** The vaccination schedule is described in Extended Data Fig. 1. PBMCs obtained on Day 1 (pre-prime) and on Day 29 (7 days post-boost for cohorts 1, 10 µg, n=11 each; 30 and 50 µg, n=10 each; 28 days post-prime for the 60 µg cohort, n=9) were enriched for CD4<sup>+</sup> or CD8<sup>+</sup> T-cell effectors and separately stimulated over night with an overlapping peptide pool representing the vaccine-encoded RBD for assessment in direct *ex vivo* IFN $\gamma$  ELISpot. Common pathogen T-cell epitope pools CEF (CMV, EBV, influenza virus HLA class I epitopes) and CEFT (CMV, EBV, influenza virus, tetanus toxoid HLA class II epitopes) served to assess general T-cell reactivity, cell culture medium served as negative control. Each data point represents the normalised mean spot count from duplicate wells for one study participant, after subtraction of the medium-only control (a, c). a, RBD-specific CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses for each dose cohort. Ratios above post-vaccination data points are the number of participants with detectable CD4<sup>+</sup> or CD8<sup>+</sup> T-cell response within the total number of tested participants per dose cohort. b, Exemplary CD4<sup>+</sup> and CD8<sup>+</sup> ELISpot image of a 10-µg cohort subject. c, RBD-specific CD4<sup>+</sup> and CD8<sup>+</sup> T cell-responses in all prime/boost vaccinated participants (n=42) with a positive response to RBD and their baseline CEFT- and CEF-specific T-cell responses. Horizontal bars indicate median values.



**Figure 4 | Cytokine polarisation of BNT162b1-induced T cells.** The vaccination schedule is described in Extended Data Fig. 1. PBMCs of vaccinated participants (7 days post-boost for cohorts 1 and 10  $\mu$ g,  $n=10$  each; 30  $\mu$ g,  $n=12$ ; 50  $\mu$ g,  $n=9$ ; 28 days post-prime for the 60  $\mu$ g cohort,  $n=11$ ) and COVID-19 recovered donors (HCS,  $n=15$ ; in (c)) were stimulated over night with an overlapping peptide pool representing the vaccine-encoded RBD and analysed by flow cytometry (a-c) and bead-based immunoassay (d). The gating strategy is depicted in Supplementary Fig. 1. a, Exemplary pseudocolor flow cytometry plots of cytokine-producing CD4<sup>+</sup> and CD8<sup>+</sup> T cells from a 10- $\mu$ g cohort participant. b, RBD-specific CD4<sup>+</sup> T cells producing the indicated cytokine as a fraction of total cytokine-producing RBD-specific CD4<sup>+</sup> T cells. Arithmetic

mean with 95% confidence interval. CD4 non-responders (<0.03% total cytokine producing T cells; 1  $\mu$ g,  $n=5$ ; 10  $\mu$ g,  $n=1$ ; 30  $\mu$ g,  $n=2$ ; 50  $\mu$ g,  $n=1$ ; 60  $\mu$ g,  $n=6$ ) were excluded. c, RBD-specific CD8<sup>+</sup> (upper panel) or CD4<sup>+</sup> (lower panel) T cells producing the indicated cytokine as a fraction of total circulating T cells of the same subset. Values above data points indicate mean fractions per dose cohort. d, Cytokine release by PBMCs from the 50- $\mu$ g cohort ( $n=5$ ; assay results from remaining samples of this and other cohorts not available at the time). Each data point represents the mean from duplicate wells subtracted by the DMSO control for one study participant. Lower limits of quantification (LLOQ) were 6.3 pg/mL for TNF, 2.5 pg/mL for IL-1 $\beta$ , 7.6 pg/mL for IL-12p70, 11.4 pg/mL for IL-4 and 5.3 pg/mL for IL-5.

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## Methods

### Clinical trial design

Study BNT162-01 (NCT04380701) is an ongoing, first-in-human, Phase 1/2, open-label dose-ranging clinical trial to assess the safety, tolerability, and immunogenicity of ascending dose levels of various intramuscularly administered BNT162 mRNA vaccine candidates in healthy men and non-pregnant women 18 to 55 years (amended to add 56–85 of age) of age. 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; and a SARS-CoV-2 NAAT-positive nasal swab; those with increased risk for severe COVID-19; and immunocompromised individuals. The primary endpoints of the study are safety and immunogenicity.

In the part of the study reported here, five dose levels (1 µg, 10 µg, 30 µg, 50 µg or 60 µg) of the BNT162b1 candidate were assessed at one site in Germany with 12 healthy participants per dose level in a dose-escalation/de-escalation design. Sentinel dosing was performed in each dose-escalation cohort. Progression in that cohort and dose escalation required data review by a safety review committee. Participants received a BNT162b1 prime dose on Day 1, and a boost dose on Day 22±2. Serum for antibody assays was obtained on Day 1 (pre-prime), 8±1 (post-prime), 22±2 (pre-boost), 29±3 and 43±4 (post-boost). PBMCs for T cell studies were obtained on Day 1 (pre-prime) and 29±3 (post-boost). Tolerability was assessed by patient diary. One subject of the 10 µg, and one subject of the 50 µg dose cohort left the study prior to the boost-immunisation due to withdrawal of consent and private reasons.

The presented data comprise the BNT162b1-immunised cohorts only and are based on a preliminary analysis with a data extraction date of 23 July 2020, focused on analysis of vaccine-induced immunogenicity (secondary endpoint) descriptively summarised at the various time points and on reactogenicity. All participants with data available were included in the immunogenicity analyses.

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 participants provided written informed consent.

