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.

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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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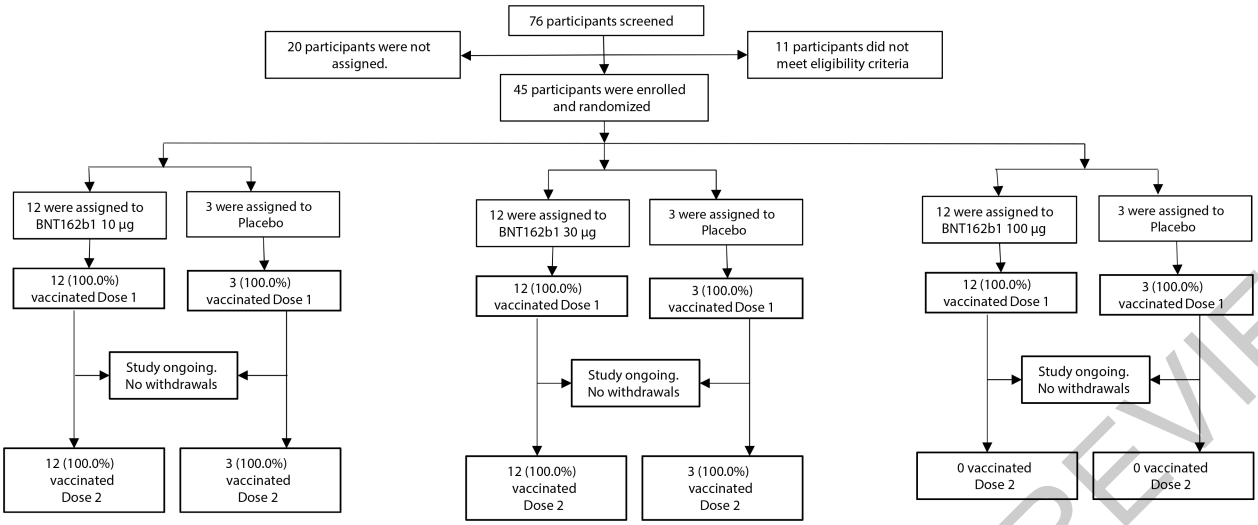


Figure 1 | Disposition of participants. Participants not assigned (n=20) were screened but not randomized because enrollmen had closed.

