16.1.11 PUBLICATIONS BASED ON THE STUDY

1. Mulligan MJ, Lyke KE, Kitchin N, et al. Phase 1/2 study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;10.1038/s41586-020-2639-4.

2. Walsh EE, Frenck FW, Falsey AR, et al. Safety and immunogenicity of two RNA-based Covid-19 vaccine candidates. N Engl J Med 2020; DOI: 10.1056/NEJMoa2027906.

3. Polack FP, Thomas SJ, Kitchin N, et al. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. N Engl J Med. 2020;383(27):2603-2615. doi:10.1056/NEJMoa2034577

4. Xie X, Liu Y, Liu J, et al. Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera [published online ahead of print, 2021 Feb 8]. Nat Med. 2021;10.1038/s41591-021-01270-4. doi:10.1038/s41591-021-01270-4

5. Liu Y, Liu J, Xia H, et al. Neutralizing Activity of BNT162b2-Elicited Serum - Preliminary Report [published online ahead of print, 2021 Feb 17]. N Engl J Med. 2021;10.1056/NEJMc2102017. doi:10.1056/NEJMc2102017

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Article

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In March 2020, the World Health Organization (WHO) declared a pandemic of coronavirus disease 2019 (COVID-19), due to severe acute respi atory syndrome coronavirus 2 (SARS-CoV-2)[1]. With rapidly accumulating cases and deaths reported globally[2], a vaccine is urgently needed. We report the a ailable safety, tolerability, and immunogenicity data from an ongoing placebo-controlled, observer-blinded dose escalation study among 45 healthy adults, 8 to 55 years of age, randomized to receive 2 doses, separated by 21 days, of 10 µg, 30 µg, or 100 µg of BNT162b1, a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine that encodes trimerized SARS-CoV-2 spike glycoprotein eceptor-binding domain (RBD). Local reactions and systemic events were dose-dependent, generally mild to moderate, and transient. A second vaccination with 100 µg was not administered due to increased reactogenicity and a lack of meaningfully increased immunogenicity after a single dose compared to the 30 µg dose. RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titers in era increased with dose level and after a second dose. Geometric mean neutral zing titers reached 1.9- to 4.6-fold that of a panel of COVID-19 convalescent human sera at least 14 days after a positive SARS-CoV-2 PCR. These results support further evaluation of this mRNA vaccine candidate. (ClinicalTrials.gov identifier: NCT04368728).

In December 2019, a pneumonia outbreak f unknown cause occurred in Wuhan, China. By January 2020, a novel coronavirus was identified as the etiologic agent. Within a month the genetic sequence of the virus became available (MN908947.3). Severe ac te respiratory syndrome coronavirus 2 (SARS-CoV-2) infec ions and the resulting disease, coronavirus disease 2019 (COVID-19), have spread globally. On 11 March 2020, the World Health Organization (WHO) declared the COVID-19 outbreak a pandemi [1]. To date, the United States has reported the most cases globally[3]. No vaccines are currently available to prevent SARS-CoV-2 infe tion or COVID-19.

The RNA vaccine platform has enabled rapid vaccine development in response to this pandemic. RNA vaccines provide flexibility in the design and expression of vaccine antigens that can mimic antigen structure and expression during natural infection. RNA is required for protein s nthesis, does not integrate into the genome, is transiently expressed, and is metabolized and eliminated by the body's natural mechanisms and, therefore, is considered safe[4–7]. RNA-based prophylactic infectious disease vaccines and RNA therapeutics have been shown o be safe and well-tolerated in clinical trials. In general, vaccination with RNA elicits a robust innate immune response. RNA directs expression of the vaccine antigen in host cells and has intrinsic adjuvant effects[8]. A strength of the RNA vaccine manufacturing platform, irrespective of the encoded pathogen antigen, is the ability to rapidly produce large quantities of vaccine doses against a new pathogen[9,10].

Vaccine RNA can be modified by incorporating 1-methyl-pseudouridine which dampens innate immune sensing and increases mRNA translation *in vivo*[11]. The BNT162b1 vaccine candidate now being studied clinically incorporates such nucleoside-modified messenger RNA (modRNA) and encodes the receptor-binding domain (RBD) of the SARS-CoV-2 spike protein, a key target of virus-neutralizing antibodies[12–14]. The RBD antigen expressed by BNT162b1 is modified by the addition of a T4 fibritin-derived foldon trimerization domain to increase its immunogenicity[15] by multivalent display[16]. The proper folding of the RBDs in the resulting protein construct has been confirmed by high resolution structural analysis (U.S., manuscript in preparation)[17]. The vaccine RNA is formulated in lipid nanoparticles (LNPs) for more efficient delivery into cells after intramuscular injection[18]. BNT162b1 is one of several RNA-based SARS-CoV-2 vaccine candidates being studied in parallel

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for selection to advance to a safety and efficacy trial. Here, we present available data, through 14 days after a second dose in adults 18 to 55 years of age, from an ongoing Phase 1/2 vaccine study with BNT162b1, which is also enrolling adults 65 to 85 years of age (ClinicalTrials.gov identifier: NCT04368728).

Study Design and Demographics

Between 04 May 2020 and 19 June 2020, 76 participants were screened, and 45 participants were randomized and vaccinated. Twelve participants per dose level (10 μg and 30 μg) were vaccinated with BNT162b1 on Days 1 and 21, and 12 participants received a 100-μg dose on Day 1. Nine participants received placebo (Figure 1). The study population consisted of healthy male and nonpregnant female participants with a mean age of 35.4 years (range: 19 to 54 years); 51.1% were male and 48.9% were female. Most participants were white (82.2%) and non-Hispanic/non-Latinx (93.3%) (Extended Data Table 1).

Safety and Tolerability

In the 7 days following either Dose 1 or 2, pain at the injection site was the most frequent solicited local reaction, reported after Dose 1 by 58.3% (7/12) in the 10-μg, 100.0% (12/12 each) in the 30-μg and 100-μg BNT162b1 groups, and 22.2% (2/9) in the placebo group. After Dose 2, pain was reported by 83.3% (10/12) and 100.0% of BNT162b1 recipients at the 10-μg and 30-μg dose levels, respectively, and by 16.7% of placebo recipients. All local reactions were mild or moderate in severity except for one report of severe pain following Dose 1 of 100 μg BNT162b1 (Figure 2; Extended Data Table 2).

The most common systemic events reported in the 7 days after each vaccination in both BNT162b1 and placebo recipients were mild to moderate fatigue and headache. Reports of fatigue and headache were more common in the BNT162b1 groups compared to the placebo group. Additionally, chills, muscle pain, and joint pain were reported among BNT162b1 recipients and not in placebo recipients. Systemic events increased with dose level and were reported in a greater number of participants after the second dose (10-μg and 30-μg groups). Following Dose 1, fever (defined as ≥38.0 °C) was reported by 8.3% (1/12) of participants in both the 10-μg and 30-μg groups and by 50.0% (6/12) of BNT162b1 recipients in the 100-μg group. Following Dose 2, 8.3% (1/12) of participants in the 10-μg group and 75.0% (9/12) of participants in the 30-μg group reported fever ≥38.0 °C. Based on the reactogenicity reported after the first dose of 100 μg and the second dose of 30 μg, participants who received an initial 100-μg dose did not receive a second 100-μg dose. Fevers generally resolved within 1 day of onset. No Grade 4 systemic events or fever were reported. (Figure 3a, b, Extended Data Table 3). Most local reactions and systemic events peaked by Day 2 after vaccination and resolved by Day 7.

Adverse events (AEs) (Extended Data Table 4) were reported by 50.0% (6/12) of participants who received either 10 μg or 30 μg of BNT162b1, 58.3% (7/12) of those who received 100 μg of BNT162b1, and 11.1% (1/9) of placebo recipients. Two participants reported a severe AE: Grade 3 fever 2 days after vaccination in the 30-μg group, and sleep disturbance 1 day after vaccination in the 100-μg group. Related AEs were reported by 25% (3/12 in the 10-μg groups) to 50% (6/12 each in 30-μg and 100-μg groups) of BNT162b1 recipients and by 11.1% (1/9) of placebo recipients. No serious adverse events (SAEs) were reported.

No Grade 1 or greater change in routine clinical laboratory values or laboratory abnormalities were observed for most participants after either of the BNT162b1 vaccinations. Of those with laboratory changes, the largest changes were decreases in lymphocyte count after Dose 1 in 8.3% (1/12), 45.5% (5/11), and 50.0% (6/12) of 10 μg, 30 μg, and 100 μg BNT162b1 recipients, respectively. One participant each in the 10-μg group (8.3% [1/12]) and 30-μg group (9.1% [1/11]) dose levels and 4 participants in the 100-μg group (33.3% [4/12]) had Grade 3 decreases in lymphocytes. These post-Dose 1 decreases in lymphocyte count, were transient and returned to normal 6 to 8 days after vaccination (Extended Data Figure 1). In addition, Grade 2 neutropenia was noted 6 to 8 days after the second dose in 1 participant each in the 10-μg and 30-μg BNT162b1 groups. These two participants continue to be followed in the study and no AEs or clinical manifestations of neutropenia have been reported to date. None of the postvaccination abnormalities observed were associated with clinical findings.

Immunogenicity

RBD-binding IgG concentrations and SARS-CoV-2 neutralizing titers were assessed at baseline, and at 7 and 21 days after the first dose and at 7 (Day 28) and 14 days (Day 35) after the second dose of BNT162b1. By 21 days after the first dose (for all three dose levels) geometric mean concentrations (GMCs) of RBD-binding IgG ranged from 534 to 1,778 U/mL (Figure 4a). In comparison, a panel of 38 SARS-CoV-2 infection/COVID-19 convalescent sera drawn at least 14 days after a polymerase chain reaction (PCR)-confirmed diagnosis from patients 18 to 83 years of age had an RBD-binding IgG GMC of 602 U/mL. (Additional information on the convalescent serum panel is presented in Methods.) By 7 days after the second dose (for the 10 μg and 30 μg dose levels) RBD-binding IgG GMCs had increased to 4,813 to 27,872 U/mL. RBD-binding antibody concentrations among participants who received one dose of 100 μg BNT162b1 did not increase beyond 21 days after the first vaccination. In the participants who received the 10 μg and 30 μg doses of BNT162b1, highly elevated RBD-binding antibody concentrations persisted to the last time point evaluated (Day 35, 14 days after the second dose). These RBD-binding antibody concentrations were 5,880 to 16,166 U/mL compared to 602 U/mL in the human convalescent serum panel.

For all doses, small increases in SARS-CoV-2 neutralizing geometric mean titers (GMTs) were observed 21 days after Dose 1 (Figure 4b). Substantially greater serum neutralizing GMTs were achieved 7 days after the second 10 μg and 30 μg dose, reaching 168 to 267. Neutralizing GMTs further increased by 14 days after the second dose to 180 at the 10 μg dose level and 437 at the 30 μg dose level, compared to 94 for the convalescent serum panel. The kinetics and durability of neutralizing titers are being monitored.

Discussion

The RNA-based SARS-CoV-2 vaccine candidate BNT162b1 administered at 10 μg, 30 μg, and 100 μg to healthy adults 18 to 55 years of age exhibited a tolerability and safety profile consistent with those previously observed for mRNA-based vaccines[5]. A clear dose-level response in elicited neutralizing titers was observed after Doses 1 and 2 in adults 18 to 55 years of age with a particularly steep dose response between the 10 μg and 30 μg dose levels.

Based on the tolerability profile of the first dose at 100 μg and the second dose at 30 μg, participants randomized to the 100-μg group did not receive a second vaccination. Reactogenicity was generally greater after the second dose in the other two dosing levels; however, symptoms were transient and resolved within a few days. Transient decreases in lymphocytes (Grades 1-3) were observed within a few days after vaccination, with lymphocyte counts returning to baseline within 6 to 8 days in all participants. These laboratory abnormalities were not associated with clinical findings. RNA vaccines are known to induce type I interferon, which has been associated with transient migration of lymphocytes into tissues[19–22].

Robust immunogenicity was observed after vaccination with BNT162b1. RBD-binding IgG concentrations were detected at 21 days after the first dose and substantially increased 7 days after the second dose given at Day 21. After the first dose, the RBD-binding IgG GMCs (10 μg dose recipients) were similar to those observed in a panel of

38 convalescent human serum samples, obtained at least 14 days after a PCR-confirmed diagnosis of SARS-CoV-2 infection/COVID-19. Post-Dose 1 GMCs were similar in the 30 µg and 100 µg groups and higher than those in the convalescent serum panel. After Dose 2 with 10 µg or 30 µg BNT162b1, the RBD-binding IgG GMCs were ~8.0-fold to ~50-fold that of the convalescent serum panel GMC.

The higher RBD-binding IgG GMC elicited by the vaccine relative to the GMC of the human convalescent serum panel may be attributed, in part, to antibodies that bind epitopes that are exposed on the RNA-expressed RBD immunogen and the recombinant RBD target antigen of the binding assay but are buried and inaccessible to antibody on the RBDs that are incorporated into the spikes of SARS-CoV-2 virions. Therefore, neutralization provides a measure of vaccine-elicited antibody response that is more relevant to potential protection. Neutralization titers were measurable after a single vaccination at Day 21 for all dose levels. At Day 28 (7 days after Dose 2), substantial SARS-CoV-2 neutralization titers were observed. The virus-neutralizing GMTs after the 10 µg and 30 µg Dose 2 were, respectively, 1.8-fold and 2.8-fold the GMT of the convalescent serum panel. By Day 35 (14 days after Dose 2), despite the decrease in RBD-binding IgG titers since Day 28, neutralizing GMTs continued to rise, to 1.9-fold and 4.6-fold the GMT of the convalescent panel for the 10 µg and 30 µg doses, respectively, consistent with affinity maturation.

Assuming that neutralization titers induced by natural infection provide protection from COVID-19 disease, comparing vaccine-induced SARS-CoV-2 neutralization titers to those from sera of convalescent humans provides a benchmark for the magnitude of the vaccine-elicited response and the vaccine's potential to provide protection. Because a protective human neutralizing titer is unknown, these findings are not proof of vaccine efficacy. Efficacy will be determined in a pivotal Phase 3 trial. Because the 100 µg dose level cohort was not boosted, no data for immunogenicity after a second vaccination at this dose level are available; however, there were no substantial differences in immunogenicity between the 30 µg and 100 µg dose levels after Dose 1. This observation suggests that a well-tolerated and immunogenic dose level may be between 10 µg and 30 µg for this vaccine candidate.

Our study had several limitations. While we used convalescent sera as a comparator, the kind of immunity (T cells versus B cells or both) and level of immunity needed to protect from COVID-19 are unknown. Further, this analysis of available data did not assess immune responses or safety beyond 2 weeks after the second dose of vaccine. Both are important to inform the public health use of this vaccine. Follow-up will continue for all participants and will include collection of SAEs for 6 months and COVID-19 infection and multiple additional immunogenicity measurements through up to two years. While our population of healthy adults 55 years of age and younger is appropriate for a Phase 1/2 study, it does not accurately reflect the population at highest risk for COVID-19. Adults 65 years of age and over have already been enrolled in this study and results will be reported as they become available. Later phases of this study will prioritize enrollment of more diverse populations, including those with chronic underlying health conditions and from racial/ethnic groups adversely affected by COVID-19[23].

The clinical testing of BNT162b1 described here has taken place in the context of a broader, ongoing COVID-19 vaccine development program. That program includes the clinical testing of three additional vaccine candidates, including candidates encoding the full-length spike, and a parallel trial in Germany, in which additional immune responses, including neutralizing responses against variant strains and cell-mediated responses, are being assessed (U.S. manuscript in preparation)[24]. The resulting comparative data will allow us to address whether a full-length spike immunogen, which presents additional epitopes, is better able than the relatively small RBD immunogen encoded by BNT162b1 to elicit high virus neutralizing titers that are robust to potential antigenic drift of SARS-CoV-2. The clinical findings for the BNT162b1 RNA-based vaccine candidate are encouraging and strongly support accelerated clinical development, including efficacy testing, and at-risk manufacturing to maximize the opportunity for the rapid production of a SARS-CoV-2 vaccine to prevent COVID-19.

Online content

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/s41586-020-2639-4.

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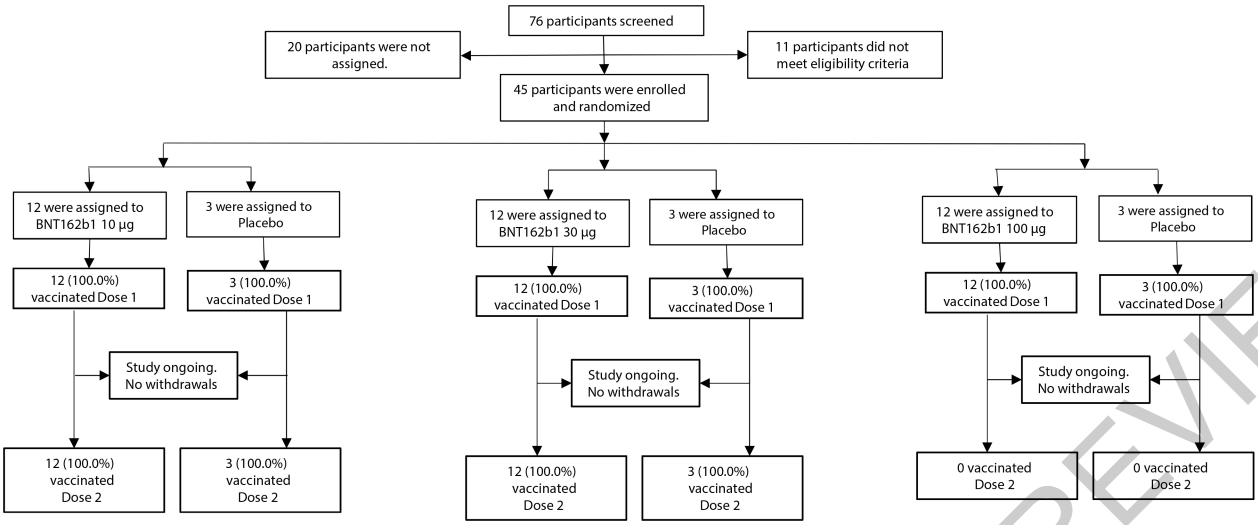


Figure 1 | Disposition of participants. Participants not assigned (n=20) were screened but not randomized because enrollmen had closed.