### Manufacturing of RNA

BNT162b1 incorporates a Good Manufacturing Practice (GMP)-grade mRNA drug substance that encodes the trimerised SARS-CoV-2 spike glycoprotein RBD antigen. The RNA is generated from a DNA template by *in vitro* transcription in the presence of 1-methylpsudouridine-5'-triphosphate (m1ΨTP; Thermo Fisher Scientific) instead of uridine-5'-triphosphate (UTP). Capping is performed co-transcriptionally using a trinucleotide cap 1 analogue ((m<sub>2</sub><sup>7,3'-O</sup>)Gppp(m<sup>2'-O</sup>)ApG; TriLink). The antigen-encoding RNA contains sequence elements that increase RNA stability and translation efficiency in human dendritic cells<sup>13,14</sup>. The mRNA is formulated with lipids to obtain the RNA-LNP drug product. The vaccine was transported and supplied as a buffered-liquid solution for IM injection and was stored at -80 °C.

### Proteins and peptides

A pool of 15-mer peptides overlapping by 11 amino acids and covering the whole sequence of the BNT162b1-encoded SARS-CoV-2 RBD, was used for ex vivo stimulation of PBMCs for flow cytometry, IFNy ELISpot and cytokine profiling. CEF (CMV, EBV, influenza virus; human leukocyte antigen (HLA) class I epitope peptide pool) and CEFT (CMV, EBV, influenza virus, tetanus toxoid; HLA class II epitope peptide pool) (both JPT Peptide Technologies) were used as controls for general T-cell reactivity.

### Human convalescent sera and PBMC panel

Human SARS-CoV-2 infection/COVID-19 convalescent sera (*n*=38) were drawn from donors 18–83 years of age at least 14 days after PCR-confirmed diagnosis and at a time when the participants were asymptomatic. The mean age of the donors was 45 years. Neutralising GMTs in subgroups of the donors were as follows: symptomatic infections – 90 (*n*=35); asymptomatic infections – 156 (*n*=3); hospitalized – 618 (*n*=1). Sera were obtained from Sanguine Biosciences (Sherman Oaks, CA), the MT Group (Van Nuys, CA) and Pfizer Occupational Health and Wellness (Pearl River, NY). Human SARS-CoV-2 infection/COVID-19 convalescent PBMC samples (*n*=15) were collected from donors 22–79 years of age 30–62 days after PCR-confirmed diagnosis when donors were asymptomatic. PBMC donors had asymptomatic or mild infections (*n*=13; clinical score 1 and 2) or had been hospitalized (*n*=2; clinical score 4 and 5). Blood samples were obtained from the Frankfurt University Hospital (Germany).

### Cell culture and primary cell isolation

Vero cells (CCL-81) and Vero E6 cells (ATCC CRL-1586) were sourced from American Type Culture Collection (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 cultured in Dulbecco's modified Eagle's medium (DMEM) with GlutaMAX™ (Gibco) supplemented with 10% fetal bovine serum (FBS) (Sigma-Aldrich). Cell lines were tested for mycoplasma contamination after receipt and before expansion and cryopreservation. PBMCs were isolated by Ficoll-Hypaque (Amersham Biosciences) density gradient centrifugation and cryopreserved prior to subsequent analysis.

### RBD-binding IgG assay

A recombinant SARS-CoV-2 RBD containing a C-terminal Avitag™ (Acro Biosystems) was bound to streptavidin-coated Luminex microspheres. Heat-inactivated participant sera were diluted 1:500, 1:5,000, and 1:50,000. Following an overnight incubation at 2–8 °C while shaking, plates were washed in a solution containing 0.05% Tween-20. A secondary R-PE labelled goat anti-human IgG polyclonal antibody (1:500; Jackson Labs) was added for 90 minutes at room temperature while shaking, before plates were washed once more in a solution containing 0.05% Tween-20. Data were captured as median fluorescent intensities (MFIs) using a Bioplex200 system (Bio-Rad) and 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. Three dilutions were used to increase the likelihood that at least one result for any sample would fall within the useable range of the standard curve. Assay results were reported in U/mL of IgG. The final assay results were expressed as the geometric mean concentration of all sample dilutions that produced a valid assay result within the assay range.

### SARS-CoV-2 neutralisation assay

The neutralisation assay used a previously described strain of SARS-CoV-2 (USA\_WA1/2020) that had been rescued by reverse genetics and engineered by the insertion of an mNeonGreen (mNG) gene into open reading frame 7 of the viral genome<sup>33</sup>. This reporter virus generates similar plaque morphologies and indistinguishable growth curves from wild-type virus. Viral master stocks (2×10<sup>7</sup> PFU/mL) were grown in Vero E6 cells as previously described<sup>33</sup>. With patient convalescent sera, the fluorescent neutralisation assay produced comparable results to the conventional plaque reduction neutralisation assay<sup>34</sup>. Serial dilutions of heat-inactivated sera were incubated with the reporter virus (2×10<sup>4</sup> PFU per well to yield a 10–30% infection rate of the Vero CCL81

monolayer) for 1 hour at 37 °C before inoculating Vero CCL81 cell monolayers (targeted to have 8,000 to 15,000 cells in a central field of each well at the time of seeding, 24 hours before infection) in 96-well plates to allow accurate quantification of infected cells. Total cell counts per well were enumerated by nuclear stain (Hoechst 33342) and fluorescent virally infected foci were detected 16-24 hours after inoculation with a Cytaction 7 Cell Imaging Multi-Mode Reader (BioTek) with Gen5 Image Prime version 3.09. 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 (VNT<sub>50</sub>) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.

#### VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay

Vesicular stomatitis virus (VSV)-SARS-CoV-2-S pseudoparticle generation and neutralisation assays were performed as previously described<sup>21</sup>. Briefly, human codon optimized SARS-CoV-2 spike (GenBank: MN908947.3) was synthesised (Genscript) and cloned into an expression plasmid. SARS-CoV-2 complete genome sequences were downloaded from GISAID nucleotide database (<https://www.gisaid.org>) on 20 March 2020, as described previously<sup>21</sup>. Sequences were curated and genetic diversity of the spike-encoding gene was assessed across high quality genome sequences using custom pipelines. Amino acid substitutions were cloned into the spike expression plasmid using site-directed mutagenesis. HEK293T cells (ATCC CRL-3216) were seeded (culture medium: DMEM high glucose [Life Technologies] supplemented with 10% heat-inactivated FBS [Life Technologies], 90.1 units/mL penicillin, 90.1 µg/mL streptomycin and 0.26 mg/mL L-glutamine [Life Technologies]) and transfected the following day with spike expression plasmid using Lipofectamine LTX (Life Technologies) following the manufacturer's protocol. At 24 hours post-transfection at 37 °C, cells were infected with the VSVΔG:mNeon/VSV-G diluted in Opti-MEM (Life Technologies) at a multiplicity of infection of 1. Cells were incubated 1 hour at 37 °C, washed to remove residual input virus and overlaid with infection medium (DMEM high glucose supplemented with 0.7% Low IgG BSA [Sigma], 1 mM sodium pyruvate [Life Technologies] and 0.05 µg/mL Gentamicin [Life Technologies]). After 24 hours at 37 °C, the supernatant containing VSV-SARS-CoV-2-S pseudoparticles was collected, centrifuged at 3000xg for 5 minutes to clarify and stored at -80 °C until further use.

For pseudovirus neutralisation assays, Vero cells (ATCC CCL-81) were seeded in 96-well plates in culture medium and allowed to reach approximately 85% confluence before use in the assay (24 hours later). Sera were serially diluted 1:2 in infection medium starting with a 1:40 dilution. VSV-SARS-CoV-2-S pseudoparticles were diluted 1:1 in infection medium for a fluorescent focus unit (ffu) count in the assay of ~1000. Serum dilutions were mixed 1:1 with pseudoparticles for 30 minutes at room temperature prior to addition to Vero cells and incubation at 37 °C for 24 hours. Supernatants were removed and replaced with PBS (Gibco), and fluorescent foci were quantified using the SpectraMax i3 plate reader with MiniMax imaging cytometer (Molecular Devices). Neutralisation titers were calculated in GraphPad Prism version 8.4.2 by generating a 4-parameter logistical (4PL) fit of the percent neutralisation at each serial serum dilution. The 50% pseudovirus neutralisation titre (pVNT<sub>50</sub>) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.

#### IFNγ ELISpot

IFNγ ELISpot analysis was performed *ex vivo* (without further *in vitro* culturing for expansion) using PBMCs depleted of CD4<sup>+</sup> and enriched for CD8<sup>+</sup> T cells (CD8<sup>+</sup> effectors), or depleted of CD8<sup>+</sup> and enriched for CD4<sup>+</sup> T cells (CD4<sup>+</sup> effectors). Tests were performed in duplicate and with a positive control (anti-CD3 monoclonal antibody [1:1,000; Mabtech]). Multiscreen filter plates (Merck Millipore) pre-coated with IFNγ-specific antibodies (ELISpotPro kit, Mabtech) were washed with

PBS and blocked with X-VIVO 15 medium (Lonza) containing 2% human serum albumin (CSL-Behring) for 1-5 hours. Per well, 3.3 × 10<sup>5</sup> effector cells were stimulated for 16-20 hours with an overlapping peptide pool representing the vaccine-encoded RBD. Bound IFNγ was visualized using a secondary anti-IFNγ antibody directly conjugated with alkaline phosphatase (1:250; ELISpotPro kit, Mabtech) followed by incubation with 5-bromo-4-chloro-3'-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate (ELISpotPro kit, Mabtech). Plates were scanned using an AID Classic Robot ELISPOT Reader and analysed by AID ELISPOT 7.0 software (AID Autoimmun Diagnostika). Spot counts were summarised as mean values of each duplicate. T-cell responses stimulated by peptides were compared to effectors incubated with 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.<sup>35,36</sup>, to provide sensitivity while maintaining control over false positives.

To account for varying sample quality reflected in the number of spots in response to anti-CD3 antibody stimulation, a normalisation method was applied to enable direct comparison of spot counts/strength of response between individuals. This dependency was modelled in a log-linear fashion with a Bayesian model including a noise component (unpublished). For a robust normalisation, each normalisation was sampled 10,000 times from the model and the median taken as normalised spot count value. Likelihood of the model:  $\log \lambda_E = \alpha \log \lambda_p + \log \beta_j + \sigma \epsilon$ , where  $\lambda_E$  is the normalized spot count of the sample,  $\alpha$  is a stable factor (normally distributed) common among all positive controls  $\lambda_p$ ,  $\beta_j$  a sample  $j$  specific component (normally distributed) and  $\sigma \epsilon$  is the noise component, of which  $\sigma$  is Cauchy distributed and  $\epsilon$  is Student's-t distributed.  $\beta_j$  ensures that each sample is treated as a different batch.