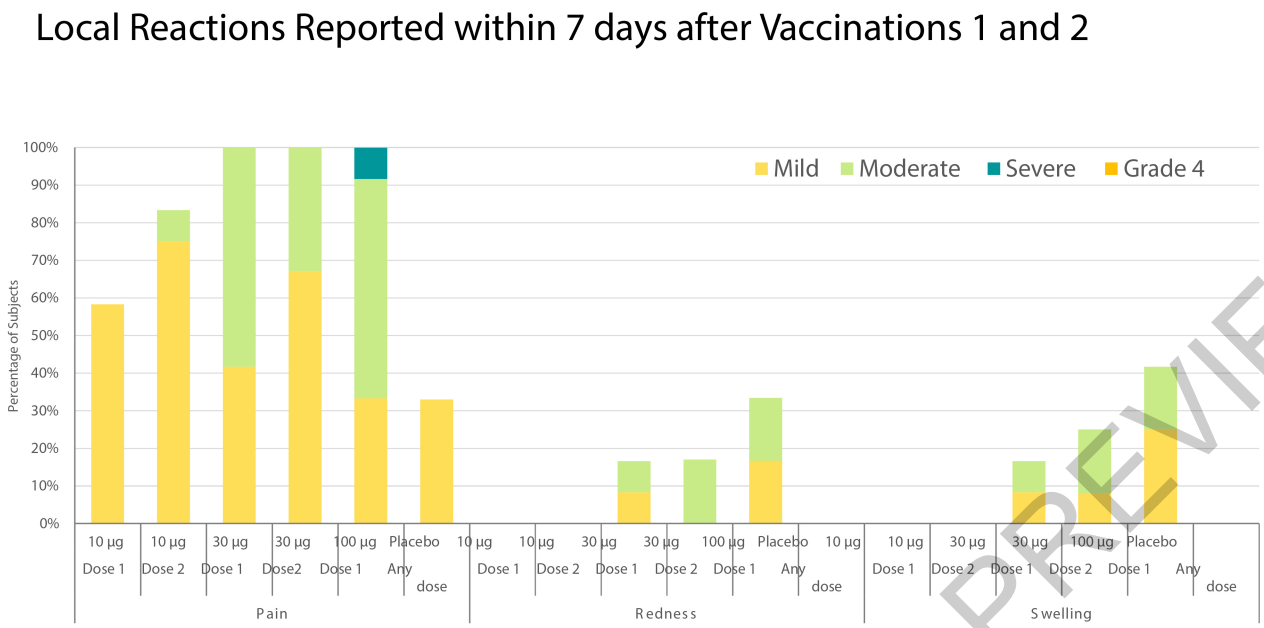
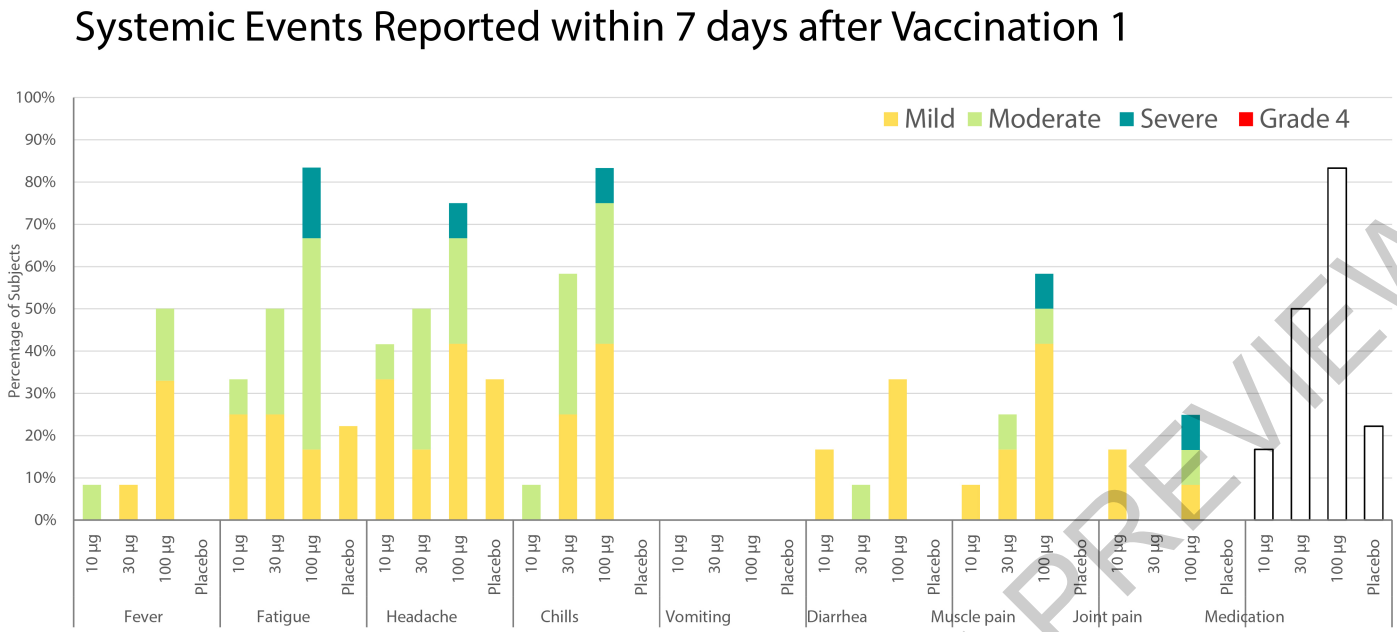


Figure 2 | Local reactions reported within 7 days of vaccination for all dose levels. Solicited injection-site (local) reactions were: pain at injection site (mild: does not interfere with activity; moderate: interferes with activity; severe: prevents daily activity; Grade 4: emergency room visit or 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.