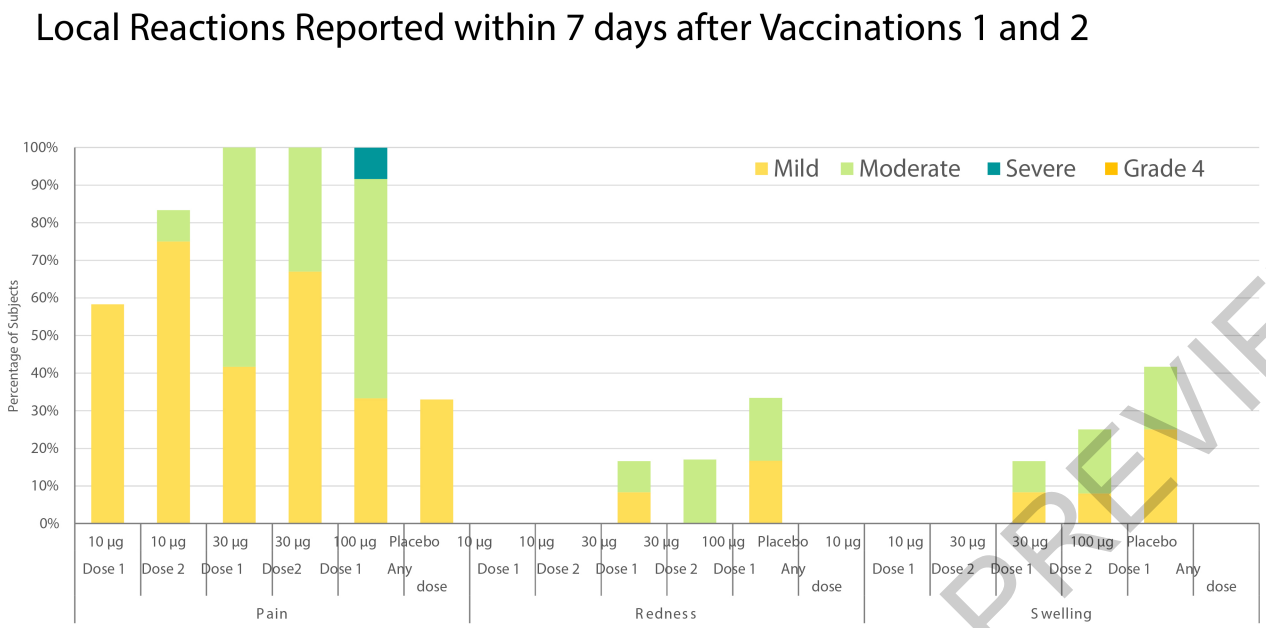
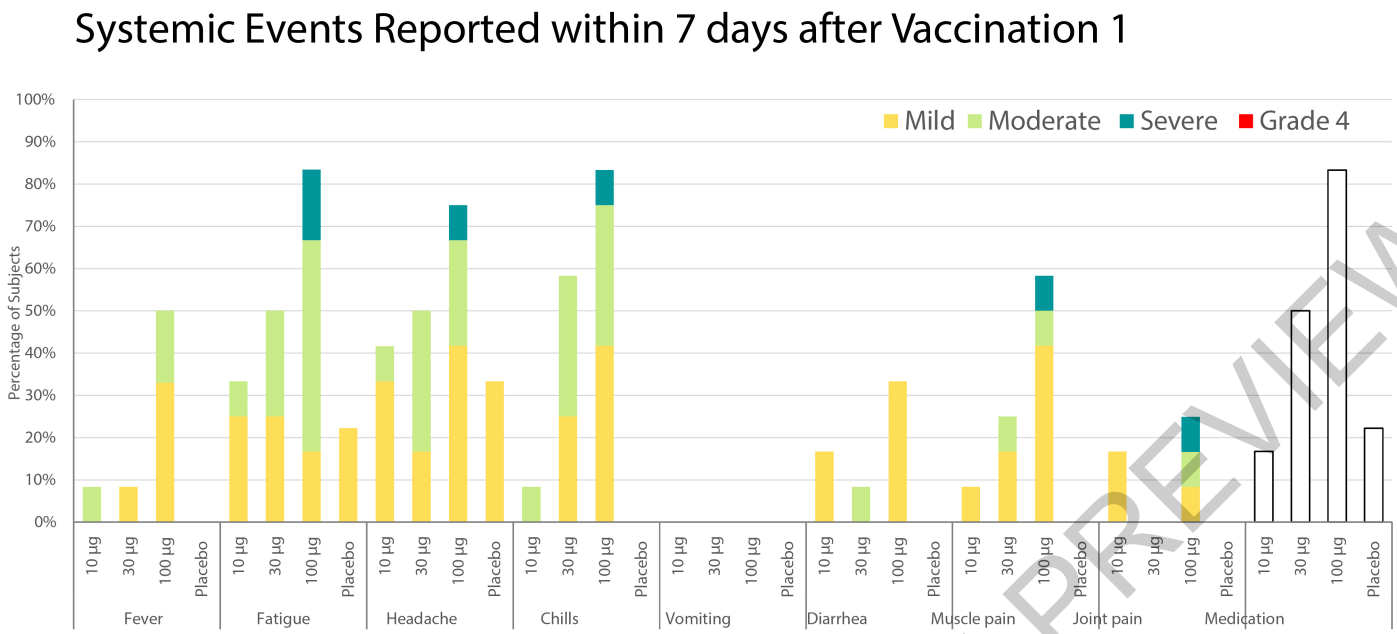


Figure 2 | Local reactions reported within 7 days of vaccination for all dose levels. Solicited injection-site (local) reactions were: pain at injection site (mild: does not interfere with activity; moderate: interferes with activity; severe: prevents daily activity; Grade 4: emergency room visit or hospitalization) and redness and swelling (mild: 2.0 to 5.0 cm in diameter; moderate: >5.0 to 10.0 cm in diameter; severe: >10.0 cm in diameter; Grade 4: necrosis or exfoliative de mat tis for redness, and necrosis for swelling). Data were collected with the use of electronic diaries for 7 days after each vaccination.



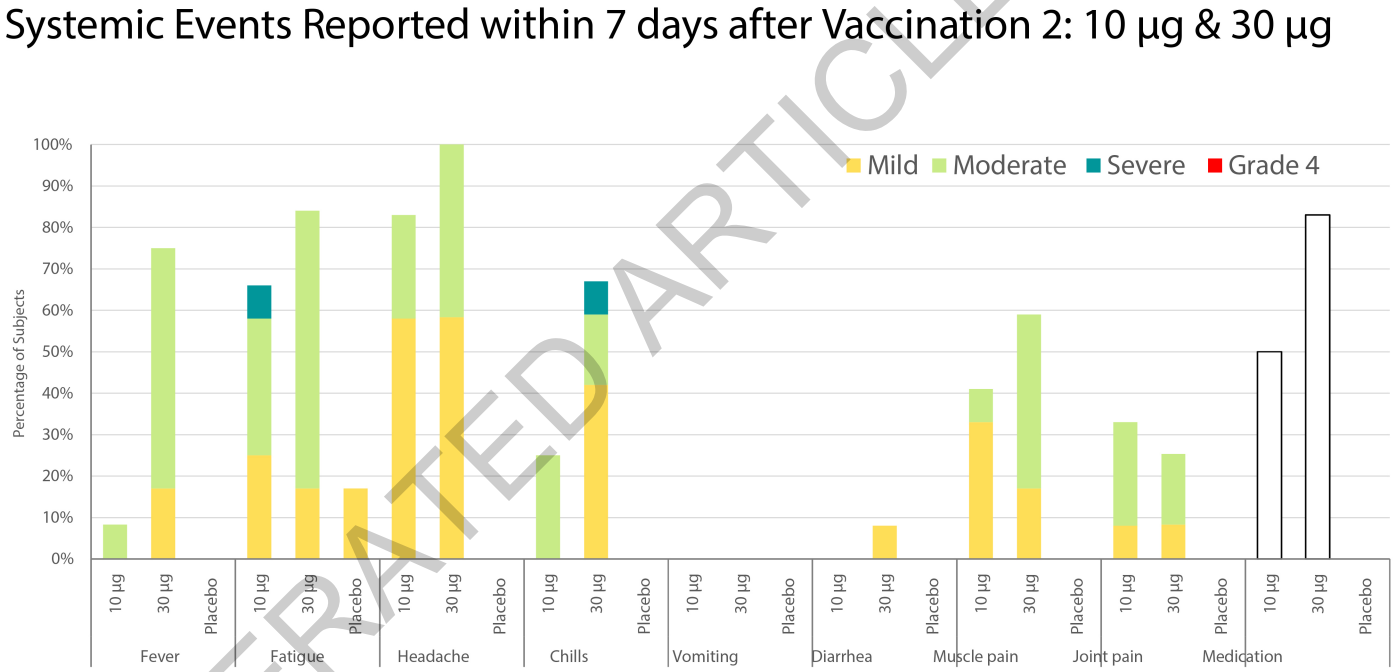


Figure 3 | a. Systemic events and medication use reported within 7 days after Vaccination 1 for all dose levels and b. After Vaccination 2 for the 10-µg and 30-µg dose level. Solicited systemic events were: fatigue, headache, chills, new or worsened muscle pain, new or worsened joint pain (mild: does not interfere with activity; moderate: some interference with activity; severe: prevents daily activity), vomiting (mild: 1 to 2 times in 24 hours; moderate: >2 times in 24 hours; severe: requires intravenous hydration), diarrhea (mild: 2 to 3 loose stools in 24 hours; moderate: 4 to 5 loose stools in 24 hours; severe: 6 or more loose stools in 24 hours); Grade 4 for all events: emergency room visit or hospitalization; and fever (mild: 38.0 °C to 38.4 °C; moderate: 38.5 °C to 38.9 °C; severe: 39.0 °C to 40.0 °C; Grade 4: >40.0 °C). Medication: proportion of participants reporting use of antipyretic or pain medication. Data were collected with the use of electronic diaries for 7 days after each vaccination.

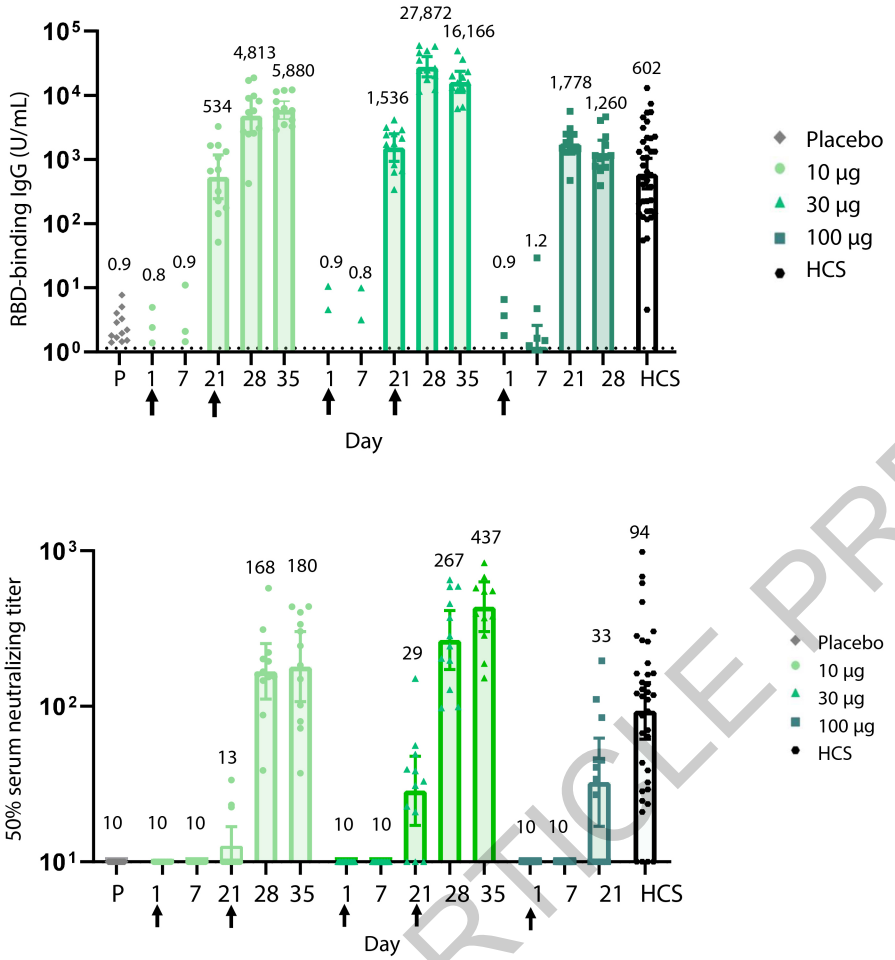


Figure 4 | Immunogenicity of BNT162b1. Participants in groups of 15 were vaccinated with the indicated dose levels of BNT162b1 (n=12) or with placebo (n=3) on Days 1 (all dose levels and placebo) and 21 (10 µg and 30 µg dose levels and placebo). Reponses in placebo recipients for each of the dosing groups are combined. The 28-day bleed is 7 days after the second vaccination. Sera were obtained before vaccination (Day 1) and 7, 21, and 28 days after the first vaccination. Human COVID-19 convalescent sera (HCS, n=38) were obtained at least 14 days after PCR-confirmed diagnosis and at a time when the donors were asymptomatic. **a**. GMCs of recombinant RBD-binding IgG. Because Luminex assay measured antibody concentrations are in arbitrary units, they cannot be directly translated into concentrations on a molar or mass basis. Lower limit of quantitation is 1.15. **b**. 50% SARS-CoV-2 neutralizing GMTs. Each data point represents a serum sample, and each vertical bar represents a geometric mean with 95% CI. The number above the bars are either the GMC or GMT for the group. Arrows indicate timing of vaccination (blood draws were conducted prior to vaccination on vaccination days).

Methods

Study design

This study was conducted in healthy men and nonpregnant women 18 to 55 years of age to assess the safety, tolerability, and immunogenicity of ascending dose levels of various BNT162 mRNA vaccine candidates. In the part of the study reported here, assessment of three dose levels (10-µg, 30-µg, or 100-µg) of the BNT162b1 candidate was conducted at two sites in the United States. This study utilized a sentinel cohort design with progression and dose escalation taking place after review of data from the sentinel cohort at each dose level.

Eligibility

Key exclusion criteria included individuals with known infection with human immunodeficiency virus, hepatitis C virus, or hepatitis B virus; immunocompromised individuals and those with a history of autoimmune disease; and those with increased risk for severe COVID-19, previous clinical or microbiological diagnosis of COVID-19, receipt of medications intended 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 nucleic acid amplification test (NAAT)-positive nasal swab within 24 hours before study vaccination.

The final protocol and informed consent document were approved by institutional review boards for each of the participating investigational centers. This study was conducted in compliance with all International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the ethical principles of the Declaration of Helsinki. A signed and dated informed consent form was required before any study-specific activity was performed.

Endpoints

In this report, results from the following study primary endpoints are presented: the proportion of participants reporting solicited local reactions, systemic events, and use of antipyretic and/or pain medi ation within 7 days after vaccination, AEs and SAEs (available through up to ~45 days after Dose 1), and the proportion of participants with clin cal laboratory abnormalities 1 and 7 days after vaccinat on and grading shifts in laboratory assessments between baseline and 1 and 7 days after Dose 1 and between Dose 2 and 7 days after Dose 2. Secondary endpoints included: SARS-CoV-2 neutralizing GMTs and SARS CoV-2 RBD-binding IgG GMCs 7 and 21 days after Dose 1 and 7 and 14 days after Dose 2.

Procedures

Study participants were randomly assigned to a vaccine group using an interactive web-based response technology system with each group comprising 15 participants (12 ac ve vaccine recipients and 3 placebo recipients). Participants were to receive two 0.5-mL doses of either BNT162b1 or placebo administered by intramuscular injection into the deltoid muscle.

BNT162b1 incorporates a Good Manufacturing Practice (GMP)-grade mRNA drug ubsta ce that encodes the trimerized SARS-CoV-2 spike glycoprotein RBD antigen. The coding sequence for the antigen has been deposited with GenBank, accession code MN908947.3. The mRNA is fo mulated with lipids as the mRNA-LNP drug product. The vaccine was supplied as a buffered-liquid solution for intramuscular injection and was stored at -80 °C. The placebo was a sterile saline solution for inject on (0.9% sodium chloride injection, in a 0.5-mL dose).

Safety assessments

Safety assessments included a 4-hour observation after vaccination (for the first 5 participants vaccinated in each group), or a 30-minute observation (for the remainder of participants) for immediate AEs. The safety assessments also included self-reporting of solicited local reactions (redness, swelling, and pain at the injection site), systemic events (fever, fatigue, headache, chills, vomiting, diarrhea, muscle pain, and joint pain), the use of antipyretic and/or pain medication in an electronic diary for 7 days after vaccination, and the reporting of unsolicited AEs and SAEs after vaccination. Hematology and chemistry assessments were conducted at screening, 1 and 7 days after Dose 1, and 7 days after Dose 2.

There were protocol-specified safety stopping rules for all sentinel cohort participants. Both an internal review committee and an external data monitoring committee reviewed all safety data. No stopping rules were met prior to the publication of this report.

Human convalescent serum panel

The 38 human SARS-CoV-2 infection/COVID-19 convalescent sera were drawn from participants 18 to 83 years of age, at least 14 days after PCR-confirmed diagnosis, and at a time when participants were asymptomatic. The mean age of the donors was 45 years of age. Neutralizing GMTs in subgroups of the donors were as follows: ≤ 55 years of age - 82 (n=29); > 55 years of age – 142 (n=9); symptomatic infections – 90 (n=35); asymptomatic infections – 156 (n=3). The antibody titer for the one hospitalized individual was 618. The sera were obtained from Sanguine Biosciences (Sherman Oaks, CA), the MT Group (Van Nuys, CA), and Pfizer Occupational Health and Wellness (Pearl River, NY).

Immunogenicity assessments

50 mL of blood was colle ted for immunogenicity assessments before each study vaccination, at 7 and 21 days after Dose 1, and at 7 and 14 days after Dose 2. In the RBD-binding IgG assay, a recombinant SARS-CoV-2 RBD contai ing a C terminal Avitag™ (Acro Biosystems Cat# SPD-C82E9) and no foldon domain was bound to streptavidin-coated Luminex® mic ospheres. Briefly, 1.25×10^7 microspheres/mL were coated with streptavidin by 1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC) reaction. Recombinant RBD Avitag was coupled to s reptavidin beads by incubating for 90 minutes at room temperature with shaking (35 RPM). Beads were blocked in 1% BSA buffer for 30 minutes at room temperature. Heat-inactivated subject serum was diluted 1:500, 1:5000, and 1:50000 in assay buffer (PBS with 0.5% BSA, 0.05% Tween, and 0.02% sodium azide). Following a 16- to 20-hour incubation at 2-8 °C with shaking (300 RPM), plates were washed three times in a solution containing 0.05% Tween-20. An R-Phycoerythrin-conjugated goat anti-human polyclonal antibody (Jackson Labs) was then added to plates for 90 minutes at room temperature with shaking (300 RPM). Plates were then washed a final time in a solution containing 0.05% Tween-20. Data were captured as median fluorescent intensities using a Luminex reader and converted to U/mL antibody concentrations using a reference standard curve with arbitrary assigned concentrations of 100 U/mL and accounting for the serum dilution factor. The reference standard was composed of a pool of five COVID-19 convalescent serum samples (>14 days post PCR diagnosis). Three dilutions are used to increase the likelihood that at least one result for any sample will fall within the usable range of the standard curve. Assay results were reported in U/mL of IgG. The final assay results are expressed as the GMC of all sample dilutions that produced a valid assay result within the assay range.

The SARS-CoV-2 neutralization 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 gene into open reading frame 7 of the viral genome[25]. This reporter virus generates similar plaque morphologies and indistinguishable growth curves from the wild-type virus. Viral master stocks (2×10^7 PFU/mL) used for the neutralization assay were grown in Vero E6 cells as previously described[25]. When testing patient convalescent serum specimens, the fluorescent neutralization assay produced comparable results as the conventional plaque reduction neutralization assay[26]. Briefly, serial dilutions of heat inactivated sera were incubated with the reporter virus to yield approximately a 10% to 30% infection rate of the Vero

monolayer) for 1 hour at 37 °C before inoculating Vero CCL81 cell monolayers (targeted to have 8,000 to 15,000 cells per well) 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 to 24 hours after inoculation with a Cytation™ 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 neutralization at each serial serum dilution. The 50% neutralization titer (VNT_{50}) was reported as the interpolated reciprocal of the dilution yielding a 50% reduction in fluorescent viral foci.

Statistical analysis

The sample size for the reported part of the study was not based on statistical hypothesis testing. The primary safety objective was evaluated by descriptive summary statistics for local reactions, systemic events, abnormal hematology and chemistry laboratory parameters, AEs, and SAEs after each vaccine dose for each vaccine group. The secondary immunogenicity objectives were descriptively summarized at the various time points. All participants with data available were included in the safety and immunogenicity analyses.

Reporting summary

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

Data availability

Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions, and exceptions, Pfizer may also provide access to the related individual anonymized participant data. See https://www.pfizer.com/science/clinical-trials/trial-data-and-results for more information. These data are interim data from an ongoing study, with the database not locked. Data have not yet been source verified or subjected to standard quality check procedures that would occur at the time of database lock and may therefore be subject to change.