#### Flow cytometry

Cytokine-producing T cells were identified by intracellular cytokine staining. PBMCs thawed and rested for 4 hours in OptiMizer medium supplemented with 2 µg/mL DNase I (Roche), were restimulated with a peptide pool representing the vaccine-encoded SARS-CoV-2 RBD (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 (CD3 BV421, 1:250; CD4 BV480, 1:50; CD8 BB515, 1:100; all BD Biosciences) in flow buffer (DPBS [Gibco] supplemented with 2% FBS [Biochrom], 2 mM ethylenediaminetetraacetic acid (EDTA; [Sigma-Aldrich]) for 20 minutes at 4 °C. Afterwards, samples were fixed and permeabilised using the Cytofix/Cytoperm kit according to manufacturer's instructions (BD Biosciences). Intracellular staining was performed in Perm/Wash buffer for 30 minutes at 4 °C (CD3 BV421, 1:250; CD4 BV480, 1:50; CD8 BB515, 1:100; IFNγ PE-Cy7, 1:50; IL-2 PE, 1:10; IL-4 APC, 1:500; all BD Biosciences). Samples were acquired on a fluorescence-activated cell sorter (FACS) VERSE instrument (BD Biosciences) using BD FACSuite™ software version 1.0.6 and analysed with FlowJo software version 10.5.3 (FlowJo LLC, BD Biosciences). RBD-specific cytokine production was corrected for background by subtraction of values obtained with dimethyl sulfoxide (DMSO)-containing medium. Negative values were set to zero. Cytokine production in Fig. 4b was calculated by summing up the fractions of all CD4<sup>+</sup> T cells positive for either IFNγ, IL-2 or IL-4, setting this sum to 100% and calculating the fraction of each specific cytokine-producing subset thereof. Pseudocolor plot axes are in log10 scale.

#### Cytokine profiling

Human PBMCs were restimulated for 48 hours with SARS-CoV-2 RBD peptide pool (2 µg/mL final concentration per peptide). Stimulation with DMSO-containing medium served as negative controls. Concentrations of tumour necrosis factor (TNF), IL-1β, IL-12p70, IL-4 and IL-5 in supernatants were determined using a bead-based, 11-plex T<sub>H</sub>1/T<sub>H</sub>2 human ProcartaPlex immunoassay (Thermo Fisher Scientific)

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according to the manufacturer's instructions. Fluorescence was measured with a Bioplex200 system (Bio-Rad) and analysed with ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific). RBD-specific cytokine production was corrected for background by subtraction of values obtained with DMSO-containing medium. Negative values were set to zero.

## Statistical analysis

The sample size for the reported part of the study was not based on statistical hypothesis testing. All participants with data available were included in the safety and immunogenicity analyses. The statistical method of aggregation used for the analysis of antibody concentrations and titers is the geometric mean and the corresponding 95% confidence interval. Employing the geometric mean allows to account for non-normal distribution of antibody concentrations and titers spanning several orders of magnitude. Spearman correlation was used to evaluate the monotonic relationship between non-normally distributed data sets.

All statistical analyses were performed using GraphPad Prism software version 8.4.2.

## Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

## 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 20 March 2020, as described previously<sup>21</sup>. Upon completion of this clinical trial, summary-level results will be made public and shared in line with data sharing guidelines.

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**Acknowledgements** We thank Mikael Dolsten, Pfizer Chief Scientific Officer, for advice during drafting of the manuscript. We thank C. Anders, C. Anft, N. Beckmann, K. Bissinger, G. Boros, P. Cienskowski, K. Clarke, C. Ecker, A. Engelmann, Y. Feuchter, L. Heesen, M. Hossainzadeh, S. Jägle, L. Jeck, O. Kahl, M. Knezovic, T. Kotur, M. Kretschmer, O. Pfante, J. Reinholz, L.-M. Schmid, R. Schulz, B. Stock, C. Müller, S. Murphy, G. Szabó and M. Vehreschild for technical support, project management and advice, and A. Finlayson and M. Rao for providing editorial assistance. We thank P. Koch and F. Groher for data management and analysis. We thank S. Liebscher and O. Kistner for expert advice. We thank Judith Absalon for manuscript advice. We thank the CRS Team (Mannheim and Berlin) for study conduct: S. Baumann, M. Berse, M. Casjens, B. Ehrlich, F. Seitz. We thank the Pfizer Vaccines Clinical Assays Team and the Pfizer Aviation Team for technical and logistical support of serology analyses. We thank GISAID Nucleotide database for sharing of SARS-CoV-2 complete genome sequences. BioNTech is the Sponsor of the study and responsible for the design, data collection, data analysis, data interpretation, and writing of the report. Pfizer advised on the study and the manuscript, generated serological data, and contracted for the generation of serological data. The corresponding authors had full access to all the data in the study and had final responsibility for the decision to submit the data for publication. All study data were available to all authors. This study was not supported by any external funding at the time of submission.

**Author contributions** U.S. conceived and conceptualised the work and strategy supported by Ö.T. Experiments were planned or supervised by E.D., C. F.-G., C.A.K., L.M.K., U.L., A.M., J.Q., P.-Y.S., and I.V.. A.B., D.C., M.C., C. F.-G., W.K., K.P., J.Q., I.S. and P.-Y.S. performed experiments. D.B., S.B., E.D., P.R.D., J.G., K.U.J., A.-K.E., L.M.K., M.-C.K., V.L., A.M., J.Q., I.S., I.V. and M.V. analysed data. D.M. planned and supervised dashboards for analysis of clinical trial data. R.H. was responsible for data normalization and adaption. C.B. and C.R. were responsible for biomarker and R&D program management. K.K. optimized the mRNA. M.B., S.B., B.F., A.K.-B., D.L. and T.P., A.S., coordinated operational conduct of the clinical trial. J.L.P. advised on the trial, and J.L. and K.A.S advised on experiments. U.S., Ö.T., supported by M.B., E.D., P.R.D., K.U.J., L.M.K., A.M., I.V. and M.V., interpreted data and wrote the manuscript. All authors supported the review of the manuscript.