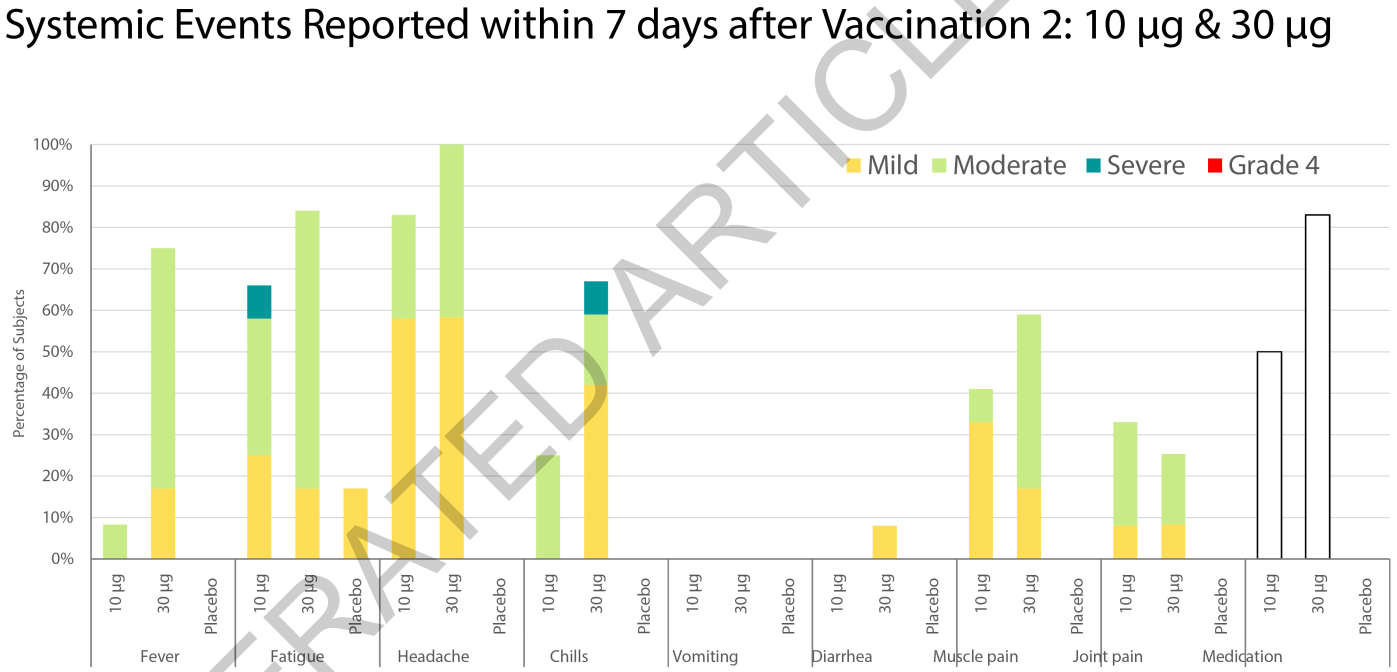


Figure 3 | a. Systemic events and medication use reported within 7 days after Vaccination 1 for all dose levels and b. After Vaccination 2 for the 10-µg and 30-µg dose level. Solicited systemic events were: fatigue, headache, chills, new or worsened muscle pain, new or worsened joint pain (mild: does 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.