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Author contributions KUJ, PRD, CG, NK SL, AG, RB, and US were involved in the design of the overall study and strategy. KN, MJM, EEW, RF, and ARF provided feedback on the study design. WK, DC, KAS, KRT CFG and PYS performed the immunological analyses. MJM, KN, EEW, RF, ARF, KEL, and VR olle ted data as study investigators. PL and KK developed the statistical desi n and oversaw the data analysis. JA, KUJ, PRD, and WCG drafted the initial version of the manuscript All authors reviewed and edited the manuscript and approved the final version.

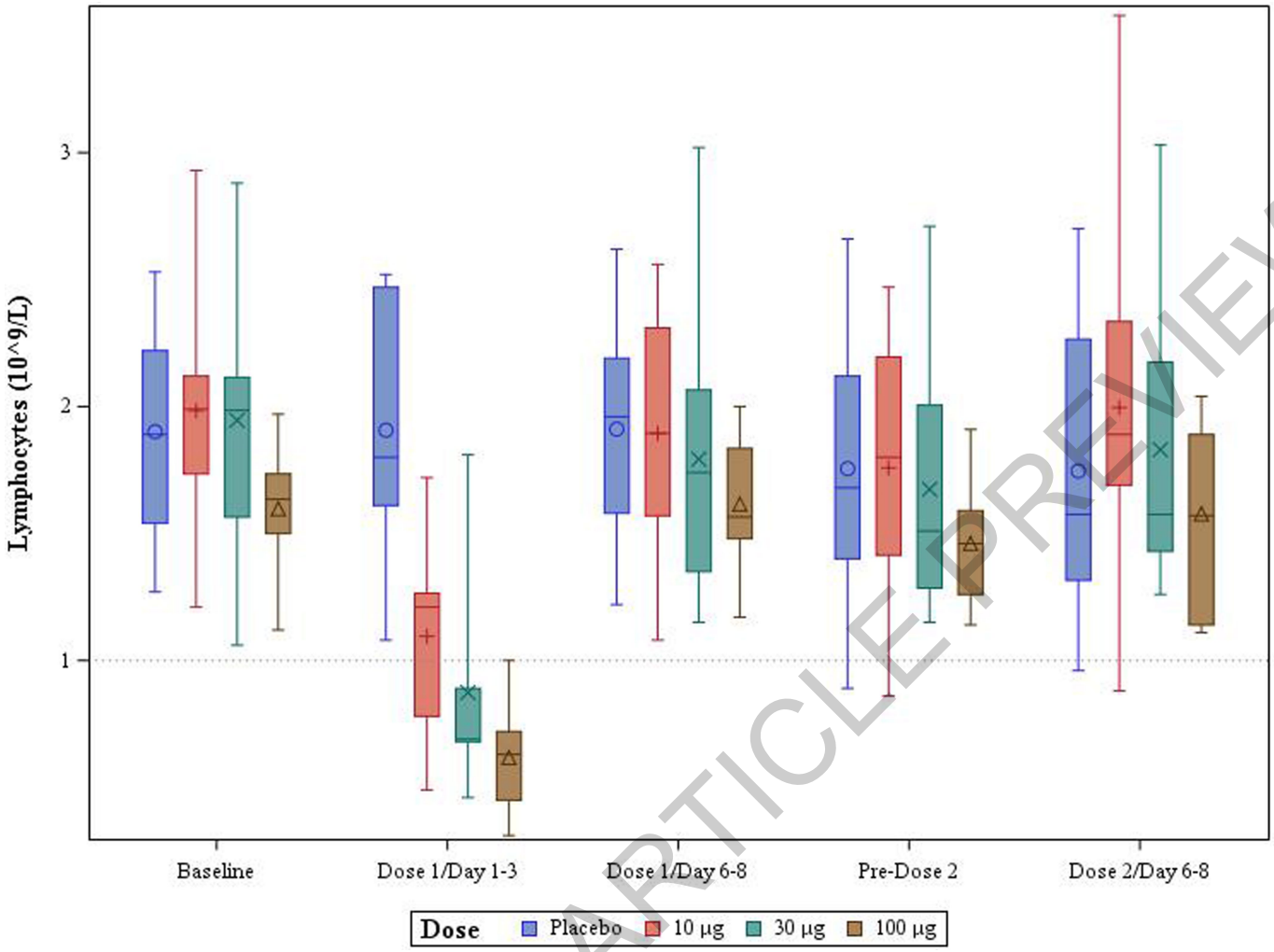
Compe ing interests NK, JA, AG, SL, RB, KAS, PL, KK, WK, DC, KRT, PRD, WCG, and KUJ are mployee of Pfizer and may hold stock options. US and ÖT are stock owners, management board members, and employees at BioNTech SE (Mainz, Germany) and are nv tors on patents and patent applications related to RNA technology. MJM, KEL, KN, EEW, ARF, RF, and VR received compensation from Pfizer for their role as study nvestigators. CFG and PYS received compensation from Pfizer to perform the neutralization assay.

Additional information

Supplementary information is available for this paper at https://doi.org/10.1038/s41586-020-2639-4.

Correspondence and requests for materials should be addressed to J.A.

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



Extended Data Figure 1 | Post Vaccination Changes in Lymphocyte Coun Over Time. Figure represents box-and-whisker plots for obs rved values at the following timepoints: Dose 1/Day 1-3: -1 day after Dose 1; Dose 2/Day 6 8: -7 days after Dose 1; Pre-Dose 2: before Dose 2; Dose 2/Day 6-8: 7 days after Dose 2. Symbols denote group means – O: placebo; +: 10 µg; X: 30 µg; Δ: 100 µg. Center line of box denotes median; lower and upper edges denote first and third quartiles; lower and upper whiskers denote minimum and maximum.

Extended Data Table 1 | Demographic Characteristics.

	10 µg (N=12) n (%)	30 µg (N=12) n (%)	100 µg (N=12) n (%)	Placebo (N=9) n (%)	Total (N=45) n (%)
Sex					
Male	7 (58.3)	6 (50.0)	5 (41.7)	5 (55.6)	23 (51.1)
Female	5 (41.7)	6 (50.0)	7 (58.3)	4 (44.4)	22 (48.9)
Race					
White	8 (66.7)	10 (83.3)	11 (91.7)	8 (88.9)	37 (82.2)
Black or African American	1 (8.3)	0	0	0	1 (2.2)
Asian	3 (25.0)	2 (16.7)	1 (8.3)	1 (11.1)	7 (15.6)
Ethnicity					
Hispanic/Latino	1 (8.3)	1 (8.3)	0	0	2 (4.4)
Non-Hispanic/non-Latino	11 (91.7)	10 (83.3)	12 (100.0)	9 (100.0)	42 (93.3)
Not reported	0	1 (8.3)	0	0	1 (2.2)
Age at vaccination (years)					
Mean (SD)	29.4 (6.39)	35.8 (9.96)	38.3 (9.34)	39.0 (11.16)	35.4 (9.71)
Median	26.5	33.5	38.0	41.0	33.0
Min, max	(24, 42)	(23, 52)	(25, 53)	(19, 54)	(19, 54)

N = number of subjects in the specified group, or the total sample. This value is the denominator for e percen age calculations. n = Number of subjects with the specified characteristic.

Extended Data Table 2 | Adverse Events.

	10 µg (N=12)	30 µg (N=12)	100 µg (N=12)	Placebo (N=9)
Adverse Event	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Any event	6 (50.0)	6 (50.0)	7 (58.3)	1 (11.1)
Related	3 (25.0)	6 (50.0)	6 (50.0)	1 (11.1)
Severe	0	1 (8.3)	1 (8.3)	0
Life-threatening	0	0	0	0
Any serious adverse event	0	0	0	0
Related	0	0	0	0
Severe	0	0	0	0
Life-threatening	0	0	0	0
Any adverse event leading to withdrawal	0	0	0	0
Related	0	0	0	0
Severe	0	0	0	0
Life-threatening	0	0	0	0
Death	0	0	0	0

N: number of subjects in the specified group or the total sample. This value is the denominator for the percentage calculations. n: number of subjects reporting at least 1 occurrence of the specified adverse event category. For "any event", n: the number of subjects reporting at least 1 occurrence of any adverse event; Related: Assessed by the investigator as related to investigational product.

nature research

Corresponding author(s): Judith Absalon

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Reporting Summary

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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
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☐	☒	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.*
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☐	☒	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	Inform (for data collected in the case report form) and electronic diary (Signant Health platform) for participant self reported reactogenicity
Data analysis	SAS 9.4

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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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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Upon request, and subject to review, Pfizer will provide the data that support the findings of this study. Subject to certain criteria, conditions and exceptions, Pfizer may also provide access to the related individual anonymized participant data. See https://www.pfizer.com/science/clinical trials/trial data and results for more information

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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	The sample size for this interim report was not based on statistical hypothesis testing. A total of 45 participants were enrolled in this part of the study. For the purposes of tolerability and dose escalation study a total of 15 participants (12 receiving vaccine and 3 receiving placebo) was deemed sufficient for a dosing finding phase study.
Data exclusions	All safety and immunogenicity data that were available at the time of the data snapshot were included in the interim report. No data were excluded from the analyses.
Replication	This is an interim report of an ongoing human clinical trial. There was no attempt at replication of study findings
Randomization	This is an randomized controlled trial. Study participants were randomly assigned to a vaccine group using an interactive web based response technology system with each group comprising 15 participants (12 active vaccine recipients and 3 placebo recipients).
Blinding	This is an observer blinded study which is investigator blinded but Sponsor unblinded during Stage 1 (the stage from which data in the manuscript are presented). Investigators were unblinded to group level data but not subject level data for the purposes of interpretation and summary of the results included in this interim report.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	Study participants were healthy men or women 18 55 years of age. Key exclusion criteria included individuals with known infection with human immunodeficiency virus, hepatitis C virus, or hepatitis B virus; immunocompromised individuals and those with a history of autoimmune disease; those with increased risk for severe COVID 19; previous clinical or microbiological diagnosis of COVID 19; receipt of medications intended 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.
Recruitment	Study participants were recruited at the two individual sites and recruitment strategies were at the discretion of individual sites and could include identification of interested individuals from the sites local database or through advertising in the local community. Once recruited participants were screened for eligibility based on pre specified protocol criteria. Eligible participants were then randomized to vaccine or placebo in a blinded manner. These processes therefore did not led themselves to enrollment biases however participants who did not know about the study may have had less of an opportunity to participate.
Ethics oversight	The study protocol was approved by the western institutional review board for one site and by the Langone Health New York University Institutional IRB prior to enrollment of any participants

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: NCT04368728
Study protocol	Details of protocol elements can be accessed from clinicaltrials.gov
Data collection	Data were collected at screening (up to 14 days before vaccination) and for randomized participants at the investigative site at baseline, 1 day, 7 days and 21 days, after Dose 1, 7 days after dose 2 and up to 14 days after dose 2. Both safety and/or serum collection for immunogenicity assessments were collected for all stated time points. In addition, reactogenicity data were assessed through participant self reports via an electronic diary for 7 days after dose 1.
Outcomes	In this interim report, the following study primary endpoints are presented: the proportion of participants reporting prompted local reactions, systemic events, and use of antipyretic and/or pain medication within 7 days after vaccination, AEs and serious adverse events (SAEs) (available through up to ~45 days after Dose 1), and the proportion of participants with clinical laboratory abnormalities 1 and 7 days after vaccination and grading shifts in laboratory assessments between baseline and 1 and 7 days after Dose 1 and between Dose 2 and 7 days after Dose 2. Secondary endpoints included: SARS CoV 2 neutralizing geometric mean titers (GMTs); SARS CoV 2 RBD binding IgG geometric mean concentrations (GMCs) 7 and 21 days after Dose 1 and 7 and 14 days after Dose 2

ORIGINAL ARTICLE

Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates

Edward E. Walsh, M.D., Robert W. Frenck, Jr., M.D., Ann R. Falsey, M.D., Nicholas Kitchin, M.D., Judith Absalon, M.D., Alejandra Gurtman, M.D., Stephen Lockhart, D.M., Kathleen Neuzil, M.D., Mark J. Mulligan, M.D., Ruth Bailey, B.Sc., Kena A. Swanson, Ph.D., Ping Li, Ph.D., Kenneth Koury, Ph.D., Warren Kalina, Ph.D., David Cooper, Ph.D., Camila Fontes-Garfias, B.Sc., Pei-Yong Shi, Ph.D., Özlem Türeci, M.D., Kristin R. Tompkins, B.Sc., Kirsten E. Lyke, M.D., Vanessa Raabe, M.D., Philip R. Dormitzer, M.D., Kathrin U. Jansen, Ph.D., Uğur Şahin, M.D., and William C. Gruber, M.D.

ABSTRACT

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections and the resulting disease, coronavirus disease 2019 (Covid-19), have spread to millions of persons worldwide. Multiple vaccine candidates are under development, but no vaccine is currently available. Interim safety and immunogenicity data about the vaccine candidate BNT162b1 in younger adults have been reported previously from trials in Germany and the United States.

METHODS

In an ongoing, placebo-controlled, observer-blinded, dose-escalation, phase 1 trial conducted in the United States, we randomly assigned healthy adults 18 to 55 years of age and those 65 to 85 years of age to receive either placebo or one of two lipid nanoparticle–formulated, nucleoside-modified RNA vaccine candidates: BNT162b1, which encodes a secreted trimerized SARS-CoV-2 receptor–binding domain; or BNT162b2, which encodes a membrane-anchored SARS-CoV-2 full-length spike, stabilized in the prefusion conformation. The primary outcome was safety (e.g., local and systemic reactions and adverse events); immunogenicity was a secondary outcome. Trial groups were defined according to vaccine candidate, age of the participants, and vaccine dose level (10 μg, 20 μg, 30 μg, and 100 μg). In all groups but one, participants received two doses, with a 21-day interval between doses; in one group (100 μg of BNT162b1), participants received one dose.

RESULTS

A total of 195 participants underwent randomization. In each of 13 groups of 15 participants, 12 participants received vaccine and 3 received placebo. BNT162b2 was associated with a lower incidence and severity of systemic reactions than BNT162b1, particularly in older adults. In both younger and older adults, the two vaccine candidates elicited similar dose-dependent SARS-CoV-2–neutralizing geometric mean titers, which were similar to or higher than the geometric mean titer of a panel of SARS-CoV-2 convalescent serum samples.

CONCLUSIONS

The safety and immunogenicity data from this U.S. phase 1 trial of two vaccine candidates in younger and older adults, added to earlier interim safety and immunogenicity data regarding BNT162b1 in younger adults from trials in Germany and the United States, support the selection of BNT162b2 for advancement to a pivotal phase 2–3 safety and efficacy evaluation. (Funded by BioNTech and Pfizer; ClinicalTrials.gov number, NCT04368728.)

From the University of Rochester and Rochester General Hospital, Rochester (E.E.W., A.R.F.), Vaccine Research and Development, Pfizer, Pearl River (J.A., A.G., K.A.S., K.K., W.K., D.C., K.R.T., P.R.D., K.U.J., W.C.G.), and New York University Langone Vaccine Center and Grossman School of Medicine, New York (M.J.M., V.R.) — all in New York; Cincinnati Children's Hospital, Cincinnati (R.W.F.); Vaccine Research and Development, Pfizer, Hurley, United Kingdom (N.K., S.L., R.B.); the University of Maryland School of Medicine, Center for Vaccine Development and Global Health, Baltimore (K.N., K.E.L.); Vaccine Research and Development, Pfizer, Collegeville, PA (P.L.); the University of Texas Medical Branch, Galveston (C.F.-G., P.-Y.S.); and BioNTech, Mainz, Germany (Ö.T., U.Ş.). Address reprint requests to Dr. Absalon at Pfizer, 401 N. Middletown Rd., Pearl River, NY 10965, or at judith.absalon@pfizer.com.

Drs. Walsh and Frenck contributed equally to this article.

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Since the first cases of coronavirus disease 2019 (Covid-19) in Wuhan, China, in December 2019, pandemic illness has spread to millions of persons worldwide. An increased risk of severe disease and death has been noted among the elderly and among persons with preexisting medical conditions. No Covid-19 vaccines are currently available, and they are urgently needed to combat escalating cases and deaths worldwide.[1]

In response, BioNTech and Pfizer launched a coordinated program to compare four RNA-based Covid-19 pandemic vaccine candidates in umbrella-type clinical studies conducted in Germany (BNT162-01) and the United States (C4591001). The program was designed to support the selection of a single vaccine candidate and dose level for a pivotal international safety and efficacy trial. On the basis of initial clinical-trial results in Germany,[2] two lipid nanoparticle–formulated,[3] nucleoside-modified RNA (modRNA)[4] vaccine candidates against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) were evaluated in the phase 1 portion of the trial in the United States.[5] One of these candidates, BNT162b1, encodes the SARS-CoV-2 receptor–binding domain, trimerized by the addition of a T4 fibritin foldon domain to increase its immunogenicity through multivalent display.[6-8] The other candidate, BNT162b2, encodes the SARS-CoV-2 full-length spike, modified by two proline mutations to lock it in the prefusion conformation[9] and more closely mimic the intact virus with which the elicited virus-neutralizing antibodies must interact.[10]

Previous articles have described the assessment of BNT162b1, at multiple dose levels, in healthy adults 18 to 55 years of age.[2,5] These studies indicated that dose levels of BNT162b1 that elicited an acceptable level of reactogenicity also efficiently elicited titers that were as high as those in a panel of SARS-CoV-2 human convalescent serum samples and that were broadly neutralizing across a panel of 17 SARS-CoV-2 pseudoviruses representing a diversity of circulating strains. BNT162b1 also elicited CD4+ type 1 helper T (Th1) cell responses and strong interferon-γ–producing and interleukin-2–producing CD8+ cytotoxic T-cell responses. This ability to elicit both humoral and cell-mediated antiviral mechanisms makes BNT162b1 a promising vaccine candidate.

Here, we report the full set of safety and immunogenicity data from the phase 1 portion of an ongoing randomized, placebo-controlled, observer-blinded, dose-escalation trial in the United States that was used to select the final vaccine candidate, as well as the comparison of the safety and immunogenicity of both vaccine candidates and additional phase 1 data that have been collected since candidate selection. These data include evaluation of the 10-μg, 20-μg, and 30-μg dose levels of BNT162b1 and BNT162b2 in adults 18 to 55 years of age and adults 65 to 85 years of age.

METHODS

TRIAL OBJECTIVES, PARTICIPANTS, AND OVERSIGHT

We assessed the safety and immunogenicity of three dose levels of BNT162b1 and BNT162b2. Healthy adults 18 to 55 years of age or 65 to 85 years of age were eligible for inclusion. Key exclusion criteria were known infection with human immunodeficiency virus, hepatitis C virus, or hepatitis B virus; an immunocompromised condition; a history of autoimmune disease; a previous clinical or microbiologic diagnosis of Covid-19; the receipt of medications intended to prevent Covid-19; any previous coronavirus vaccination; positive test for SARS-CoV-2 IgM or IgG at the screening visit; and positive nasal-swab results on a SARS-CoV-2 nucleic acid amplification test within 24 hours before the receipt of trial vaccine or placebo.

BioNTech was the regulatory sponsor of the trial. Pfizer was responsible for the trial design; for the collection, analysis, and interpretation of the data; and for the writing of the report. The corresponding author had full access to all the data in the trial and had final responsibility for the decision to submit the manuscript for publication. All the trial data were available to all the authors.

TRIAL PROCEDURES

Using an interactive Web-based response technology system, we randomly assigned trial participants to groups defined according to the vaccine candidate, dose level, and age range. Groups of participants 18 to 55 years of age and 65 to 85 years of age were to receive doses of 10 μg, 20 μg, or 30 μg of BNT162b1 or BNT162b2 (or placebo) on a two-dose schedule; one group of participants

18 to 55 years of age was assigned to receive 100-μg doses of BNT162b1 or placebo. All the participants were assigned to receive two 0.5-ml injections of active vaccine (BNT162b1 or BNT162b2) or placebo into the deltoid, administered 21 days apart.