**Competing interests** All authors have completed the International Committee of Medical Journal Editors (ICMJE) uniform disclosure form at [www.icmje.org/coi\\_disclosure.pdf](http://www.icmje.org/coi_disclosure.pdf) and declare: U.S. and Ö.T. are management board members and employees at BioNTech SE (Mainz, Germany); D.B., C.B., S.B., E.D., A.-K.E., B.F., J.G., R.H., M.-C.K., U.L., V.L., D.M., C.R., J.S. and T.P. are employees at BioNTech SE; K.K., L.M.K., I.V., A.M., J.Q. and M.V. are employees at BioNTech RNA Pharmaceuticals GmbH; M.B. is an employee at Bexon Clinical Consulting LLC. A.B., C.A.K. and K.P. are employees of Regeneron Pharmaceuticals Inc; K.K., A.M., U.S. and Ö.T. are inventors on patents and patent applications related to RNA technology and COVID-19 vaccine; D.B., C.B., S.B., E.D., J.G., K.K., R.H., A.K.-B., L.M.K., D.L., U.L., A.M., C.R., U.S., Ö.T., I.V. and M.V. have securities from BioNTech SE; D.C., M.C., P.R.D., K.U.J., W.K., J.L., J.L.P., I.L.S. and K.A.S. are employees at Pfizer and may have securities from Pfizer; C.A.K. is an officer at Regeneron Pharmaceuticals, Inc; A.B., C.A.K. and K.P. have securities from Regeneron Pharmaceuticals, Inc; C.F.-G. and P.-Y.S. received compensation from Pfizer to perform the neutralisation assay; no other relationships or activities that could appear to have influenced the submitted work.

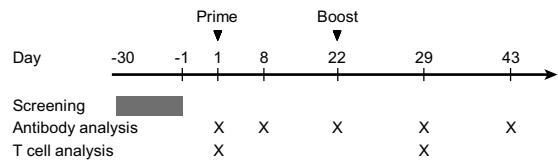
## Additional information

**Supplementary information** is available for this paper at <https://doi.org/10.1038/s41586-020-2814-7>.

**Correspondence and requests for materials** should be addressed to U.S.

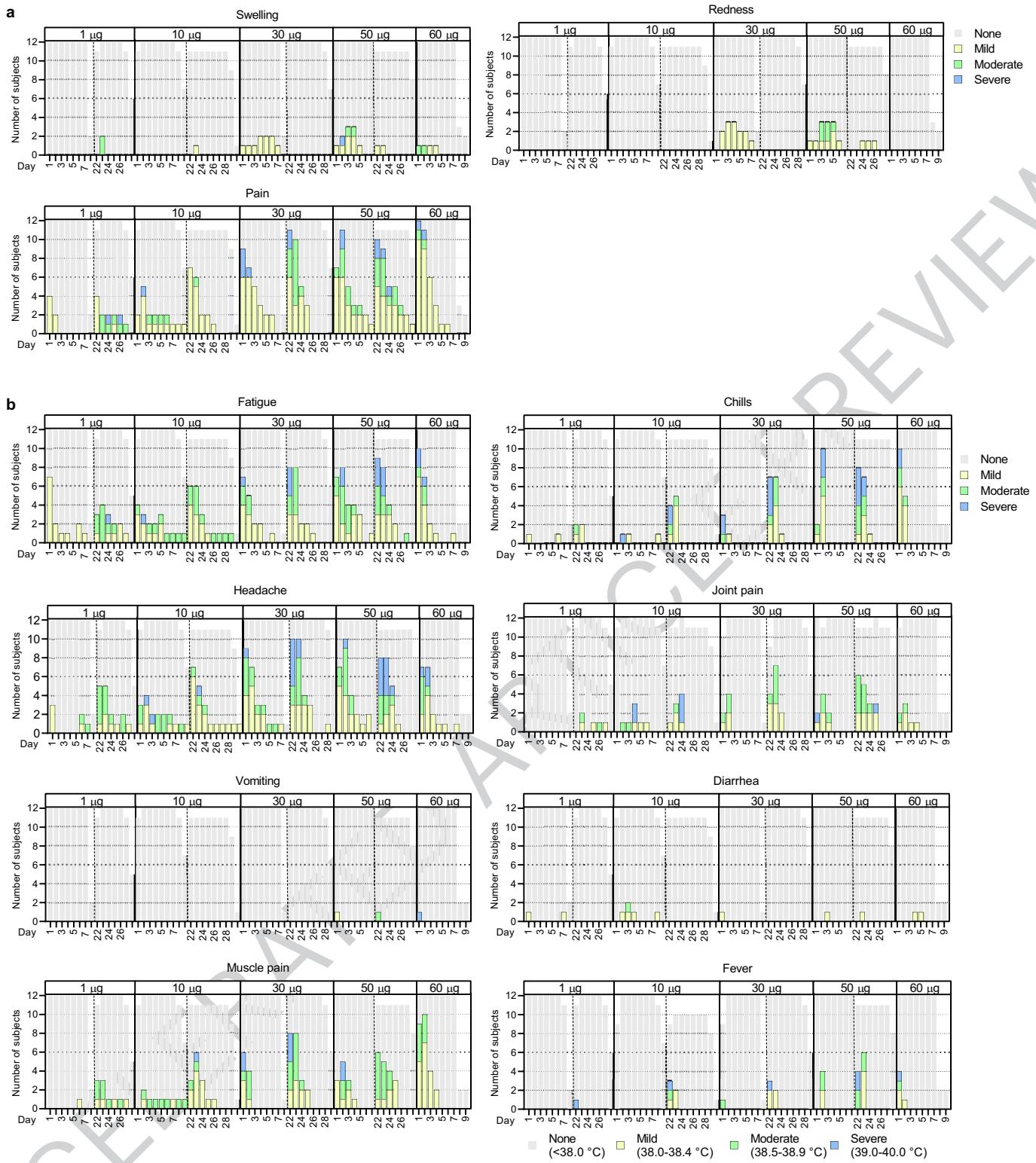
**Peer review information** *Nature* thanks Barbra Richardson and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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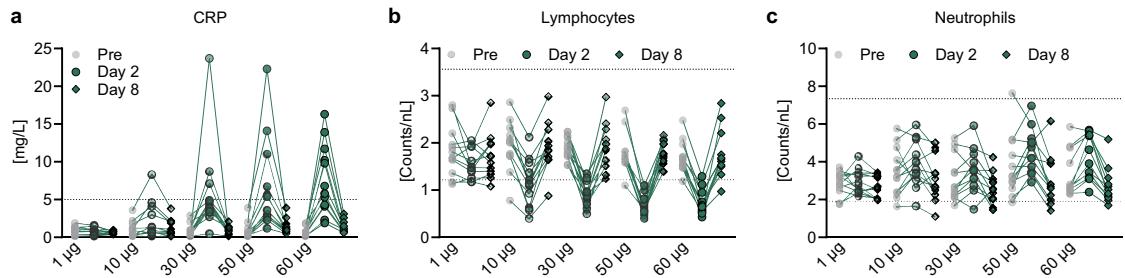
**Extended Data Fig. 1 | Schedule of vaccination and assessment.** Study participants received a prime immunisation with BNT162b1 on Day 1 (all dose levels), and a boost immunisation on Day  $22 \pm 2$  (all dose levels except 60  $\mu$ g). Serum was obtained on Day 1 (pre-prime),  $8 \pm 1$  (post-prime),  $22 \pm 2$  (pre-boost),  $29 \pm 3$  and  $43 \pm 4$  (post-boost). PBMCs were obtained on Day 1 (pre-prime) and  $29 \pm 3$  (post-boost).