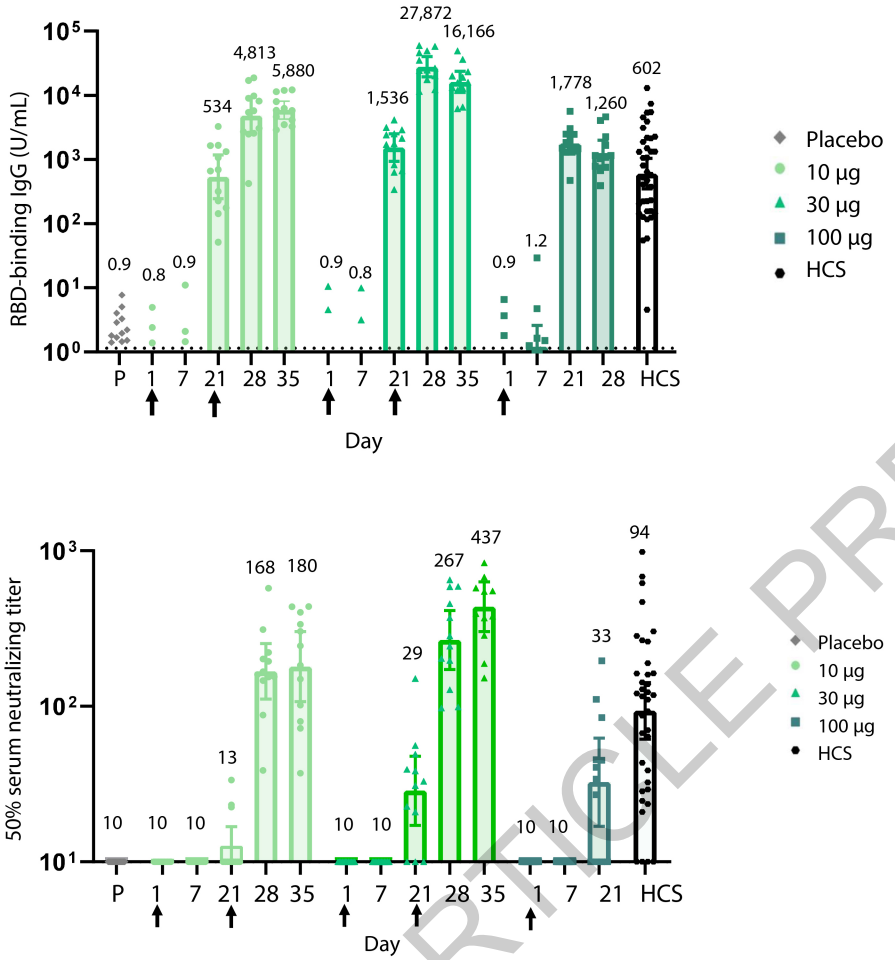


Figure 4 | Immunogenicity of BNT162b1. Participants in groups of 15 were vaccinated with the indicated dose levels of BNT162b1 (n=12) or with placebo (n=3) on Days 1 (all dose levels and placebo) and 21 (10 µg and 30 µg dose levels and placebo). Reponses in placebo recipients for each of the dosing groups 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.

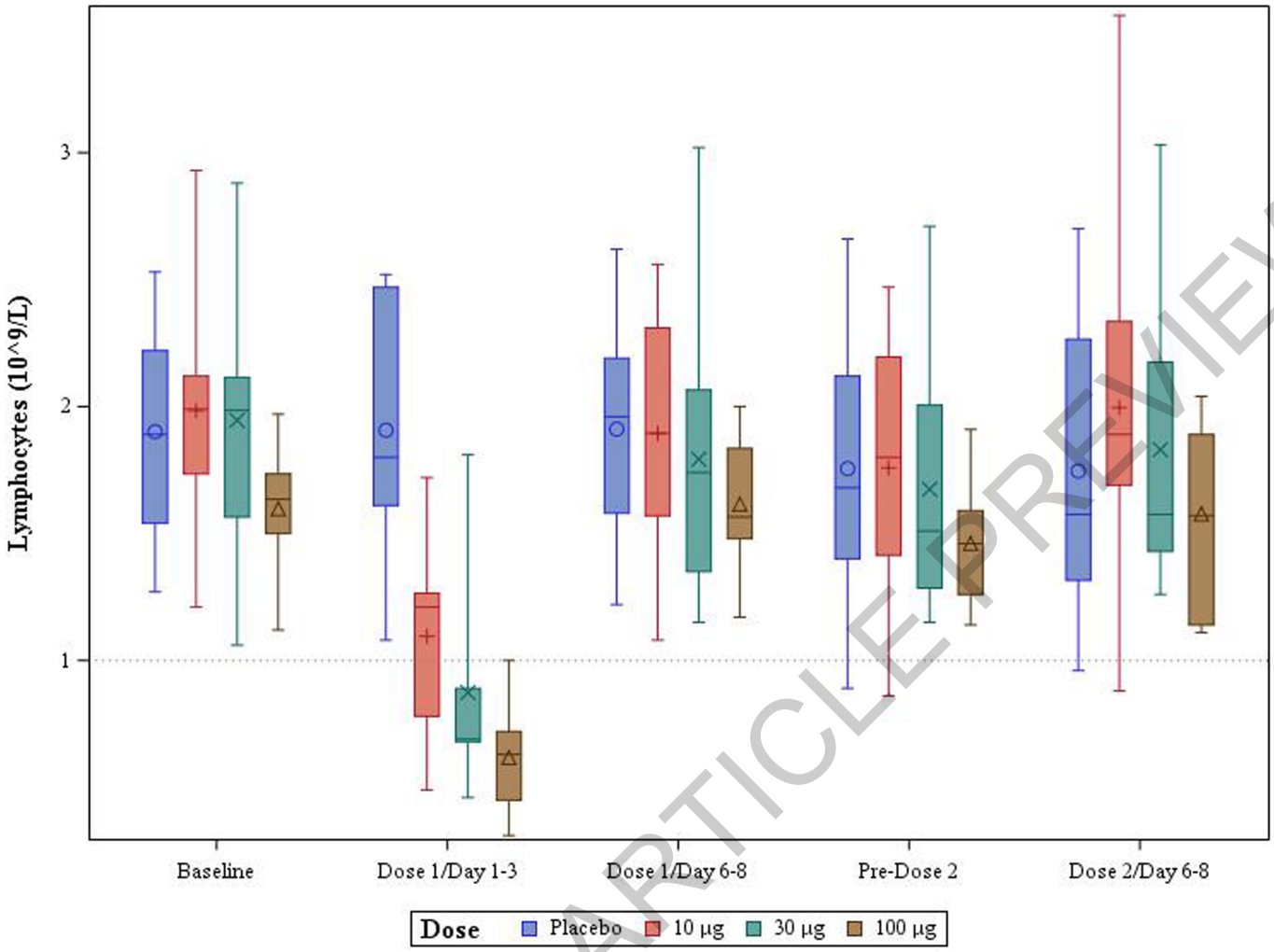
Compe ing interests NK, JA, AG, SL, RB, KAS, PL, KK, WK, DC, KRT, PRD, WCG, and KUJ are mployee of Pfizer and may hold stock options. US and ÖT are stock owners, management board members, and employees at BioNTech SE (Mainz, Germany) and are nv tors on patents and patent applications related to RNA technology. 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