The first five participants in each new dose level or age group (with a randomization ratio of 4:1 for active vaccine:placebo) were observed for 4 hours after the injection to identify immediate adverse events. All the other participants were observed for 30 minutes. Blood samples were obtained for safety and immunogenicity assessments.

SAFETY

The primary end points in phase 1 of this trial were solicited local reactions (i.e., specific local reactions as prompted by and recorded in an electronic diary), systemic events, and use of antipyretic or pain medication within 7 days after the receipt of vaccine or placebo, as prompted by and recorded in an electronic diary; unsolicited adverse events and serious adverse events (i.e., those reported by the participants, without electronic-diary prompts), assessed from the receipt of the first dose through 1 month and 6 months, respectively, after the receipt of the second dose; clinical laboratory abnormalities, assessed 1 day and 7 days after the receipt of vaccine or placebo; and grading shifts in laboratory assessments between baseline and 1 day and 7 days after the first dose and between 2 days and 7 days after the second dose. Protocol-specified safety stopping rules were in effect for all the participants in the phase 1 portion of the trial. The full protocol, including the statistical analysis plan, is available with the full text of this article at NEJM.org. An internal review committee and an external data and safety monitoring committee reviewed all safety data.

IMMUNOGENICITY

Immunogenicity assessments (SARS-CoV-2 serum neutralization assay and receptor-binding domain [RBD]–binding or S1-binding IgG direct Luminex immunoassays) were conducted before the administration of vaccine or placebo, at 7 days and 21 days after the first dose, and at 7 days (i.e., day 28) and 14 days (i.e., day 35) after the second dose. The neutralization assay, which also generated previously described virus-neutralization data from trials of the BNT162 candidates,[2,5] used a previously described strain of SARS-CoV-2 (USA_WA1/2020) that had been generated by reverse genetics and engineered by the insertion of an mNeonGreen gene into open reading frame 7 of the viral genome.[11,12] The 50% neutralization titers and 90% neutralization titers were reported as the interpolated reciprocal of the dilutions yielding 50% and 90% reductions, respectively, in fluorescent viral foci. Any serologic values below the lower limit of quantitation were set to 0.5 times the lower limit of quantitation. Available serologic results were included in the analysis.

Immunogenicity data from a human convalescent serum panel were included as a benchmark. A total of 38 serum samples were obtained from donors 18 to 83 years of age (median age, 42.5 years) who had recovered from SARS-CoV-2 infection or Covid-19; samples were obtained at least 14 days after a polymerase chain reaction–confirmed diagnosis and after symptom resolution. Neutralizing geometric mean titers (GMTs) in subgroups of the donors were as follows: 90, among 35 donors with symptomatic infections; 156, among 3 donors with asymptomatic infection; and 618, in 1 donor who was hospitalized. Each serum sample in the panel was from a different donor. Thus, most of the serum samples were obtained from persons with moderate Covid-19 who had not been hospitalized. The serum samples were obtained from Sanguine Biosciences, the MT Group, and Pfizer Occupational Health and Wellness.

STATISTICAL ANALYSIS

We report descriptive results of safety and immunogenicity analyses, and the sample size was not based on statistical hypothesis testing. Results of the safety analyses are presented as counts, percentages, and associated Clopper–Pearson 95% confidence intervals for local reactions, systemic events, and any adverse events after the administration of vaccine or placebo, according to terms in the *Medical Dictionary for Regulatory Activities*, version 23.0, for each vaccine group. Summary statistics are provided for abnormal laboratory values and grading shifts. Given the small number of participants in each group, the trial was not powered for formal statistical comparisons between dose levels or between age groups.

Immunogenicity analyses of SARS-CoV-2 serum neutralizing titers, S1-binding IgG and RBD-binding IgG concentrations, GMTs, and geometric

mean concentrations (GMCs) were computed along with associated 95% confidence intervals. The GMTs and GMCs were calculated as the mean of the assay results after the logarithmic transformation was made; we then exponentiated the mean to express results on the original scale. Two-sided 95% confidence intervals were obtained by performing logarithmic transformations of titers or concentrations, calculating the 95% confidence interval with reference to Student's t-distribution, and then exponentiating the limits of the confidence intervals.

RESULTS

DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS

Between May 4, 2020, and June 22, 2020, a total of 332 healthy adults (men and nonpregnant women) underwent screening at four sites in the United States (two sites per vaccine candidate). A total of 195 participants were randomly assigned to 13 groups comprising 15 participants each; in each group, 12 participants received vaccine and 3 received placebo (Fig. 1). In all groups

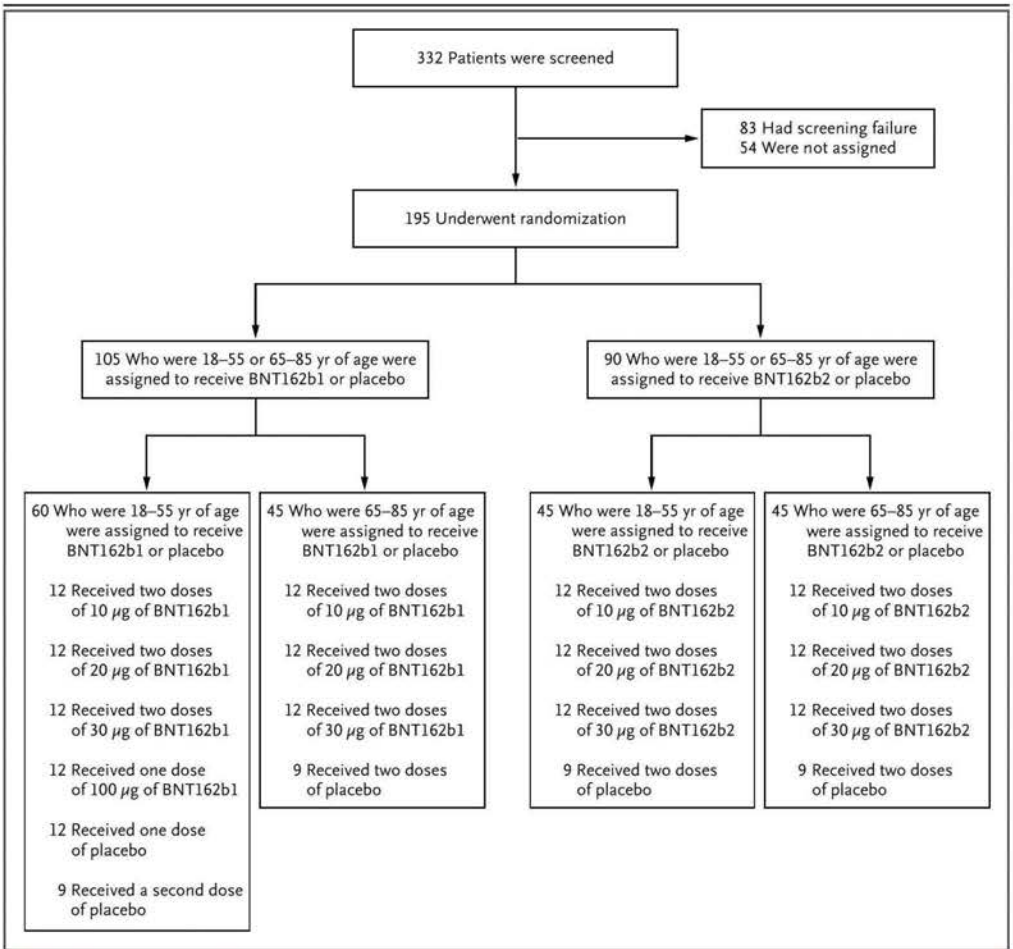

Figure 1. Screening and Randomization of the Participants.

The 54 participants who were not assigned to a trial group were screened but did not undergo randomization because trial enrollment had closed. All the participants received two doses of the vaccine (BNT162b1 or BNT162b2) or placebo, except for the participants who were assigned to receive 100 μg of BNT162b1 or placebo, who received one dose.

Table 1. Demographic Characteristics of the Participants, According to Vaccine Candidate and Age Group.*

Variable	Participants 18–55 Years of Age						Participants 65–85 Years of Age				
	10 μg	20 μg	30 μg	100 μg	Placebo	Total	10 μg	20 μg	30 μg	Placebo	Total
BNT162b1											
No. of participants	12	12	12	12	12	60	12	12	12	9	45
Sex — no. (%)											
Male	7 (58)	9 (75)	6 (50)	5 (42)	7 (58)	34 (57)	4 (33)	4 (33)	4 (33)	1 (11)	13 (29)
Female	5 (42)	3 (25)	6 (50)	7 (58)	5 (42)	26 (43)	8 (67)	8 (67)	8 (67)	8 (89)	32 (71)
Race — no. (%)†											
White	8 (67)	11 (92)	10 (83)	11 (92)	11 (92)	51 (85)	12 (100)	11 (92)	10 (83)	9 (100)	42 (93)
Black	1 (8)	1 (8)	0	0	0	2 (3)	0	1 (8)	0	0	1 (2)
Asian	3 (25)	0	2 (17)	1 (8)	1 (8)	7 (12)	0	0	2 (17)	0	2 (4)
Hispanic ethnic group — no. (%)†	1 (8)	0	1 (8)	0	0	2 (3)	0	0	0	1 (11)	1 (2)
Age — yr‡											
Mean	29.4±6.4	44.8±8.3	35.8±10.0	38.3±9.3	36.3±11.3	36.9±10.2	69.7±5.4	70.6±4.9	69.9±3.6	68.2±3.0	69.7±4.3
Median (range)	26.5 (24–42)	49.0 (30–54)	33.5 (23–52)	38.0 (25–53)	35.0 (19–54)	35.0 (19–54)	68.5 (65–82)	69.0 (65–81)	69.0 (65–77)	68.0 (65–73)	69.0 (65–82)
BNT162b2											
No. of participants	12	12	12	0	9	45	12	12	12	9	45
Sex — no. (%)											
Male	5 (42)	6 (50)	3 (25)	—	5 (56)	19 (42)	2 (17)	5 (42)	6 (50)	4 (44)	17 (38)
Female	7 (58)	6 (50)	9 (75)	—	4 (44)	26 (58)	10 (83)	7 (58)	6 (50)	5 (56)	28 (62)
Race — no. (%)†											
White	11 (92)	10 (83)	9 (75)	—	9 (100)	39 (87)	12 (100)	12 (100)	12 (100)	9 (100)	45 (100)
Black	0	2 (17)	1 (8)	—	0	3 (7)	0	0	0	0	0
Asian	1 (8)	0	2 (17)	—	0	3 (7)	0	0	0	0	0
Hispanic ethnic group — no. (%)†	1 (8)	1 (8)	0	—	0	2 (4)	0	0	0	0	0
Age — yr‡											
Mean	36.8±12.2	37.6±10.1	37.3±9.8	—	34.4±13.2	36.7±11.0	68.0±2.9	71.0±5.8	68.5±2.8	70.0±3.8	69.3±4.1
Median (range)	37.0 (21–53)	38.0 (23–53)	36.5 (23–54)	—	30.0 (19–53)	37.0 (19–54)	67.0 (65–73)	68.5 (65–81)	68.0 (65–74)	69.0 (65–77)	68.0 (65–81)

* Plus–minus values are means ±SD. Percentages may not total 100 because of rounding.
† Race and ethnic group were reported by the participant.
‡ The age of the participants was the age at the time of the injection.

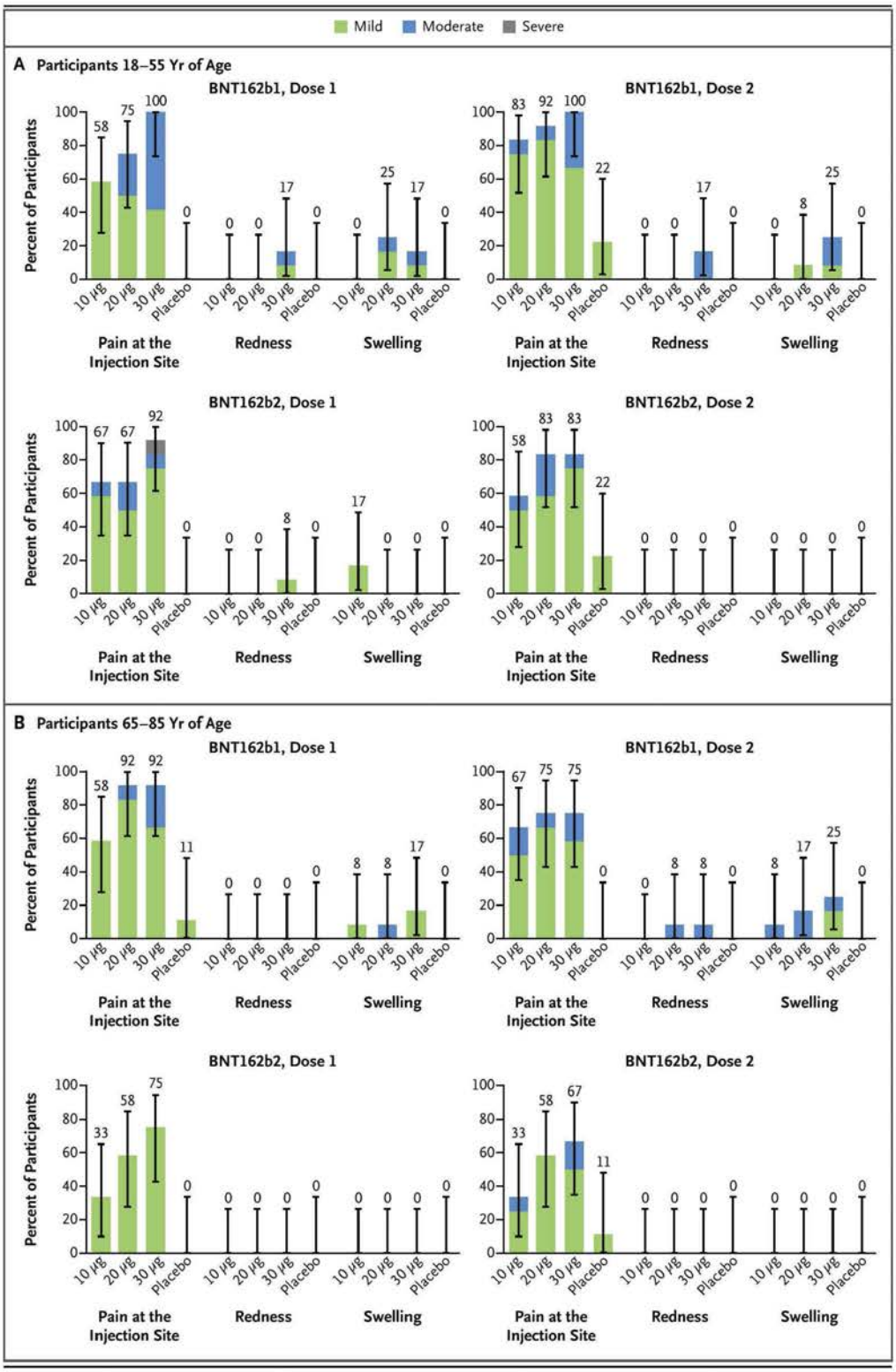

Figure 2 (facing page). Local Reactions Reported within 7 Days after the Administration of Vaccine or Placebo, According to Age Group.

Panel A shows local reactions in participants 18 to 55 years of age, and Panel B those in participants 65 to 85 years of age. Injection-site (local) reactions were recorded in electronic diaries for 7 days after each injection. Pain at the injection site was graded as mild (does not interfere with activity), moderate (interferes with activity), severe (prevents daily activity), or grade 4 (led to an emergency department visit or hospitalization). Redness and swelling were graded as mild (2.0 to 5.0 cm in diameter), moderate (>5.0 to 10.0 cm in diameter), severe (>10.0 cm in diameter), or grade 4 (necrosis or exfoliative dermatitis for redness and necrosis for swelling). I bars represent 95% confidence intervals. The numbers above the I bars show the overall percentage of the participants in each group who reported the specified local reaction. No participant who received either vaccine candidate reported a grade 4 local reaction.

but one, all the participants who underwent randomization received the assigned two doses of vaccine or placebo. Participants 18 to 55 years of age who had been assigned to receive 100 μg of BNT162b1 or placebo received one dose; the second dose was not administered because of reactogenicity in the participants who received active vaccine.[5]

The majority of participants were White (67 to 100%) and non-Hispanic (89 to 100%) (Table 1). More older women than older men participated. The median age among the younger participants was 35 years in the BNT162b1 group and 37 years in the BNT162b2 group; the median age among the older participants was 69 years and 68 years, respectively.

SAFETY

Local Reactions

Participants 18 to 55 years of age who received 10 μg, 20 μg, or 30 μg of BNT162b1 reported mild-to-moderate local reactions, primarily pain at the injection site, within 7 days after an injection; the local reactions were more frequent after the second dose.[2,5] BNT162b1 elicited local reactions in similar proportions of the participants in the younger age group and in the older age group. Among the older participants, mild-to-moderate injection-site pain was reported by 92% after the first dose and by 75% after the second dose (Fig. 2). A similar pattern was observed after vaccination with BNT162b2. No older participant who received BNT162b2 reported redness or swelling. No participant who received either BNT162 vaccine candidate reported a grade 4 local reaction.

Systemic Events

Participants 18 to 55 years of age who received 10 μg, 20 μg, or 30 μg of BNT162b1 frequently had mild-to-moderate fever and chills, with 75% of the participants reporting a temperature of 38.0°C or higher after the second 30-μg dose (Fig. 3; and Fig. S1 in the Supplementary Appendix, available at NEJM.org).[5] In participants 65 to 85 years of age who received BNT162b1, systemic events were milder than in the younger participants, although many older participants reported fatigue and headache after the first or second dose, and 33% reported a temperature of 38°C or higher after the second dose, including one older participant who reported a fever of 38.9 to 40.0°C (Fig. 3 and Fig. S2). As was observed with local reactions, systemic events were dose-dependent (greater after the second dose than after the first dose) and transient. Symptoms generally peaked by day 2 after vaccination and resolved by day 7.

Systemic events in response to BNT162b2 were milder than those in response to BNT162b1 (Fig. 3 and Figs. S1 and S2). For example, 17% of the participants 18 to 55 years of age and 8% of those 65 to 85 years of age reported fever (≥38.0 to 38.9°C) after the second dose of 30 μg of BNT162b2. Severe systemic events (fatigue, headache, chills, muscle pain, and joint pain) were reported in small numbers of younger recipients of BNT162b2, but no severe systemic events were reported by older recipients of this vaccine candidate. No participant who received either BNT162 vaccine candidate reported a grade 4 systemic event. After the first dose, systemic events that were reported by participants 65 to 85 years of age who received BNT162b2 were similar to those reported by participants who received placebo.

In both age groups and for both vaccine candidates, the use of antipyretic or pain medication increased with increasing dose level and with the number of doses administered. Fewer BNT162b2 recipients than BNT162b1 recipients reported using antipyretic or pain medication.