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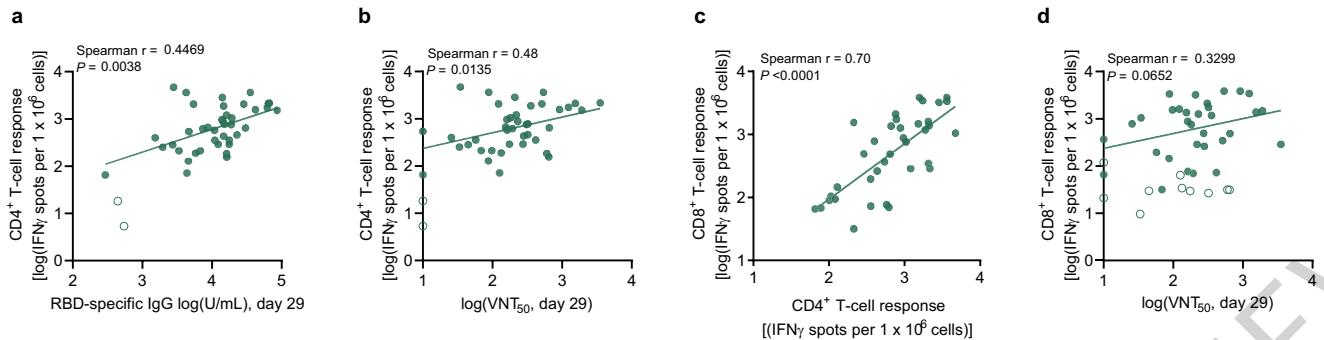
**Extended Data Fig. 2 | Solicited adverse events.** Number of participants with local (a) or systemic solicited adverse events (AEs) (b). Participants were immunised with BNT162b1 on Days 1 (all dose levels) and 22 (all dose levels except 60 µg). n=12 subjects were injected per group, from Day 22 on n=11 for the 10 µg and 50 µg cohort due to discontinuation of patients due to non-vaccine related reasons. Grey shading indicates number of participants at

each time point. As per protocol, AEs were recorded up to 7 days after each immunisation (Days 1-7 and 22-28) to determine reactogenicity; for some participants 1-2 additional days of follow-up were available. Grading of AEs was performed according to U.S. Food and Drug Administration (FDA) recommendations<sup>37</sup>.



**Extended Data Fig. 3 | Pharmacodynamic markers.** Participants were immunised with BNT162b1 on Days 1 (all dose levels) and 22 (all dose levels except 60 µg) ( $n=12$  per group, from Day 22 on  $n=11$  for the 10 µg and 50 µg cohort). a, Kinetics of C-reactive protein (CRP) level. b, Kinetics of lymphocyte

counts. c, Kinetics of neutrophil counts. Dotted lines indicate upper and lower limit of reference range. For values below the lower limit of quantification (LLOQ)=0.3, LLOQ/2 values were plotted (a).


**Extended Data Fig. 4 | Correlation of antibody and T-cell responses.**

Participants were immunised with BNT162b1 on Days 1 (all dose levels) and 22 (all dose levels except 60 µg) ( $n=12$  per group, from Day 22 on  $n=11$  for the 10 µg and 50 µg cohort). Data are plotted for all prime/boost vaccinated participants (cohorts 1, 10, 30 and 50 µg) with data points for participants with no detectable T cell response (open circles; a, b, d) excluded from correlation analysis. Nonparametric Spearman correlation. a, Correlation of RBD-specific

IgG responses (as in Fig. 1) with CD4<sup>+</sup> T-cell responses on Day 29 (as in Fig. 3). r=0.4829, P=0.0014. b, Correlation of VNT<sub>50</sub> (as in Fig. 2a) with CD4<sup>+</sup> T-cell responses (as in Fig. 3). r=0.48, P=0.0057. c, Correlation of CD4<sup>+</sup> with CD8<sup>+</sup> T-cell responses ( $n=51$  as in Fig. 3a) from Day 29 in dose cohorts 1 to 60 µg. r=0.7, P<0.0001. d, Correlation of VNT<sub>50</sub> (as in Fig. 2a) with CD8<sup>+</sup> T-cell responses (as in Fig. 3) on Day 29. r=0.3299, P=0.0652.