Field-specific reporting

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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

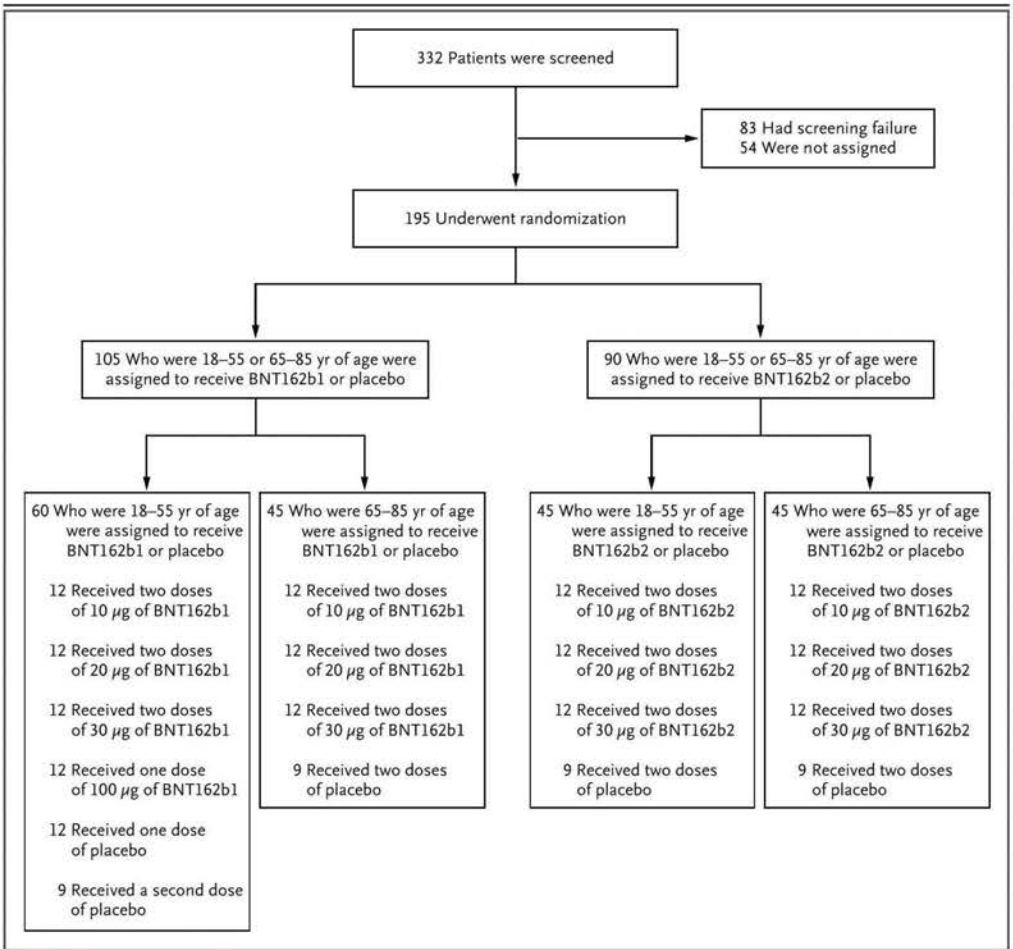

Figure 1. Screening and Randomization of the Participants.