Adverse Events and Shifts in Laboratory Values

Through 1 month after the receipt of the second dose, adverse events that were considered by the

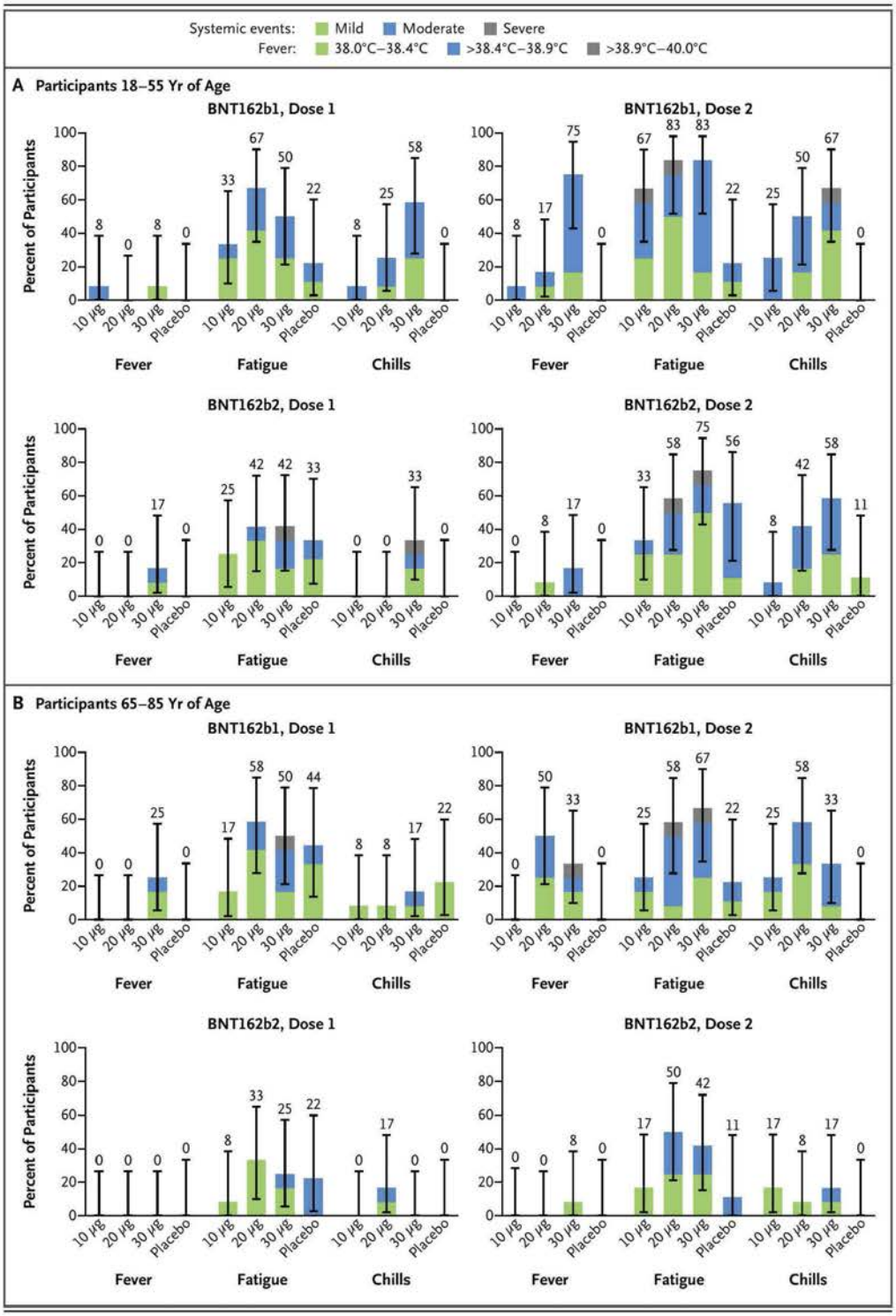

Figure 3 (facing page). Selected Systemic Events Reported within 7 Days after the Administration of Vaccine or Placebo, According to Age Group.

Panel A shows systemic reactions in participants 18 to 55 years of age, and Panel B those in participants 65 to 85 years of age. Data on fever, chills, and fatigue are reported here. (Data on headache, vomiting, diarrhea, muscle pain, and joint pain are reported in Fig. S1.) Data on systemic events were recorded in electronic diaries for 7 days after each injection. The fever scale is shown in the key. Chills and fatigue were graded as being mild (does not interfere with activity), moderate (interferes somewhat with activity), severe (prevents daily activity), or grade 4 (led to an emergency department visit or hospitalization). I bars represent 95% confidence intervals. The numbers above the I bars show the overall percentage of participants in each group who reported the specified systemic event. No participant who received either vaccine candidate reported a grade 4 systemic event or a temperature higher than 40.0°C.

investigators to be related to vaccine or placebo were reported by 50% of the participants 18 to 55 years of age who received 30 μg of BNT162b1, as compared with 8% of those who received placebo.[5] Adverse events that were considered to be related to vaccine were reported by 17% of the participants 65 to 85 years of age who received 30 μg of BNT162b1 and by 25% of the participants 18 to 55 years of age who received 30 μg of BNT162b2. No participant 65 to 85 years of age who received 30 μg of BNT162b2 reported a related adverse event (Table S1).

No serious adverse events were reported, and no stopping rules were met as of the time of this report. The largest changes from baseline in laboratory values were transient decreases in lymphocyte counts, which resolved within 1 week after vaccination (Fig. S3) and which were not associated with clinical manifestations.

IMMUNOGENICITY

The serologic responses elicited by BNT162b1 and BNT162b2 were similar (Fig. 4). Two serum samples, both from the group of participants 18 to 55 years of age who received 30 μg of BNT162b2, were obtained outside the specified time windows (one each at day 28 and day 35) and thus were excluded from the reported immunogenicity analysis. Antigen-binding IgG and virus-neutralizing responses to vaccination with 10 μg to 30 μg of BNT162b1 or BNT162b2 were boosted by the second dose in both the younger adults[2,5] and the older adults. Both vaccines elicited generally lower antigen-binding IgG and virus-neutralizing responses in participants 65 to 85 years of age than in those 18 to 55 years of age. Higher doses appeared to elicit somewhat higher antibody responses.

The highest neutralization titers were measured in samples obtained on day 28 (i.e., 7 days after the second dose) or on day 35 (i.e., 14 days after the second dose). Similar trends were observed for the 50% and 90% neutralizing titers (Fig. S4). The 50% neutralizing GMTs for the two vaccine candidates at the 30-μg dose level on day 28 or day 35 ranged from 1.7 to 4.6 times the GMT of the convalescent serum panel among participants 18 to 55 years of age and from 1.1 to 2.2 times the GMT of the convalescent serum panel among those 65 to 85 years of age. With 10 to 12 valid results per assay from samples that could be evaluated for each group at each time point, pair-wise comparisons are subject to error and have no clear interpretation.

DISCUSSION

Previously reported data from vaccination with 10 μg or 30 μg of BNT162b1 in adults 18 to 55 years of age suggested that it could be a promising Covid-19 vaccine candidate.[2,5] Consistent with our strategy to evaluate several RNA vaccine candidates and make a data-driven decision to advance the candidate with the best safety and immunogenicity profile, we compared clinical data obtained after vaccination with BNT162b1,[2,5] which encodes the RBD, with data obtained after vaccination with BNT162b2, which encodes the full-length spike. The data presented here include those that guided our decision to advance BNT162b2 at the 30-μg dose level to the phase 2–3, international trial to evaluate its safety and efficacy in participants 18 to 85 years of age.

The primary consideration driving this decision was the milder systemic reactogenicity profile of BNT162b2, particularly in older adults, in the context of the similar antibody responses elicited by the two candidate vaccines. Short-lived decreases in postvaccination lymphocyte counts had no associated clinical effect, were observed

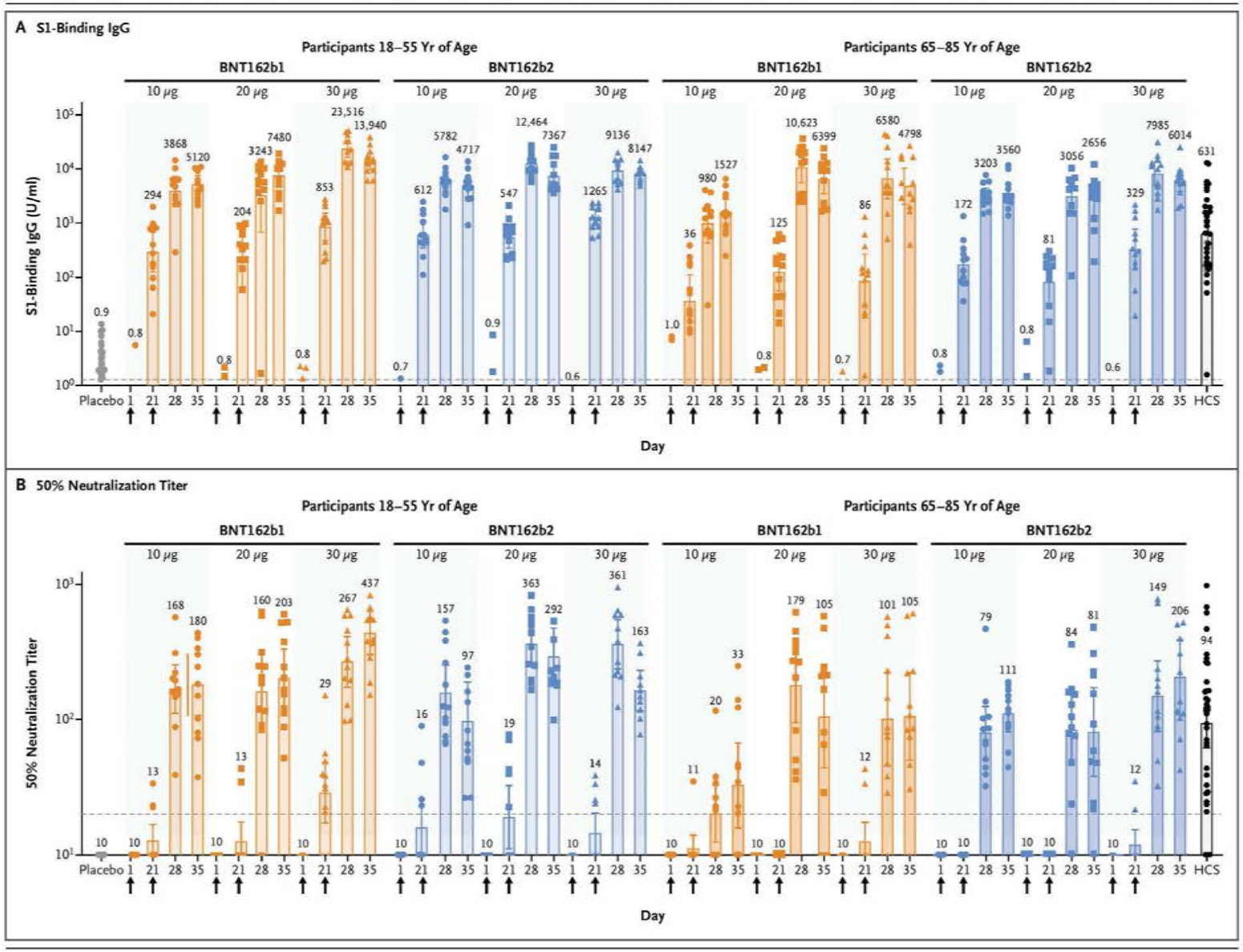
A S1-Binding IgG
Participants 18–55 Yr of Age
Participants 65–85 Yr of Age
BNT162b1
BNT162b2
10 μg
20 μg
30 μg
S1-Binding IgG (U/ml)
Placebo
HCS
Day
B 50% Neutralization Titer
50% Neutralization Titer
Day

Figure 4 (facing page). Immunogenicity of BNT162b1 and BNT162b2.

Participants in groups of 15 received an injection with the indicated dose levels of one of either of the BNT162 vaccine candidates (12 participants) or placebo (3 participants) on days 1 and 21. Arrows indicate days of vaccination. Responses in the placebo recipients in each of the dose-level groups are combined. Serum samples were obtained before injection (on day 1) and on days 21, 28, and 35 after the first dose. The blood samples obtained on days 28 and 35 are those obtained 7 days and 14 days, respectively, after the second dose. Human coronavirus disease 2019 (Covid-19) or SARS-CoV-2 infection convalescent serum (HCS) samples were obtained from 38 donors at least 14 days after polymerase chain reaction–confirmed diagnosis and at a time when the donors were asymptomatic. Panel A shows the geometric mean concentrations of recombinant S1-binding IgG (lower limit of quantitation, 1.267; dashed line), and Panel B the 50% SARS-CoV-2–neutralizing geometric mean titers (lower limit of quantitation, 20; dashed line). On days that vaccine or placebo was administered, samples were obtained before the injection. Each data point represents a serum sample, and the top of each vertical bar represents the geometric mean with the 95% confidence interval (I bar). Data points associated with placebo, HCS samples, or the 10-μg dose of vaccine are shown as circles, those for the 20-μg dose as squares, and those for the 30-μg dose as triangles. The numbers above the bars show the geometric mean concentration or geometric mean titer in the group. All the vaccine groups had 12 valid results from samples that could be evaluated at each time point except for the following: among participants who received BNT162b2, 11 results from day 28 in younger participants who received 30 μg, 10 results from day 35 in younger participants who received 30 μg, and 11 results from day 35 in older participants who received 10 μg.

across the age groups, and probably reflect a temporary redistribution of lymphocytes from the bloodstream to lymphoid tissues as a functional response to immune stimulation by the vaccine.[13-16] The immune response and toxicity profile at the selected, relatively low, 30-μg dose level indicate that the BNT162b2 modRNA vaccine candidate has a favorable balance of reactogenicity and immunogenicity.[17,18]

The composition of the lipid nanoparticles, the formulation components, or the sequence selection for the vaccine RNA could influence the side-effect profile. The reason for the lower reactogenicity of BNT162b2 than of BNT162b1 is not certain, given that the two vaccine candidates share the same modRNA platform, RNA production and purification processes, and formulation of lipid nanoparticles. They differ in the nucleotide sequences that encode the vaccine antigens and in the overall size of the RNA constructs, which results in a number of RNA molecules in 30 μg of BNT162b1 that is approximately 5 times as high as that in 30 μg of BNT162b2. The nucleotide composition of RNA has been reported to affect its immune stimulatory activity and reactogenicity profile, and this is a possible explanation for the differences in these vaccine candidates.[19]

The immune responses elicited by BNT162b1 and BNT162b2 were similar. As has been observed with other vaccines and as is probably associated with immunosenescence,[20,21] the immunogenicity of the two vaccine candidates decreased with age, eliciting lower overall humoral responses in adults 65 to 85 years of age than in those 18 to 55 years of age. Nevertheless, at 7 days and 14 days after the second dose, the 50% and 90% neutralizing GMTs that were elicited by 30 μg of BNT162b2 in older adults exceeded those of the convalescent serum panel. Antibody responses in both younger and older adults showed a clear benefit of a second dose.

This trial and interim report have several limitations. First, the relative importance of humoral and cellular immunity with regard to protection from Covid-19 has not yet been fully characterized. Although strong cell-mediated immune responses (Th1-biased CD4+ and CD8+) elicited by BNT162b1 have been observed and reported in the German trial,[2] the cellular immune responses elicited by BNT162b2 are still being studied. Second, although the serum neutralizing responses that were elicited by the vaccine candidates relative to those elicited by natural infection are highly encouraging, the degree of protection against Covid-19 provided by this or any other benchmark is unknown. Third, the phase 1 portion of this trial tested many hypotheses and was not powered to make formal statistical comparisons. Fourth, the human convalescent serum panels that have been used by different vaccine developers are not standardized among laboratories, and each represents a unique distribution of donor characteristics and times of collection. Therefore, the serum panel that we used does not provide a well-controlled benchmark for comparisons of the serologic responses elicited by these two BNT162 vaccine candidates with those elicited by other Covid-19 vaccine candi-

dates. Finally, the participants in this early-stage clinical trial were healthy and had limited racial and ethnic diversity as compared with the general population.

Many of the limitations cited above are being addressed in the international, phase 2–3 portion of this trial. In this later, pivotal part of the trial, we are assessing the safety and efficacy of two doses of 30 μg of BNT162b2 in up to 44,000 participants (randomly assigned in a 1:1 ratio to receive vaccine or placebo) from diverse backgrounds, including persons with stable chronic underlying health conditions, persons at increased risk owing to occupational exposure, and persons from racial and ethnic backgrounds at higher risk for severe Covid-19.[22] We are conducting outreach to recruit trial participants from many backgrounds and are using U.S. Census data to locate trial sites in diverse communities.

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Disclosure forms provide by the authors are available with the full text of the article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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ORIGINAL ARTICLE

Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine

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ABSTRACT

BACKGROUND

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and the resulting coronavirus disease 2019 (Covid-19) have afflicted tens of millions of people in a worldwide pandemic. Safe and effective vaccines are needed urgently.

METHODS

In an ongoing multinational, placebo-controlled, observer-blinded, pivotal efficacy trial, we randomly assigned persons 16 years of age or older in a 1:1 ratio to receive two doses, 21 days apart, of either placebo or the BNT162b2 vaccine candidate (30 μg per dose). BNT162b2 is a lipid nanoparticle–formulated, nucleoside-modified RNA vaccine that encodes a prefusion stabilized, membrane-anchored SARS-CoV-2 full-length spike protein. The primary end points were efficacy of the vaccine against laboratory-confirmed Covid-19 and safety.

RESULTS

A total of 43,548 participants underwent randomization, of whom 43,448 received injections: 21,720 with BNT162b2 and 21,728 with placebo. There were 8 cases of Covid-19 with onset at least 7 days after the second dose among participants assigned to receive BNT162b2 and 162 cases among those assigned to placebo; BNT162b2 was 95% effective in preventing Covid-19 (95% credible interval, 90.3 to 97.6). Similar vaccine efficacy (generally 90 to 100%) was observed across subgroups defined by age, sex, race, ethnicity, baseline body-mass index, and the presence of coexisting conditions. Among 10 cases of severe Covid-19 with onset after the first dose, 9 occurred in placebo recipients and 1 in a BNT162b2 recipient. The safety profile of BNT162b2 was characterized by short-term, mild-to-moderate pain at the injection site, fatigue, and headache. The incidence of serious adverse events was low and was similar in the vaccine and placebo groups.

CONCLUSIONS

A two-dose regimen of BNT162b2 conferred 95% protection against Covid-19 in persons 16 years of age or older. Safety over a median of 2 months was similar to that of other viral vaccines. (Funded by BioNTech and Pfizer; ClinicalTrials.gov number, NCT04368728.)

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A Quick Take is available at NEJM.org

Coronavirus disease 2019 (COVID-19) has affected tens of millions of people globally[1] since it was declared a pandemic by the World Health Organization on March 11, 2020.[2] Older adults, persons with certain coexisting conditions, and front-line workers are at highest risk for Covid-19 and its complications. Recent data show increasing rates of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection and Covid-19 in other populations, including younger adults.[3] Safe and effective prophylactic vaccines are urgently needed to contain the pandemic, which has had devastating medical, economic, and social consequences.

We previously reported phase 1 safety and immunogenicity results from clinical trials of the vaccine candidate BNT162b2,[4] a lipid nanoparticle–formulated,[5] nucleoside-modified RNA (modRNA)[6] encoding the SARS-CoV-2 full-length spike, modified by two proline mutations to lock it in the prefusion conformation.[7] Findings from studies conducted in the United States and Germany among healthy men and women showed that two 30-μg doses of BNT162b2 elicited high SARS-CoV-2 neutralizing antibody titers and robust antigen-specific CD8+ and Th1-type CD4+ T-cell responses.[8] The 50% neutralizing geometric mean titers elicited by 30 μg of BNT162b2 in older and younger adults exceeded the geometric mean titer measured in a human convalescent serum panel, despite a lower neutralizing response in older adults than in younger adults. In addition, the reactogenicity profile of BNT162b2 represented mainly short-term local (i.e., injection site) and systemic responses. These findings supported progression of the BNT162b2 vaccine candidate into phase 3.

Here, we report safety and efficacy findings from the phase 2/3 part of a global phase 1/2/3 trial evaluating the safety, immunogenicity, and efficacy of 30 μg of BNT162b2 in preventing Covid-19 in persons 16 years of age or older. This data set and these trial results are the basis for an application for emergency use authorization.[9] Collection of phase 2/3 data on vaccine immunogenicity and the durability of the immune response to immunization is ongoing, and those data are not reported here.