**Extended Data Table 1 | Demographic characteristics**

Cohort		1 µg (N=12) n (%)	10 µg (N=12) n (%)	30 µg (N=12) n (%)	50 µg (N=12) n (%)	60 µg (N=12) n (%)	Total (N=60) n (%)
Sex	Male	5 (41.7)	4 (33.3)	8 (66.7)	6 (50.0)	7 (58.3)	30 (50.0)
	Female	7 (58.3)	8 (66.7)	4 (33.3)	6 (50.0)	5 (41.7)	30 (50.0)
Race	Caucasian	12 (100)	11 (91.7)	12 (100)	12 (100)	11 (91.7)	58 (96.7)
	African American	0	1 (8.3)	0	0	0	1 (1.7)
	Asian	0	0	0	0	1 (8.3)	1 (1.7)
Age at vaccination (years)	Mean (SD)	38.21 (10.48)	43.62 (11.03)	35.74 (8.60)	33.88 (10.72)	35.81 (12.50)	37.5 (10.91)
	Median	39.8	46.6	35.2	30.8	30.1	36.0
	Min, Max	21.4, 55.8	25.1, 55.0	23.9, 54.0	19.9, 47.8	20.9, 53.2	19.9, 55.8

N, number of subjects in the specified group. This value is the denominator for the percentage calculations. n, number of subjects with the specified characteristics.

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**Extended Data Table 2 | Subject disposition and analysis sets**

Cohort	BNT162b1 vaccinated		Antibody analysis					T-cell analysis	
	Prime	Boost	Day 1	Day 8±1	Day 22±2	Day 29±3	Day 43±4	Day 1	Day 29±3
1 µg	12	12	12	12	12	12	12	10* (10)	11 (10)
10 µg	12	11	12	12	12	11	11	11 (10)	11 (10)
30 µg	12	12	12	12	12	12	12	10 (12)	10 (12)
50 µg	12	11	12	12	12	11	11	10 (9)	10 (9)
60 µg	12	N/A	12	12	11	12	12	9 (11)	9 (11)

Antibody analysis: Values indicate number of participants for whom virus neutralisation assays and RBD binding IgG antibody assays were performed. T-cell analysis: Values indicate number of participants for whom IFNy ELISpot and flow cytometry (values in parentheses) were performed. N/A, not applicable. \*9 for CD4<sup>+</sup> response data.

**Extended Data Table 3 | BNT162b1-induced geometric mean RBD-binding IgG concentrations and 95% confidence intervals**

Dose	-	1 µg				10 µg				30 µg				50 µg				60 µg				HCS	
Day	Pre	8	22	29	43	8	22	29	43	8	22	29	43	8	22	29	43	8	22	29	43	-	
N		60	12	12	12	12	12	12	11	11	12	12	12	12	12	12	11	11	12	11	12	12	38
Geometric mean		1.6	2.6	265	2,015	3,920	1.1	826	9,107	6,707	1.9	1,273	17,051	12,431	1.3	1,672	25,006	18,289	1.2	909	1,058	755	602
Lower 95% CI		1.1	1.3	104	948	1,915	0.58	295	5,290	3,383	0.89	655	10,534	7,900	0.61	992	14,228	10,029	0.57	438	590	521	350
Upper 95% CI		2.1	5.2	676	4,284	8,023	2.3	2,316	15,676	13,296	3.9	2,475	27,600	19,559	2.9	2,818	43,950	33,350	2.5	1,887	1,899	1,094	1,034

Geometric mean RBD-binding IgG concentration values and 95% confidence intervals by cohort and sampling time-point as displayed in Fig. 1. CI, confidence interval; N, Sample number; HCS, Human COVID-19 convalescent sample.

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**Extended Data Table 4 | BNT162b1-induced virus geometric mean 50% neutralisation titers and 95% confidence intervals**

Dose	-	1 µg				10 µg				30 µg				50 µg				60 µg				HCS
Day	Pre	8 22 29 43				8 22 29 43				8 22 29 43				8 22 29 43				8 22 29 43				-
N	60	12 12 12 12				12 12 11 11				12 12 12 12				12 12 11 11				12 11 12 12				38
Geometric mean	10	10 12 36 62				10 21 11 11				10 28 308 157				10 32 578 333				10 19 20 17				94
Lower 95% CI	10	10 8.9 18 31				10 12 90 57				10 15 165 86				10 19 331 176				10 12 13 12				62
Upper 95% CI	10	10 17 71 123				10 36 278 276				10 51 574 288				10 54 1,009 631				10 28 30 25				142

Geometric mean 50% virus neutralisation titer values ( $VNT_{50}$ ) and 95% confidence intervals by cohort and sampling time-point as displayed in Fig. 2a. CI, confidence interval; N, Sample number; HCS, Human COVID-19 convalescent sample.

**Extended Data Table 5 | BNT162b1-induced geometric mean 50% pseudovirus neutralisation titers and 95% confidence intervals**

Variant	WT	Q321L	V341I	A348T	N354D	S359N	V367F	K378R	R408I	Q409E	A435S	K458R	I472V	G476S	V483A	Y508H	H519P	D614G
N	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
GMT	2,948	1,898	1,561	3,302	3,411	4,563	7,668	3,280	5,879	2,872	3,776	3,711	2,055	3,641	3,824	1,916	5,352	3,822
Lower 95% CI	1,413	867	767	1,417	1,556	1,737	3,441	1,414	2,601	1,115	1,552	1,850	907	1,547	1,368	896	1,784	1,460
Upper 95% CI	6,151	4,155	3,175	7,692	7,479	11,982	17,088	7,611	13,285	7,400	9,187	7,444	4,655	8,570	10,695	4,093	16,059	10,010

Geometric mean 50% pseudovirus neutralisation titer (pVNT<sub>50</sub>) values and 95% confidence intervals by tested SARS-CoV-2 spike protein variants as displayed in Fig. 2c. CI, confidence interval; N, Sample number; HCS, Human COVID-19 convalescent sample; Variant, SARS-CoV-2 spike protein variant.