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.

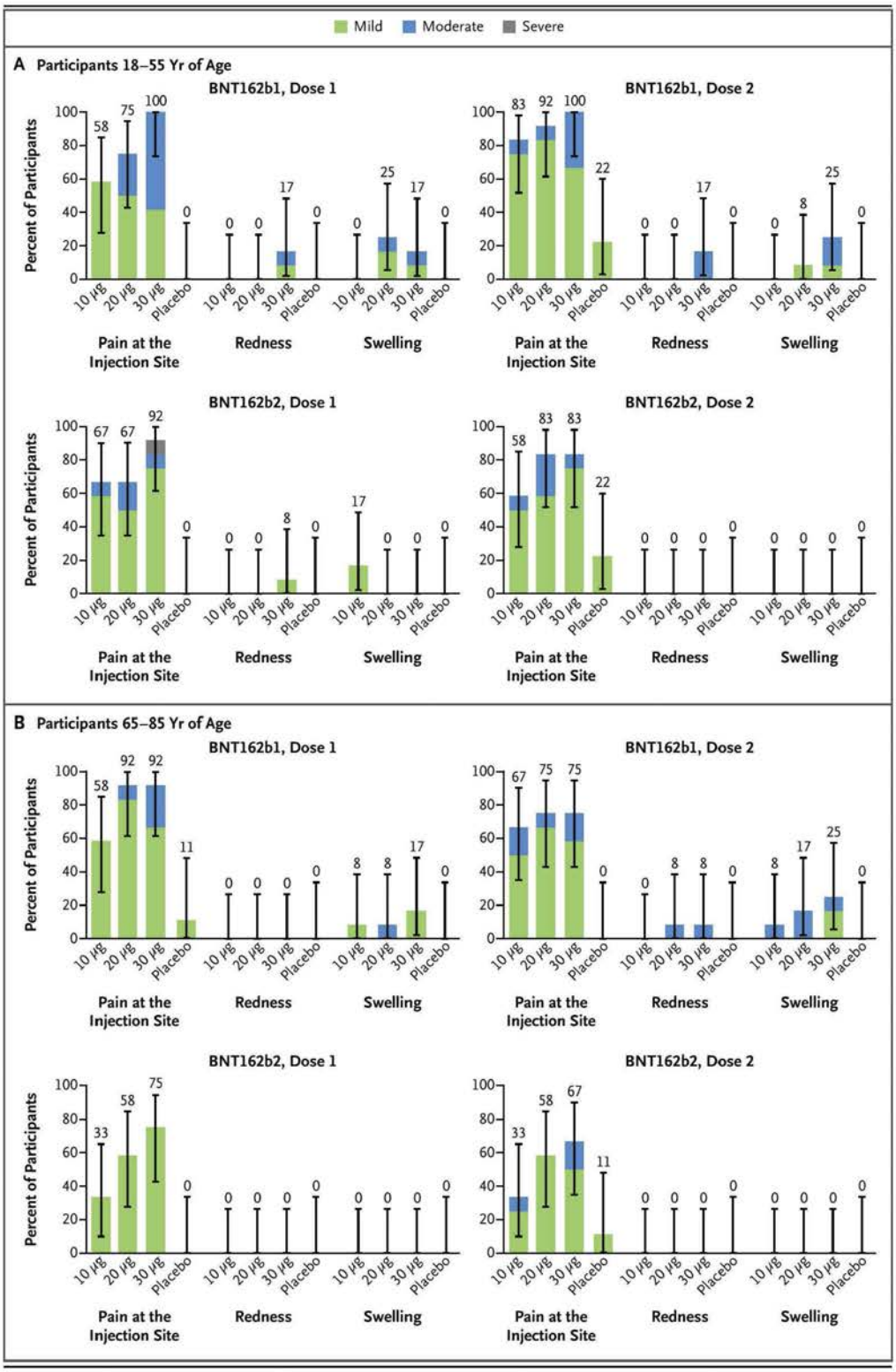

Figure 2 (facing page). Local Reactions Reported within 7 Days after the Administration of Vaccine or Placebo, According to Age Group.