METHODS

TRIAL OBJECTIVES, PARTICIPANTS AND OVERSIGHT

We assessed the safety and efficacy of two 30-μg doses of BNT162b2, administered intramuscularly 21 days apart, as compared with placebo. Adults 16 years of age or older who were healthy or had stable chronic medical conditions, including but not limited to human immunodeficiency virus (HIV), hepatitis B virus, or hepatitis C virus infection, were eligible for participation in the trial. Key exclusion criteria included a medical history of Covid-19, treatment with immunosuppressive therapy, or diagnosis with an immunocompromising condition.

Pfizer was responsible for the design and conduct of the trial, data collection, data analysis, data interpretation, and the writing of the manuscript. BioNTech was the sponsor of the trial, manufactured the BNT162b2 clinical trial material, and contributed to the interpretation of the data and the writing of the manuscript. All the trial data were available to all the authors, who vouch for its accuracy and completeness and for adherence of the trial to the protocol, which is available with the full text of this article at NEJM.org. An independent data and safety monitoring board reviewed efficacy and unblinded safety data.

TRIAL PROCEDURES

With the use of an interactive Web-based system, participants in the trial were randomly assigned in a 1:1 ratio to receive 30 μg of BNT162b2 (0.3 ml volume per dose) or saline placebo. Participants received two injections, 21 days apart, of either BNT162b2 or placebo, delivered in the deltoid muscle. Site staff who were responsible for safety evaluation and were unaware of group assignments observed participants for 30 minutes after vaccination for any acute reactions.

SAFETY

The primary end points of this trial were solicited, specific local or systemic adverse events and use of antipyretic or pain medication within 7 days after the receipt of each dose of vaccine or placebo, as prompted by and recorded in an electronic diary in a subset of participants (the reactogenicity subset), and unsolicited adverse events (those reported by the participants without prompts from the electronic diary) through 1 month after the second dose and unsolicited serious adverse events through 6 months after the second dose. Adverse event data through approximately 14 weeks after the second dose are included in this report. In this report, safety

data are reported for all participants who provided informed consent and received at least one dose of vaccine or placebo. Per protocol, safety results for participants infected with HIV (196 patients) will be analyzed separately and are not included here.

During the phase 2/3 portion of the study, a stopping rule for the theoretical concern of vaccine-enhanced disease was to be triggered if the one-sided probability of observing the same or a more unfavorable adverse severe case split (a split with a greater proportion of severe cases in vaccine recipients) was 5% or less, given the same true incidence for vaccine and placebo recipients. Alert criteria were to be triggered if this probability was less than 11%.

EFFICACY

The first primary end point was the efficacy of BNT162b2 against confirmed Covid-19 with onset at least 7 days after the second dose in participants who had been without serologic or virologic evidence of SARS-CoV-2 infection up to 7 days after the second dose; the second primary end point was efficacy in participants with and participants without evidence of prior infection. Confirmed Covid-19 was defined according to the Food and Drug Administration (FDA) criteria as the presence of at least one of the following symptoms: fever, new or increased cough, new or increased shortness of breath, chills, new or increased muscle pain, new loss of taste or smell, sore throat, diarrhea, or vomiting, combined with a respiratory specimen obtained during the symptomatic period or within 4 days before or after it that was positive for SARS-COV-2 by nucleic acid amplification–based testing, either at the central laboratory or at a local testing facility (using a protocol-defined acceptable test).

Major secondary end points included the efficacy of BNT162b2 against severe Covid-19. Severe Covid-19 is defined by the FDA as confirmed Covid-19 with one of the following additional features: clinical signs at rest that are indicative of severe systemic illness; respiratory failure; evidence of shock; significant acute renal, hepatic, or neurologic dysfunction; admission to an intensive care unit; or death. Details are provided in the protocol.

An explanation of the various denominator values for use in assessing the results of the trial is provided in Table S1 in the Supplementary Appendix, available at NEJM.org. In brief, the safety population includes persons 16 years of age or older; a total of 43,448 participants constituted the population of enrolled persons injected with the vaccine or placebo. The main safety subset as defined by the FDA, with a median of 2 months of follow-up as of October 9, 2020, consisted of 37,706 persons, and the reactogenicity subset consisted of 8183 persons. The modified intention-to-treat (mITT) efficacy population includes all age groups 12 years of age or older (43,355 persons; 100 participants who were 12 to 15 years of age contributed to person-time years but included no cases). The number of persons who could be evaluated for efficacy 7 days after the second dose and who had no evidence of prior infection was 36,523, and the number of persons who could be evaluated 7 days after the second dose with or without evidence of prior infection was 40,137.

STATISTICAL ANALYSIS

The safety analyses included all participants who received at least one dose of BNT162b2 or placebo. The findings are descriptive in nature and not based on formal statistical hypothesis testing. Safety analyses are presented as counts, percentages, and associated Clopper–Pearson 95% confidence intervals for local reactions, systemic events, and any adverse events after vaccination, according to terms in the *Medical Dictionary for Regulatory Activities* (MedDRA), version 23.1, for each vaccine group.

Analysis of the first primary efficacy end point included participants who received the vaccine or placebo as randomly assigned, had no evidence of infection within 7 days after the second dose, and had no major protocol deviations (the population that could be evaluated). Vaccine efficacy was estimated by $100 \times (1 - \text{IRR})$, where IRR is the calculated ratio of confirmed cases of Covid-19 illness per 1000 person-years of follow-up in the active vaccine group to the corresponding illness rate in the placebo group. The 95.0% credible interval for vaccine efficacy and the probability of vaccine efficacy greater than 30% were calculated with the use of a Bayesian beta-binomial model. The final analysis uses a success boundary of 98.6% for probability of vaccine efficacy greater than 30% to compensate for the interim analysis and to control the overall type 1 error rate at 2.5%.

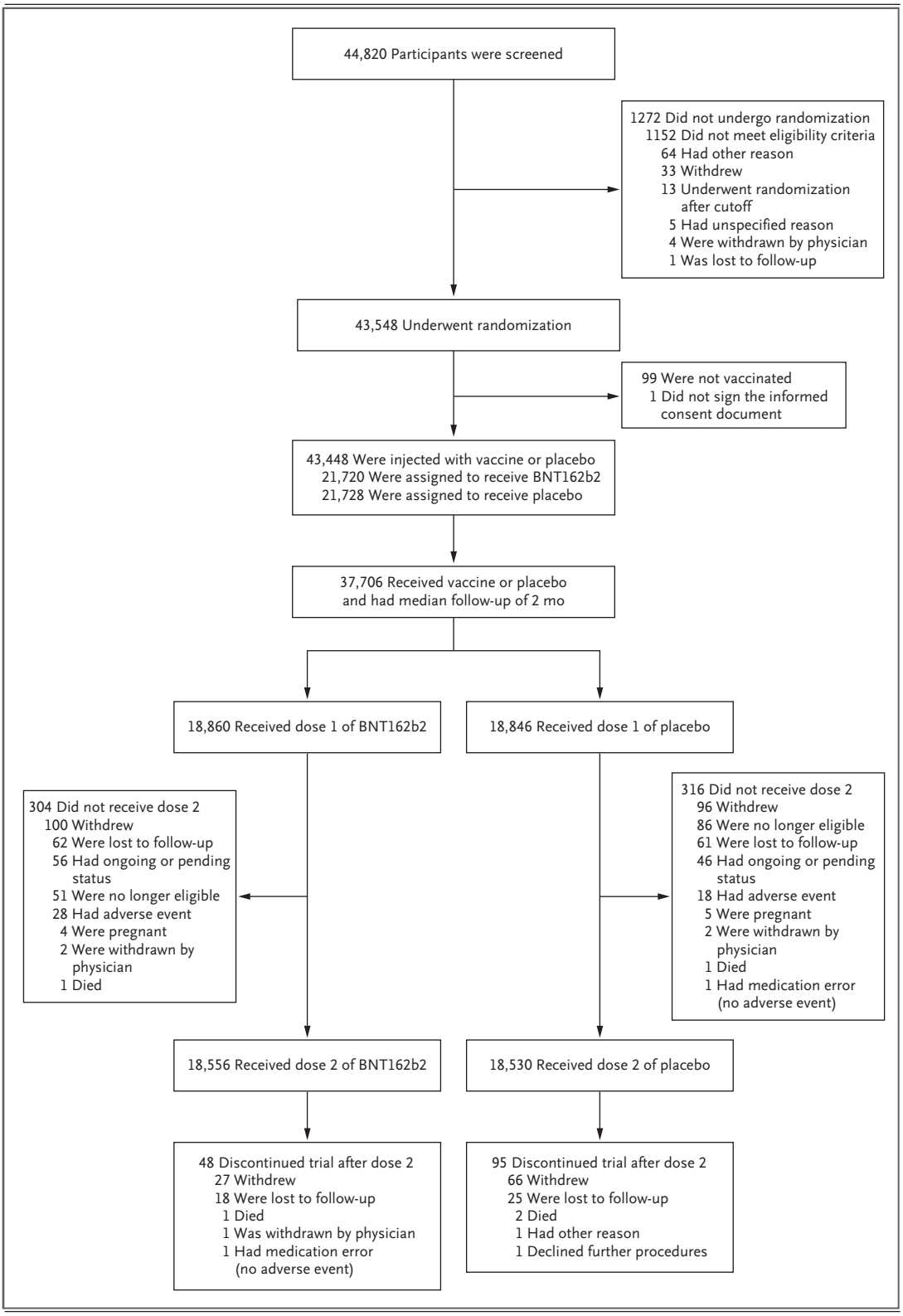


Figure 1 (facing page). Enrollment and Randomization.
The diagram represents all enrolled participants through November 14, 2020. The safety subset (those with a median of 2 months of follow-up, in accordance with application requirements for Emergency Use Authorization) is based on an October 9, 2020, data cutoff date. The further procedures that one participant in the placebo group declined after dose 2 (lower right corner of the diagram) were those involving collection of blood and nasal swab samples.

Moreover, primary and secondary efficacy end points are evaluated sequentially to control the familywise type 1 error rate at 2.5%. Descriptive analyses (estimates of vaccine efficacy and 95% confidence intervals) are provided for key subgroups.

RESULTS

PARTICIPANTS

Between July 27, 2020, and November 14, 2020, a total of 44,820 persons were screened, and 43,548 persons 16 years of age or older underwent randomization at 152 sites worldwide (United States, 130 sites; Argentina, 1; Brazil, 2; South Africa, 4; Germany, 6; and Turkey, 9) in the phase 2/3 portion of the trial. A total of

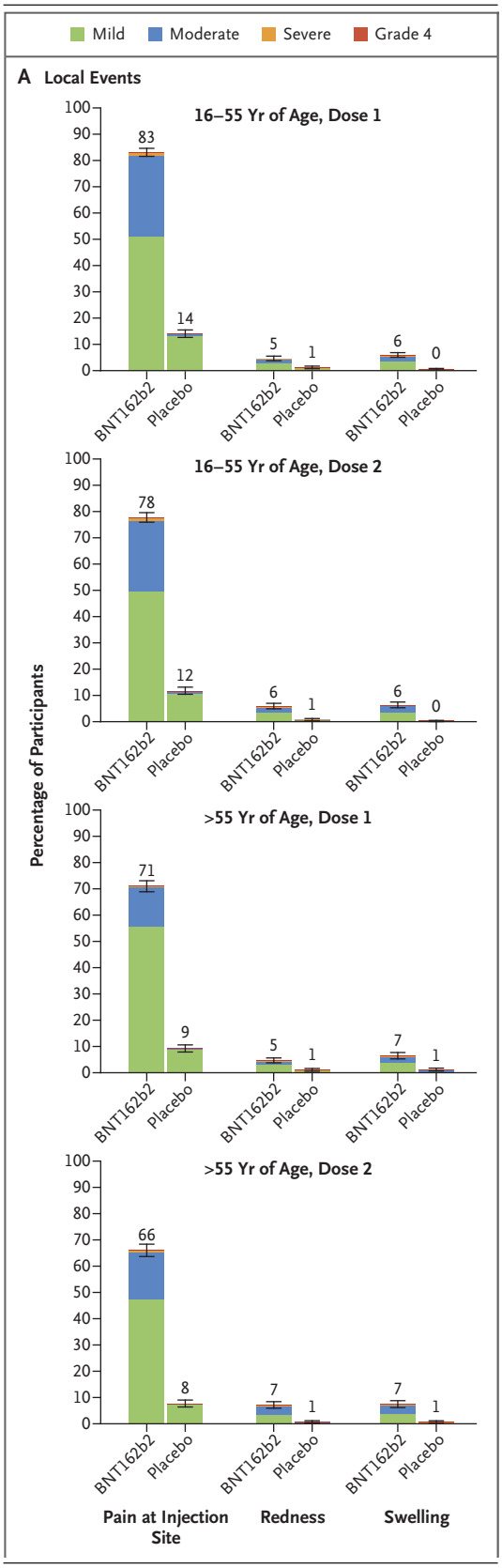
Table 1. Demographic Characteristics of the Participants in the Main Safety Population.*

Characteristic	BNT162b2 (N=18,860)	Placebo (N=18,846)	Total (N=37,706)
Sex — no. (%)			
Male	9,639 (51.1)	9,436 (50.1)	19,075 (50.6)
Female	9,221 (48.9)	9,410 (49.9)	18,631 (49.4)
Race or ethnic group — no. (%)†			
White	15,636 (82.9)	15,630 (82.9)	31,266 (82.9)
Black or African American	1,729 (9.2)	1,763 (9.4)	3,492 (9.3)
Asian	801 (4.2)	807 (4.3)	1,608 (4.3)
Native American or Alaska Native	102 (0.5)	99 (0.5)	201 (0.5)
Native Hawaiian or other Pacific Islander	50 (0.3)	26 (0.1)	76 (0.2)
Multiracial	449 (2.4)	406 (2.2)	855 (2.3)
Not reported	93 (0.5)	115 (0.6)	208 (0.6)
Hispanic or Latinx	5,266 (27.9)	5,277 (28.0)	10,543 (28.0)
Country — no. (%)			
Argentina	2,883 (15.3)	2,881 (15.3)	5,764 (15.3)
Brazil	1,145 (6.1)	1,139 (6.0)	2,284 (6.1)
South Africa	372 (2.0)	372 (2.0)	744 (2.0)
United States	14,460 (76.7)	14,454 (76.7)	28,914 (76.7)
Age group — no. (%)			
16–55 yr	10,889 (57.7)	10,896 (57.8)	21,785 (57.8)
>55 yr	7,971 (42.3)	7,950 (42.2)	15,921 (42.2)
Age at vaccination — yr			
Median	52.0	52.0	52.0
Range	16–89	16–91	16–91
Body-mass index‡			
≥30.0: obese	6,556 (34.8)	6,662 (35.3)	13,218 (35.1)

* Percentages may not total 100 because of rounding.
† Race or ethnic group was reported by the participants.
‡ The body-mass index is the weight in kilograms divided by the square of the height in meters.

Figure 2. Local and Systemic Reactions Reported within 7 Days after Injection of BNT162b2 or Placebo, According to Age Group.

Data on local and systemic reactions and use of medication were collected with electronic diaries from participants in the reactogenicity subset (8,183 participants) for 7 days after each vaccination. Solicited injection-site (local) reactions are shown in Panel A. Pain at the injection site was assessed according to the following scale: mild, does not interfere with activity; moderate, interferes with activity; severe, prevents daily activity; and grade 4, emergency department visit or hospitalization. Redness and swelling were measured according to the following scale: mild, 2.0 to 5.0 cm in diameter; moderate, >5.0 to 10.0 cm in diameter; severe, >10.0 cm in diameter; and grade 4, necrosis or exfoliative dermatitis (for redness) and necrosis (for swelling). Systemic events and medication use are shown in Panel B. Fever categories are designated in the key; medication use was not graded. Additional scales were as follows: fatigue, headache, chills, new or worsened muscle pain, new or worsened joint pain (mild: does not interfere with activity; moderate: some interference with activity; or severe: prevents daily activity), vomiting (mild: 1 to 2 times in 24 hours; moderate: >2 times in 24 hours; or severe: requires intravenous hydration), and diarrhea (mild: 2 to 3 loose stools in 24 hours; moderate: 4 to 5 loose stools in 24 hours; or severe: 6 or more loose stools in 24 hours); grade 4 for all events indicated an emergency department visit or hospitalization. I bars represent 95% confidence intervals, and numbers above the I bars are the percentage of participants who reported the specified reaction.

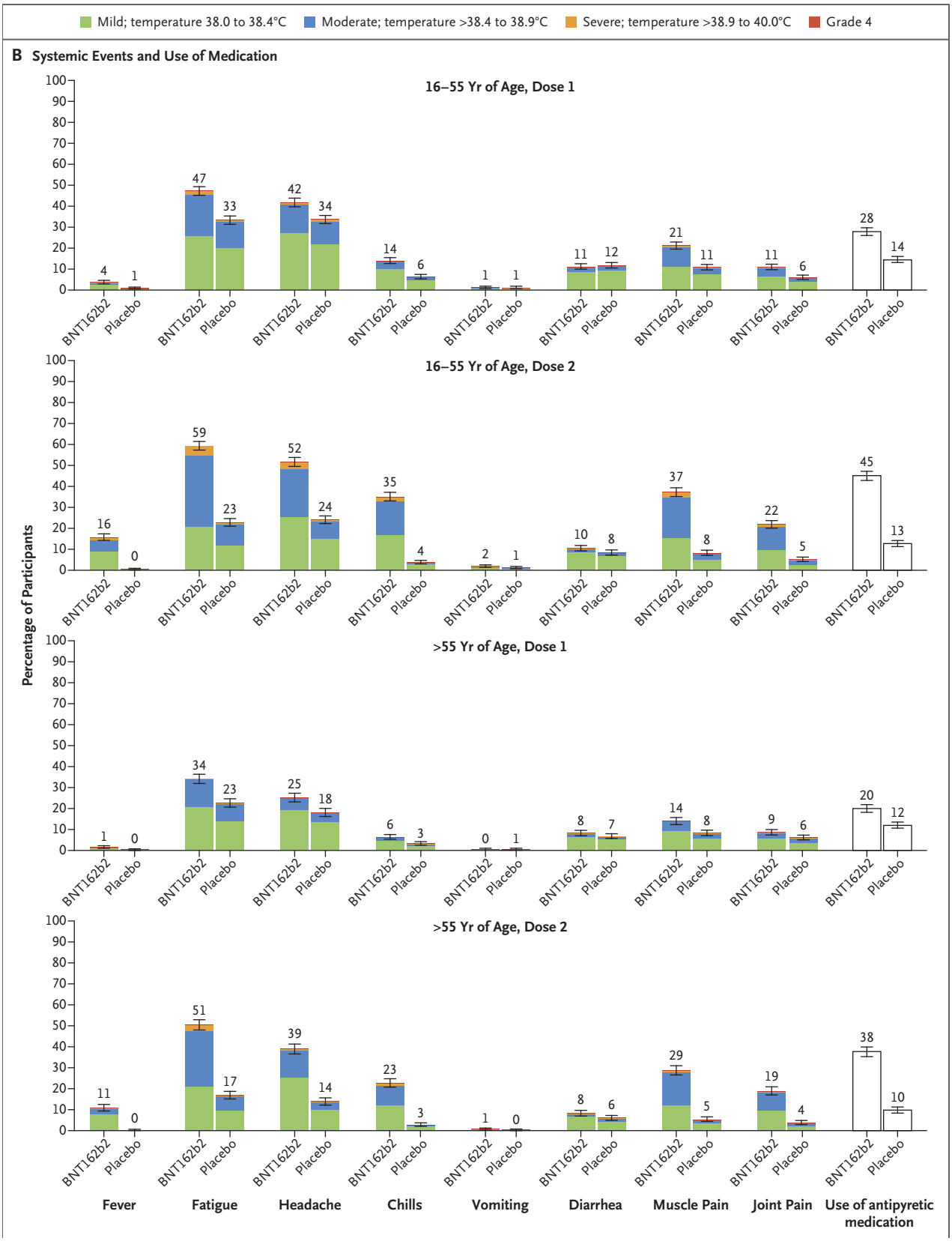


43,448 participants received injections: 21,720 received BNT162b2 and 21,728 received placebo (Fig. 1). At the data cut-off date of October 9, a total of 37,706 participants had a median of at least 2 months of safety data available after the second dose and contributed to the main safety data set. Among these 37,706 participants, 49% were female, 83% were White, 9% were Black or African American, 28% were Hispanic or Latinx, 35% were obese (body mass index [the weight in kilograms divided by the square of the height in meters] of at least 30.0), and 21% had at least one coexisting condition. The median age was 52 years, and 42% of participants were older than 55 years of age (Table 1 and Table S2).