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**Extended Data Table 6 | BNT162b1-induced mean cytokine values and 95% confidence intervals**

Dose	1 µg				10 µg				30 µg				50 µg				60 µg			
Cytokines	IFN $\gamma$ <sup>+</sup>	IL-2 <sup>+</sup>	IFN $\gamma$ <sup>+</sup> IL-2 <sup>+</sup>	IL-4 <sup>+</sup>	IFN $\gamma$ <sup>+</sup>	IL-2 <sup>+</sup>	IFN $\gamma$ <sup>+</sup> IL-2 <sup>+</sup>	IL-4 <sup>+</sup>	IFN $\gamma$ <sup>+</sup>	IL-2 <sup>+</sup>	IFN $\gamma$ <sup>+</sup> IL-2 <sup>+</sup>	IL-4 <sup>+</sup>	IFN $\gamma$ <sup>+</sup>	IL-2 <sup>+</sup>	IFN $\gamma$ <sup>+</sup> IL-2 <sup>+</sup>	IL-4 <sup>+</sup>	IFN $\gamma$ <sup>+</sup>	IL-2 <sup>+</sup>	IFN $\gamma$ <sup>+</sup> IL-2 <sup>+</sup>	IL-4 <sup>+</sup>
N	5	5	5	5	9	9	9	9	10	10	10	10	8	8	8	8	5	5	5	5
Mean	39	59	32	2.5	26	66	24	7.9	24	64	27	12	42	54	22	4.0	30	70	14	0.0
Lower 95% CI	19	38	20	0	16	53	12	0	11	48	17	0	32	43	17	1.7	0	28	3.1	0.0
Upper 95% CI	59	79	44	6.8	37	79	35	23	37	80	37	30	51	65	26	6.3	72	113	24	0.0

Mean values and 95% confidence intervals for the individual cytokines tested. CI, confidence interval; N, Sample number.

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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
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- 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

Flow cytometry data was collected using the FACS VERSE instrument (BD Biosciences) and FACSSuite software version 1.0.6 and analysed with FlowJo software version 10.5.3 (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).  
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 CytaCount 7 Cell Imaging Multi-Mode Reader (Biotek) with Gen5 Image Prime version 3.09.  
For VSV-SARS-CoV-2 spike variant pseudovirus neutralisation assay, fluorescent foci were quantified using the SpectraMax i3 plate reader with MiniMax imaging cytometer (Molecular Devices).  
Cytokine profiles in PBMC supernatants were determined using a bead-based, 11-plex TH1/TH2 human ProcartaPlex immunoassay (Thermo Fisher Scientific) according to the manufacturer's instructions and fluorescence was measured with a Bioplex200 system (Bio-Rad).  
No custom software codes have been developed.

### Data analysis

Flow cytometry data was analysed using FlowJo software version 10.5.3 (FlowJo LLC, BD Biosciences).  
ELISpot assays were analysed using AID ELISPOT 7.0 software (AID Autoimmun Diagnostika). T-cell responses stimulated by peptides were compared to T-cell responses stimulated 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.  
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, 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. Cytokine profiles in PBMC supernatants were analysed using ProcartaPlex Analyst 1.0 software (Thermo Fisher Scientific). All statistical analyses were performed using GraphPad Prism software version 8.4.2.

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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
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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

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## 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, 30 µg, 50 µg or 60 µg) of the BNT162b1 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 data available until data extraction date of 13JUL2020 were included. All participants with data available were included in the safety and immunogenicity analyses. For serology/cell-mediated immunity correlation analyses (Ext. Data Fig. 4 a/b/d), data were only plotted for prime/boost vaccinated participants (excluding the 60 µg dose level cohort) with detectable T-cell response. All participants with sufficient PBMC material available were included in the ICS analyses. In Fig. 4b, CD4 non-responders (<0.03% total cytokine producing T cells; 1 µg, n=5; 10 µg, n=1; 30 µg, n=2; 50 µg, n=1; 60 µg, n=6) were excluded. All participants with sufficient PBMC material available were included in the ELISPOT analyses. In Fig. 3c, participants without a T-cell response were excluded, and data from the 60 µg cohort were excluded. In Ext. Data Fig. 4c, only data from participants with both CD4+ and CD8+ T-cell responses were included. For cytokine analysis in Fig. 4d, only assay results from n=5 participants of the 50 µg cohort were available by the time of submission/re-submission. The remaining samples from this and other cohorts were prioritized for other analyses. 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 (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 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input checked="" type="checkbox"/> Human research participants
<input type="checkbox"/>	<input checked="" type="checkbox"/> Clinical data

## Methods

n/a	Involved in the study
	<input checked="" type="checkbox"/> ChIP-seq
	<input type="checkbox"/> <input checked="" type="checkbox"/> Flow cytometry
	<input checked="" type="checkbox"/> MRI-based neuroimaging

## Antibodies

### Antibodies used

Flow cytometry (specificity/host+reactivity/fluorochrome/clone/manufacturer/catalogue number/lot number/dilution/extracellular/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  
 IFN $\gamma$ /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

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

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 (amended to add 56-85 of age) 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 rosters 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 is not published online, but 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 and 43±4 (post-boost). PBMCs for T cell studies were obtained on day 1 (pre-prime) and 29±3 (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 increase 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 OptiMizer medium supplemented with 2 µg/mL DNaseI (Roche), were restimulated with a peptide pool representing the vaccine-encoded SARS-CoV-2 RBD (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 ((DPBS (Gibco) supplemented with 2% FCS (Biochrom), 2 mM EDTA (Sigma-Aldrich)) 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 for 30 minutes at 4 °C.

**Instrument**

Samples were acquired on a FACS VERSE instrument (BD Biosciences).

**Software**

For data analysis FlowJo software version 10.5.3 (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.

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