SAFETY

Local Reactogenicity

The reactogenicity subset included 8183 participants. Overall, BNT162b2 recipients reported more local reactions than placebo recipients. Among BNT162b2 recipients, mild-to-moderate pain at



the injection site within 7 days after an injection was the most commonly reported local reaction, with less than 1% of participants across all age groups reporting severe pain (Fig. 2). Pain was reported less frequently among participants older than 55 years of age (71% reported pain after the first dose; 66% after the second dose) than among younger participants (83% after the first dose; 78% after the second dose). A noticeably lower percentage of participants reported injection-site redness or swelling. The proportion of participants reporting local reactions did not increase after the second dose (Fig. 2A), and no participant reported a grade 4 local reaction. In general, local reactions were mostly mild-to-moderate in severity and resolved within 1 to 2 days.

Systemic Reactogenicity

Systemic events were reported more often by younger vaccine recipients (16 to 55 years of age) than by older vaccine recipients (more than 55 years of age) in the reactogenicity subset and more often after dose 2 than dose 1 (Fig. 2B). The most commonly reported systemic events were fatigue and headache (59% and 52%, respectively, after the second dose, among younger vaccine recipients; 51% and 39% among older recipients), although fatigue and headache were also reported by many placebo recipients (23% and 24%, respectively, after the second dose, among younger vaccine recipients; 17% and 14% among older recipients). The frequency of any severe systemic event after the first dose was 0.9% or less. Severe systemic events were reported in less than 2% of vaccine recipients after either dose, except for fatigue (in 3.8%) and headache (in 2.0%) after the second dose.

Fever (temperature, ≥38°C) was reported after the second dose by 16% of younger vaccine recipients and by 11% of older recipients. Only 0.2% of vaccine recipients and 0.1% of placebo recipients reported fever (temperature, 38.9 to 40°C) after the first dose, as compared with 0.8% and 0.1%, respectively, after the second dose. Two participants each in the vaccine and placebo groups reported temperatures above 40.0°C. Younger vaccine recipients were more likely to use antipyretic or pain medication (28% after dose 1; 45% after dose 2) than older vaccine recipients (20% after dose 1; 38% after dose 2), and placebo recipients were less likely (10 to 14%) than vaccine recipients to use the medications, regardless of age or dose. Systemic events including fever and chills were observed with the first 1 to 2 days after vaccination and resolved shortly thereafter.

Daily use of the electronic diary ranged from 90 to 93% for each day after the first dose and from 75 to 83% for each day after the second dose. No difference was noted between the BNT162b2 group and the placebo group.

ADVERSE EVENTS

Adverse event analyses are provided for all enrolled 43,252 participants, with variable follow-up time after dose 1 (Table S3). More BNT162b2 recipients than placebo recipients reported any adverse event (27% and 12%, respectively) or a related adverse event (21% and 5%). This distribution largely reflects the inclusion of transient reactogenicity events, which were reported as adverse events more commonly by vaccine recipients than by placebo recipients. Sixty-four vaccine recipients (0.3%) and 6 placebo recipients (<0.1%) reported lymphadenopathy. Few participants in either group had severe adverse events, serious adverse events, or adverse events leading to withdrawal from the trial. Four related serious adverse events were reported among BNT162b2 recipients (shoulder injury related to vaccine administration, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia, and right leg paresthesia). Two BNT162b2 recipients died (one from arteriosclerosis, one from cardiac arrest), as did four placebo recipients (two from unknown causes, one from hemorrhagic stroke, and one from myocardial infarction). No deaths were considered by the investigators to be related to the vaccine or placebo. No Covid-19–associated deaths were observed. No stopping rules were met during the reporting period. Safety monitoring will continue for 2 years after administration of the second dose of vaccine.

EFFICACY

Among 36,523 participants who had no evidence of existing or prior SARS-CoV-2 infection, 8 cases of Covid-19 with onset at least 7 days after the second dose were observed among vaccine recipients and 162 among placebo recipients. This case split corresponds to 95.0% vaccine efficacy (95% confidence interval [CI], 90.3 to 97.6; Ta-

Table 2. Vaccine Efficacy against Covid-19 at Least 7 days after the Second Dose.*

Efficacy End Point	BNT162b2		Placebo		Vaccine Efficacy, % (95% Credible Interval)‡	Posterior Probability (Vaccine Efficacy >30%)§
	No. of Cases	Surveillance Time (n)†	No. of Cases	Surveillance Time (n)†		
		(N=18,198)		(N=18,325)		
Covid-19 occurrence at least 7 days after the second dose in participants without evidence of infection	8	2.214 (1,7411)	162	2.222 (17,511)	95.0 (90.3–97.6)	>0.9999
		(N=19,965)		(N=20,172)		
Covid-19 occurrence at least 7 days after the second dose in participants with and those without evidence of infection	9	2.332 (18,559)	169	2.345 (18,708)	94.6 (89.9–97.3)	>0.9999

* The total population without baseline infection was 36,523; total population including those with and those without prior evidence of infection was 40,137.
† The surveillance time is the total time in 1000 person-years for the given end point across all participants within each group at risk for the end point. The time period for Covid-19 case accrual is from 7 days after the second dose to the end of the surveillance period.
‡ The credible interval for vaccine efficacy was calculated with the use of a beta-binomial model with prior beta (0.700102, 1) adjusted for the surveillance time.
§ Posterior probability was calculated with the use of a beta-binomial model with prior beta (0.700102, 1) adjusted for the surveillance time.

ble 2). Among participants with and those without evidence of prior SARS CoV-2 infection, 9 cases of Covid-19 at least 7 days after the second dose were observed among vaccine recipients and 169 among placebo recipients, corresponding to 94.6% vaccine efficacy (95% CI, 89.9 to 97.3). Supplemental analyses indicated that vaccine efficacy among subgroups defined by age, sex, race, ethnicity, obesity, and presence of a coexisting condition was generally consistent with that observed in the overall population (Table 3 and Table S4). Vaccine efficacy among participants with hypertension was analyzed separately but was consistent with the other subgroup analyses (vaccine efficacy, 94.6%; 95% CI, 68.7 to 99.9; case split: BNT162b2, 2 cases; placebo, 44 cases). Figure 3 shows cases of Covid-19 or severe Covid-19 with onset at any time after the first dose (mITT population) (additional data on severe Covid-19 are available in Table S5). Between the first dose and the second dose, 39 cases in the BNT162b2 group and 82 cases in the placebo group were observed, resulting in a vaccine efficacy of 52% (95% CI, 29.5 to 68.4) during this interval and indicating early protection by the vaccine, starting as soon as 12 days after the first dose.

DISCUSSION

A two-dose regimen of BNT162b2 (30 μg per dose, given 21 days apart) was found to be safe and 95% effective against Covid-19. The vaccine met both primary efficacy end points, with more than a 99.99% probability of a true vaccine efficacy greater than 30%. These results met our prespecified success criteria, which were to establish a probability above 98.6% of true vaccine efficacy being greater than 30%, and greatly exceeded the minimum FDA criteria for authorization.[9] Although the study was not powered to definitively assess efficacy by subgroup, the point estimates of efficacy for subgroups based on age, sex, race, ethnicity, body-mass index, or the presence of an underlying condition associated with a high risk of Covid-19 complications are also high. For all analyzed subgroups in which more than 10 cases of Covid-19 occurred, the lower limit of the 95% confidence interval for efficacy was more than 30%.

The cumulative incidence of Covid-19 cases over time among placebo and vaccine recipients begins to diverge by 12 days after the first dose, 7 days after the estimated median viral incuba-

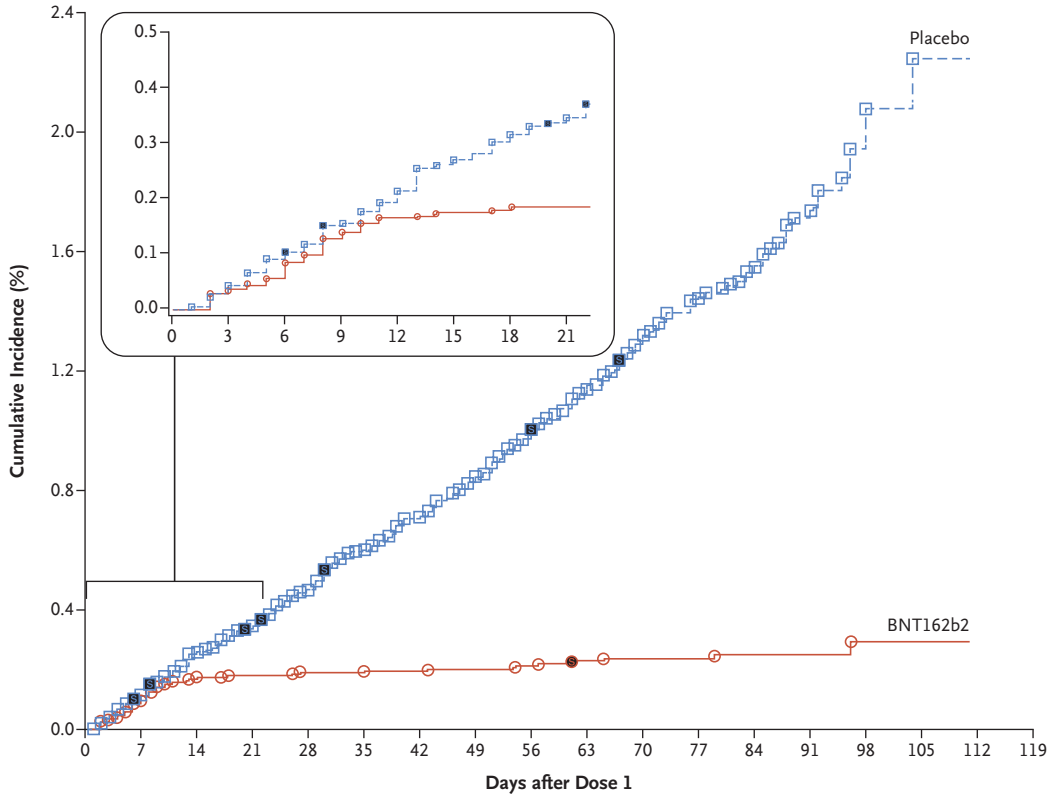
Table 3. Vaccine Efficacy Overall and by Subgroup in Participants without Evidence of Infection before 7 Days after Dose 2.

Efficacy End-Point Subgroup	BNT162b2 (N=18,198)		Placebo (N=18,325)		Vaccine Efficacy, % (95% CI)†
	No. of Cases	Surveillance Time (No. at Risk)*	No. of Cases	Surveillance Time (No. at Risk)*	
Overall	8	2.214 (17,411)	162	2.222 (17,511)	95.0 (90.0–97.9)
Age group					
16 to 55 yr	5	1.234 (9,897)	114	1.239 (9,955)	95.6 (89.4–98.6)
>55 yr	3	0.980 (7,500)	48	0.983 (7,543)	93.7 (80.6–98.8)
≥65 yr	1	0.508 (3,848)	19	0.511 (3,880)	94.7 (66.7–99.9)
≥75 yr	0	0.102 (774)	5	0.106 (785)	100.0 (–13.1–100.0)
Sex					
Male	3	1.124 (8,875)	81	1.108 (8762)	96.4 (88.9–99.3)
Female	5	1.090 (8,536)	81	1.114 (8,749)	93.7 (84.7–98.0)
Race or ethnic group‡					
White	7	1.889 (14,504)	146	1.903 (14,670)	95.2 (89.8–98.1)
Black or African American	0	0.165 (1,502)	7	0.164 (1,486)	100.0 (31.2–100.0)
All others	1	0.160 (1,405)	9	0.155 (1,355)	89.3 (22.6–99.8)
Hispanic or Latinx	3	0.605 (4,764)	53	0.600 (4,746)	94.4 (82.7–98.9)
Non-Hispanic, non-Latinx	5	1.596 (12,548)	109	1.608 (12,661)	95.4 (88.9–98.5)
Country					
Argentina	1	0.351 (2,545)	35	0.346 (2,521)	97.2 (83.3–99.9)
Brazil	1	0.119 (1,129)	8	0.117 (1,121)	87.7 (8.1–99.7)
United States	6	1.732 (13,359)	119	1.747 (13,506)	94.9 (88.6–98.2)

* Surveillance time is the total time in 1000 person-years for the given end point across all participants within each group at risk for the end point. The time period for Covid-19 case accrual is from 7 days after the second dose to the end of the surveillance period.
† The confidence interval (CI) for vaccine efficacy is derived according to the Clopper–Pearson method, adjusted for surveillance time.
‡ Race or ethnic group was reported by the participants. "All others" included the following categories: American Indian or Alaska Native, Asian, Native Hawaiian or other Pacific Islander, multiracial, and not reported.

tion period of 5 days,[10] indicating the early onset of a partially protective effect of immunization. The study was not designed to assess the efficacy of a single-dose regimen. Nevertheless, in the interval between the first and second doses, the observed vaccine efficacy against Covid-19 was 52%, and in the first 7 days after dose 2, it was 91%, reaching full efficacy against disease with onset at least 7 days after dose 2. Of the 10 cases of severe Covid-19 that were observed after the first dose, only 1 occurred in the vaccine group. This finding is consistent with overall high efficacy against all Covid-19 cases. The severe case split provides preliminary evidence of vaccine-mediated protection against severe disease, alleviating many of the theoretical concerns over vaccine-mediated disease enhancement.[11]

The favorable safety profile observed during phase 1 testing of BNT162b2[4,8] was confirmed in the phase 2/3 portion of the trial. As in phase 1, reactogenicity was generally mild or moderate, and reactions were less common and milder in older adults than in younger adults. Systemic reactogenicity was more common and severe after the second dose than after the first dose, although local reactogenicity was similar after the two doses. Severe fatigue was observed in approximately 4% of BNT162b2 recipients, which is higher than that observed in recipients of some vaccines recommended for older adults.[12] This rate of severe fatigue is also lower than that observed in recipients of another approved viral vaccine for older adults.[13] Overall, reactogenicity events were transient and resolved within a couple



Efficacy End-Point Subgroup	BNT162b2, 30 μg (N=21,669)		Placebo (N=21,686)		VE (95% CI)
	No. of participants	Surveillance time *person-yr (no. at risk)*	No. of participants	Surveillance time *person-yr (no. at risk)*	*percent*
Covid-19 occurrence					
After dose 1	50	4.015 (21,314)	275	3.982 (21,258)	82.0 (75.6–86.9)
After dose 1 to before dose 2	39		82		52.4 (29.5–68.4)
Dose 2 to 7 days after dose 2	2		21		90.5 (61.0–98.9)
≥7 Days after dose 2	9		172		94.8 (89.8–97.6)

Figure 3. Efficacy of BNT162b2 against Covid-19 after the First Dose.

Shown is the cumulative incidence of Covid-19 after the first dose (modified intention-to-treat population). Each symbol represents Covid-19 cases starting on a given day; filled symbols represent severe Covid-19 cases. Some symbols represent more than one case, owing to overlapping dates. The inset shows the same data on an enlarged y axis, through 21 days. Surveillance time is the total time in 1000 person-years for the given end point across all participants within each group at risk for the end point. The time period for Covid-19 case accrual is from the first dose to the end of the surveillance period. The confidence interval (CI) for vaccine efficacy (VE) is derived according to the Clopper–Pearson method.

of days after onset. Lymphadenopathy, which generally resolved within 10 days, is likely to have resulted from a robust vaccine-elicited immune response. The incidence of serious adverse events was similar in the vaccine and placebo groups (0.6% and 0.5%, respectively).

This trial and its preliminary report have several limitations. With approximately 19,000 participants per group in the subset of participants with a median follow-up time of 2 months after the second dose, the study has more than 83% probability of detecting at least one adverse event, if the true incidence is 0.01%, but it is not large enough to detect less common adverse events reliably. This report includes 2 months of follow-up after the second dose of vaccine for half the trial participants and up to 14 weeks' maximum follow-up for a smaller subset. Therefore, both

the occurrence of adverse events more than 2 to 3.5 months after the second dose and more comprehensive information on the duration of protection remain to be determined. Although the study was designed to follow participants for safety and efficacy for 2 years after the second dose, given the high vaccine efficacy, ethical and practical barriers prevent following placebo recipients for 2 years without offering active immunization, once the vaccine is approved by regulators and recommended by public health authorities. Assessment of long-term safety and efficacy for this vaccine will occur, but it cannot be in the context of maintaining a placebo group for the planned follow-up period of 2 years after the second dose. These data do not address whether vaccination prevents asymptomatic infection; a serologic end point that can detect a history of infection regardless of whether symptoms were present (SARS-CoV-2 N-binding antibody) will be reported later. Furthermore, given the high vaccine efficacy and the low number of vaccine breakthrough cases, potential establishment of a correlate of protection has not been feasible at the time of this report.

This report does not address the prevention of Covid-19 in other populations, such as younger adolescents, children, and pregnant women. Safety and immune response data from this trial after immunization of adolescents 12 to 15 years of age will be reported subsequently, and additional studies are planned to evaluate BNT162b2 in pregnant women, children younger than 12 years, and those in special risk groups, such as immunocompromised persons. Although the vaccine can be stored for up to 5 days at standard refrigerator temperatures once ready for use, very cold temperatures are required for shipping and longer storage. The current cold storage requirement may be alleviated by ongoing stability studies and formulation optimization, which may also be described in subsequent reports.

The data presented in this report have significance beyond the performance of this vaccine candidate. The results demonstrate that Covid-19 can be prevented by immunization, provide proof of concept that RNA-based vaccines are a promising new approach for protecting humans against infectious diseases, and demonstrate the speed with which an RNA-based vaccine can be developed with a sufficient investment of resources. The development of BNT162b2 was initiated on January 10, 2020, when the SARS-CoV-2 genetic sequence was released by the Chinese Center for Disease Control and Prevention and disseminated globally by the GISAID (Global Initiative on Sharing All Influenza Data) initiative. This rigorous demonstration of safety and efficacy less than 11 months later provides a practical demonstration that RNA-based vaccines, which require only viral genetic sequence information to initiate development, are a major new tool to combat pandemics and other infectious disease outbreaks. The continuous phase 1/2/3 trial design may provide a model to reduce the protracted development timelines that have delayed the availability of vaccines against other infectious diseases of medical importance. In the context of the current, still expanding pandemic, the BNT162b2 vaccine, if approved, can contribute, together with other public health measures, to reducing the devastating loss of health, life, and economic and social well-being that has resulted from the global spread of Covid-19.

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A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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APPENDIX

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BRIEF COMMUNICATION

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Neutralization of SARS-CoV-2 spike 69/70 deletion, E484K and N501Y variants by BNT162b2 vaccine-elicited sera

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We engineered three severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) viruses containing key spike mutations from the newly emerged United Kingdom (UK) and South African (SA) variants: N501Y from UK and SA; 69/70-deletion + N501Y + D614G from UK; and E484K + N501Y + D614G from SA. Neutralization geometric mean titers (GMTs) of 20 BTN162b2 vaccine-elicited human sera against the three mutant viruses were 0.81- to 1.46-fold of the GMTs against parental virus, indicating small effects of these mutations on neutralization by sera elicited by two BNT162b2 doses.

We previously reported that BNT162b2, a nucleoside-modified RNA vaccine that encodes the SARS-CoV-2 full-length, prefusion-stabilized spike glycoprotein, elicited dose-dependent SARS-CoV-2-neutralizing GMTs that were similar to or higher than the GMT of a panel of SARS-CoV-2 convalescent human serum samples[1]. We subsequently reported that, in a randomized, placebo-controlled trial of approximately 44,000 participants 16 years of age or older, a two-dose regimen of BNT162b2 conferred 95% protection against Coronavirus Disease 2019 (COVID-19)[2].

Since the previously reported studies were conducted, rapidly spreading variants of SARS-CoV-2 have arisen in the UK, SA and other regions[3,4]. These variants have multiple mutations in their spike glycoproteins, which are key targets of virus-neutralizing antibodies. The emerged spike mutations have raised concerns of vaccine efficacy against these new strains. The goal of this study was to examine the effect of several key spike mutations from the UK and SA strains on BNT162b2 vaccine-elicited neutralization.

Using an infectious complementary DNA (cDNA) clone of SARS-CoV-2 (ref. [5]), we engineered three spike mutant viruses on the genetic background of clinical strain USA-WA1/2020 (Supplementary Fig. 1). 1) Mutant N501Y virus contains the N501Y mutation that is shared by both the UK and SA variants. This mutation is located in the viral receptor-binding domain (RBD) for cell entry, increases binding to the angiotensin-converting enzyme 2 receptor and enables the virus to expand its host range to infect mice[5,6]. 2) Mutant Δ69/70 + N501Y + D614G virus contains two additional changes present in the UK variants: amino acid 69 and 70 deletion (Δ69/70) and D614G substitution. Amino acids 69 and 70 are located in the N-terminal domain of the spike S1 fragment; deletion of these residues might allosterically change S1 conformation[6]. The D614G mutation is dominant in circulating strains around the world[7,8]. 3) Mutant E484K + N501Y + D614G virus additionally contains the E484K substitution, which is also located in the viral RBD. The E484K substitution alone confers resistance to several monoclonal antibodies[9,10]. Compared to the wild-type (WT) USA-WA1/2020 strain, the three mutant viruses showed similar plaque morphologies on Vero E6 cells (Supplementary Fig. 2).

We tested a panel of human sera from 20 participants in the previously reported clinical trial[1,2], drawn 2 or 4 weeks after immunization with two 30-μg doses of BNT162b2 spaced 3 weeks apart (Supplementary Fig. 3). All neutralization assays were done with the same 20 sera samples, with the two experiments (as described in the Fig. 1 legend) done at different times. Each serum was tested for neutralization of WT USA-WA1/2020 strain and the three mutant viruses by a 50% plaque-reduction neutralization assay ($PRNT_{50}$; Supplementary Tables 1 and 2). All sera showed equivalent neutralization titers between the WT and mutant viruses, with differences of four-fold or less (Fig. 1). Notably, ten out of the 20 sera had neutralization titers against mutant Δ69/70 + N501Y + D614G virus that were twice their titers against the WT virus (Fig. 1b), whereas six out of the 20 sera had neutralization titers against mutant E484K + N501Y + D614G virus that were half their titers against the WT virus (Fig. 1c). The ratios of the neutralization GMTs of the sera against the N501Y, Δ69/70 + N501Y + D614G and E484K + N501Y + D614G viruses to their GMTs against the USA-WA1/2020 virus were 1.46, 1.41 and 0.81, respectively (Supplementary Fig. 4).

Consistent with other recent reports of the neutralization of SARS-CoV-2 variants or corresponding pseudoviruses by convalescent or post-immunization sera[11,12], the neutralization GMT of the serum panel against the virus with three mutations from the SA variant (E484K + N501Y + D614G) was slightly lower than the neutralization GMTs against the N501Y virus or the virus with three mutations from the UK variant (Δ69/70 + N501Y + D614G). However, the magnitude of the differences in neutralization GMTs against any of the mutant viruses in this study was small (0.81- to 1.41-fold), as compared to the greater than four-fold differences in

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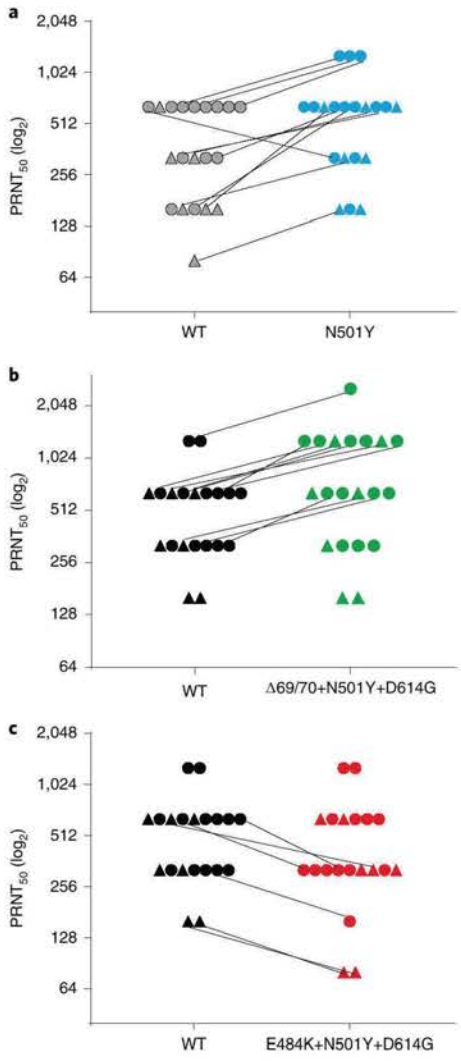

Fig. 1 | $PRNT_{50}$s of 20 BNT162b2-vaccinated human sera against WT and mutant SARS-CoV-2. a, WT (USA-WA1/2020) and mutant N501Y. **b**, WT and Δ69/70 + N501Y + D614G. **c**, WT and E484K + N501Y + D614G. Seven (triangles) and 13 (circles) sera were drawn 2 and 4 weeks after the second dose of vaccination, respectively. Sera with different $PRNT_{50}$s against WT and mutant viruses are connected by lines. Results in **a** were from one experiment; results in **b** and **c** were from another set of experiments. Each data point is the average of duplicate assay results.

hemagglutination-inhibition titers that have been used to signal potential need for a strain change in influenza vaccines[13].

A limitation of the current study is that the engineered viruses do not include the full set of spike mutations found in the UK or SA variants[3,4]. Nevertheless, preserved neutralization of N501Y, Δ69/70 + N501Y + D614G and E484K + N501Y + D614G viruses by BNT162b2 vaccine-elicited human sera is consistent with preserved neutralization of a panel of 15 pseudoviruses bearing spikes with other single mutations found in circulating SARS-CoV-2 strains[14]. The emergence of the common mutation N501Y from different geographical regions, as well as the previously emerged globally dominant D614G mutation, suggest that these mutations might improve viral fitness, as recently demonstrated for the increased viral transmission by the D614G mutation in animal models[7,15]. The biological functions of N501Y and the other mutations (such as Δ69/70 and E484K) remain to be defined for viral replication, pathogenesis and/or transmission in animal models. A second limitation of the study is that no serological correlate of protection against COVID-19 has been defined. Therefore, predictions about vaccine efficacy based on neutralization titers require assumptions about the levels of neutralization and roles of humoral and cell-mediated immunity in vaccine-mediated protection. Clinical data are needed for firm conclusions about vaccine effectiveness against variant viruses.

The ongoing evolution of SARS-CoV-2 necessitates continuous monitoring of the significance of changes for vaccine efficacy. This surveillance should be accompanied by preparations for the possibility that future mutations might necessitate changes to vaccine strains. The serological criteria for strain changes of influenza vaccine have been well accepted[16]. For COVID-19, such vaccine updates would be facilitated by the flexibility of messenger RNA-based vaccine technology.

Online content

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/s41591-021-01270-4.

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Methods

Construction of isogenic viruses. Three recombinant SARS-CoV-2 mutants (N501Y, Δ69/70-N501Y + D614G and E484K + N501Y + D614G in spike protein) were prepared on the genetic background of an infectious cDNA clone derived from clinical strain WA1 (2019-nCoV/USA_WA1/2020)[5] by following the polymerase chain reaction-based mutagenesis protocol as reported previously[7]. The full-length infectious cDNAs were in vitro ligated and used as templates to transcribe full-length viral RNA. Mutant viruses (P0) were recovered on day 2 from Vero E6 cells after electroporation of the in vitro RNA transcripts. P1 viruses were harvested as stocks by passaging the P0 virus once on Vero E6 cells. The titers of P1 viruses were determined by plaque assay on Vero E6 cells. The genome sequences of the P1 viruses were validated by Sanger sequencing. The detailed protocol was recently reported[17].

Serum specimens and neutralization assay. Serum samples were collected from BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial (ClinicalTrials.gov identifier: NCT04368728). The protocol and informed consent were approved by institutional review boards for each of the investigational centers participating in the study. The study was conducted in compliance with all International Council for Harmonisation Good Clinical Practice guidelines and the ethical principles of the Declaration of Helsinki.

The immunization and serum collection regimens are illustrated schematically in Supplementary Fig. 3. A conventional (non-fluorescent) plaque-reduction neutralization assay was performed to quantify the serum-mediated virus suppression as previously reported[18]. Briefly, each serum was two-fold serially diluted in culture medium, with the first dilution of 1:40 (dilution range of 1:40 to 1:1280). The diluted sera were incubated with 100 plaque-forming units of WT or mutant viruses at 37 °C for 1 h, after which the serum–virus mixtures were inoculated onto Vero E6 cell monolayer in six-well plates. After 1 h of infection at 37 °C, 2 ml of 2% SeaPlaque agar (Lonza) in DMEM containing 2% FBS and 1% penicillin–streptomycin was added to the cells. After 2 d of incubation, 2 ml of 2% SeaPlaque agar in DMEM containing 2% FBS, 1% penicillin–streptomycin and 0.01% neutral red (Sigma-Aldrich) were added on top of the first layer. After another 16 h of incubation at 37 °C, plaque numbers were counted. The minimal serum dilution that inhibits 50% of plaque counts is defined as the $PRNT_{50}$. Each serum was tested in duplicates. The $PRNT_{50}$ assay was performed at the Biosafety Level-3 facility with the approval from the Institutional Biosafety Committee at the University of Texas Medical Branch.

Statistics. No statistical analysis was performed in the study.

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

Data availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Author contributions

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Competing interests

The authors declare competing interests. X.X., V.D.M. and P.-Y.S. have filed a patent on the reverse genetic system. K.A.S., M.C., D.C. and P.R.D. are employees of Pfizer and might hold stock options. X.X., J.Z., C.R.F.G., H.X. and P.-Y.S. received compensation from Pfizer to perform the neutralization assay.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-021-01270-4.

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☒	☐	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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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
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No code and software used for the data collection
Data analysis	Graphpad Prism 9

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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Life sciences study design

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

Sample size	No sample size calculation was performed. Based on the availability, 20 samples were collected from BNT162b2 vaccinees participating in the phase 1 portion of the ongoing phase 1/2/3 clinical trial (ClinicalTrials.gov identifier: NCT04368728). Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.).
Data exclusions	No data was excluded in the study.
Replication	Each human serum sample was analyzed in duplication. The averaged results from the duplication were reported in this study.
Randomization	No randomization was performed. All samples were analyzed for the neutralizing activities against WT SARS-CoV-2 and variants in the same experimental settings.
Blinding	Patient information was blinded in the study. Those 20 samples had been tested as neutralizing positive against WT SARS-CoV-2 using the method according to the reference (Walsh EE, Frenck RW, Jr., Falsey AR, et al. Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. N Engl J Med 2020.).

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Vero E6 cells (ATCC® CRL-1586) were obtained from ATCC
Authentication	ATCC have comprehensively performed authentication on cell lines.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma.
Commonly misidentified lines (See ICLAC register)	not applicable

Human research participants

Policy information about studies involving human research participants

Population characteristics	Only serum samples were used in this study. Please refer to the ClinicalTrials.gov identifier: NCT04368728 for the population characterisistics.

Recruitment	Only serum samples were used in this study. Please refer to the ClinicalTrials.gov identifier: NCT04368728 for the patient recruitment requirment.
Ethics oversight	The protocol and informed consent were approved by institutional review boards for each of the investigational centers participating in the study. The study was conducted in compliance with all International Council for Harmonisation (ICH) Good Clinical Practice (GCP) guidelines and the ethical principles of the Declaration of Helsinki.

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

CORRESPONDENCE

Neutralizing Activity of BNT162b2-Elicited Serum

TO THE EDITOR: BNT162b2 is a nucleoside-modified RNA vaccine expressing the full-length prefusion spike glycoprotein (S) of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In a randomized, placebo-controlled clinical trial involving approximately 44,000 participants, immunization conferred 95% efficacy against coronavirus disease 2019 (Covid-19).[1]

New, highly transmissible SARS-CoV-2 variants that were first detected in the United Kingdom (B.1.1.7 lineage), South Africa (B.1.351 lineage), and Brazil (P.1 lineage) with mutations in the *S* gene are spreading globally. To analyze effects on neutralization elicited by BNT162b2, we engineered *S* mutations from each of the three new lineages into USA-WA1/2020, a relatively early isolate of the virus from January 2020 (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). We thereby produced three recombinant viruses representing each of these lineages and two additional ones in which we engineered subsets of mutations of the B.1.351 lineage. Thus, the first recombinant virus had all the mutations found in the *S* gene in the B.1.1.7 lineage (B.1.1.7-spike), the second had all the mutations found in the *S* gene in the P.1 lineage (P.1-spike), the third had all the mutations found in the *S* gene in the B.1.351 lineage (B.1.351-spike), the fourth had an N-terminal domain deletion found in the B.1.351 lineage and the globally dominant D614G substitution (B.1.351-Δ242-244+D614G), and the fifth had the three mutations from the B.1.351 lineage affecting amino acids in the receptor-binding site (K417N, E484K, and N501Y) and a D614G substitution (B.1.351-RBD+D614G). The mutant amino acid residues in the B.1.351-RBD+D614G recombinant virus are also among those in the P.1 lineage virus, although in the P.1 lineage virus, K417 is mutated to threonine rather than asparagine. All the mutant viruses yielded infectious viral titers exceeding 10^7 plaque-forming units per milliliter. The B.1.1.7-spike and B.1.351-spike viruses formed plaques that were smaller than those formed by the other viruses (Fig. S2).

We performed 50% plaque reduction neutralization testing ($PRNT_{50}$) using 20 serum samples that had been obtained from 15 participants in the pivotal trial[1,2] 2 or 4 weeks after the administration of the second dose of 30 μg of BNT162b2 (which occurred 3 weeks after the first immunization) (Fig. S3). All the serum samples efficiently neutralized USA-WA1/2020 and all the viruses with variant spikes. Almost all of them did so at titers higher than 1:40. Geometric mean neutralizing titers against USA-WA1/2020, B.1.1.7-spike, P.1-spike, B.1.351-spike, B.1.351-Δ242-244+D614G, and B.1.351-RBD+D614G viruses were 532, 663, 437, 194, 485, and 331, respectively (Fig. 1 and Table S1). Thus, as compared with neutralization of USA-WA1/2020, neutralization of B.1.1.7-spike and P.1-spike viruses was roughly equivalent, and neutralization of B.1.351-spike virus was robust but lower. Our data are also consistent with lower neutralization titers against the virus with the full set of B.1.351-spike mutations than against virus with either subset of mutations. Our findings also suggest that mutations that result in amino acid substitutions K417N, E484K, and N501Y in the receptor-binding site have a greater effect on neutralization than the 242–244 deletion affecting the N-terminal domain of the spike protein.

Limitations of the study include the potential for mutations to alter neutralization by affecting spike function rather than antigenicity. Therefore, each neutralization assay with a different target virus is unique, and comparisons between neutralization titers from different assays should be interpreted with caution. Neutralizing activity against the B.1.351 lineage virus was robust at a geometric mean titer that was much higher than that obtained after one dose of BNT162b2, when strong efficacy was already observed in the C4591001 efficacy trial.[1-3] T-cell immunity may also be involved in protection,[4] and BNT162b2

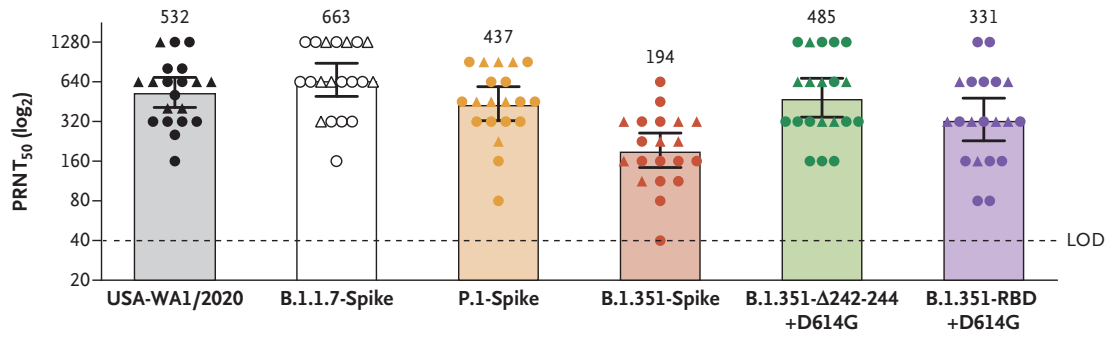

Figure 1. Serum Neutralization of Variant Strains of SARS-CoV-2 after the Second Dose of BNT162b2 Vaccine.

Shown are the results of 50% plaque reduction neutralization testing ($PRNT_{50}$) with the use of 20 samples obtained from 15 trial participants 2 weeks (circles) or 4 weeks (triangles) after the administration of the second dose of the BNT162b2 vaccine. The mutant viruses were obtained by engineering the full set of mutations in the B.1.1.7, P.1., or B.1.351 lineage or subsets of the *S* gene mutations in the B.1.351 lineage (B.1.351-Δ242-244+D614G and B.1.351-RBD+D614G) into USA-WA1/2020. Each data point represents the geometric mean $PRNT_{50}$ obtained with a serum sample against the indicated virus, including data from repeat experiments, as detailed in Table S1 in the Supplementary Appendix. The data for USA-WA1/2020 are from three experiments; for B.1.1.7-spike, B.1.351-Δ242-244+D614G, and B.1.351-RBD-D614G viruses from one experiment each; and for P.1-spike and B.1.351-spike viruses from two experiments each. In each experiment, the neutralization titer was determined in duplicate assays, and the geometric mean was taken. The heights of bars and the numbers over the bars indicate geometric mean titers. The I bars indicate 95% confidence intervals. Statistical analysis was performed with the use of the Wilcoxon signed-rank test. The statistical significance of the difference between geometric mean titers in the USA-WA1/2020 neutralization assay and in each variant virus neutralization assay with the same serum samples are as follows: P=0.02 for B.1.1.7-spike; P=0.06 for P.1-spike; P<0.001 for B.1.351-spike; P=0.99 for B.1.351-Δ242-244+D614G; and P=0.005 for B.1.351-RBD+D614G. LOD denotes limit of detection.

immunization elicits CD8+ T-cell responses that recognize multiple variants.[5] Ultimately, conclusions about vaccine-mediated protection that are extrapolated from neutralization or T-cell data must be validated by real-world evidence collected in regions where the SARS-CoV-2 variants are circulating.

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