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

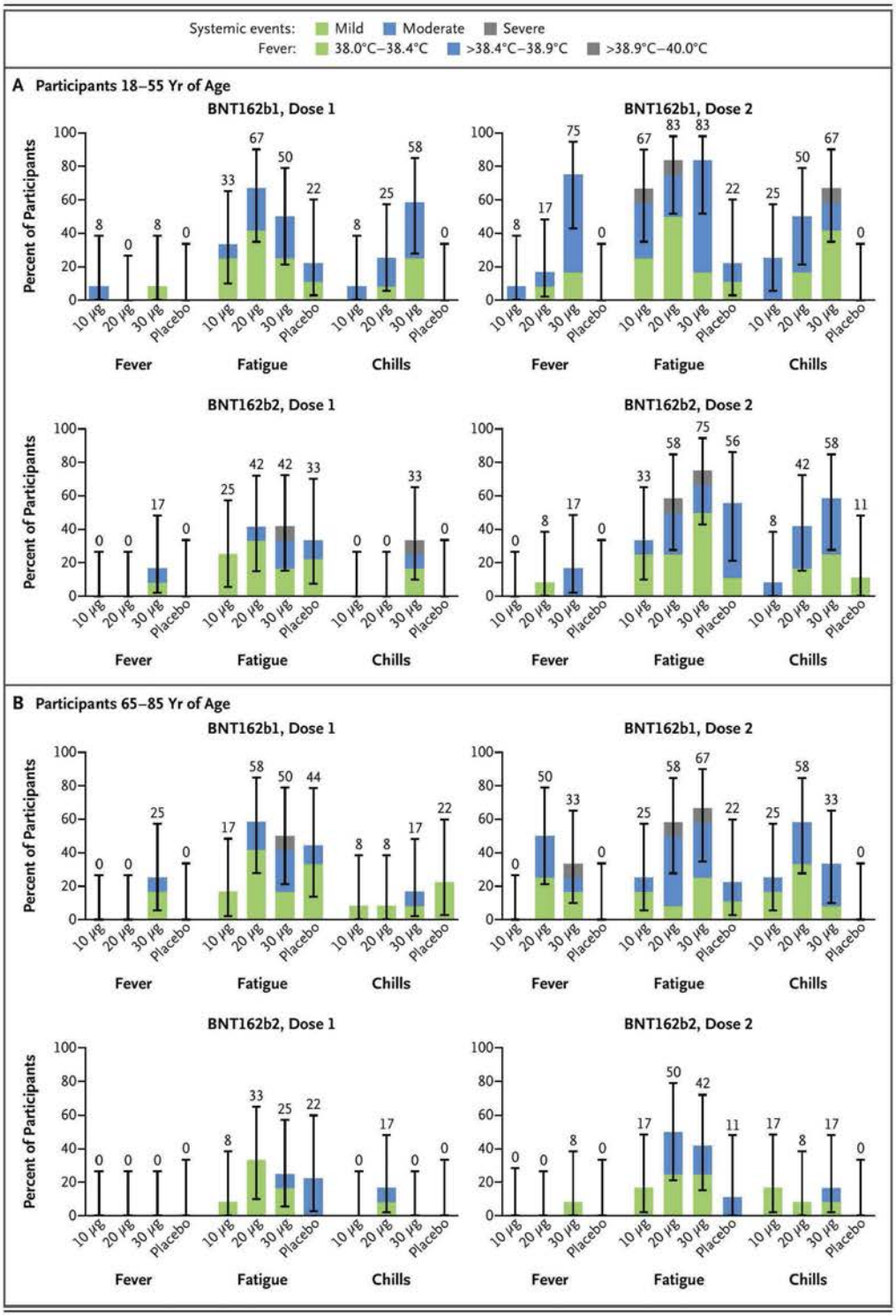

Figure 3 (facing page). Selected Systemic Events Reported within 7 Days after the Administration of Vaccine or Placebo, According to Age Group.

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

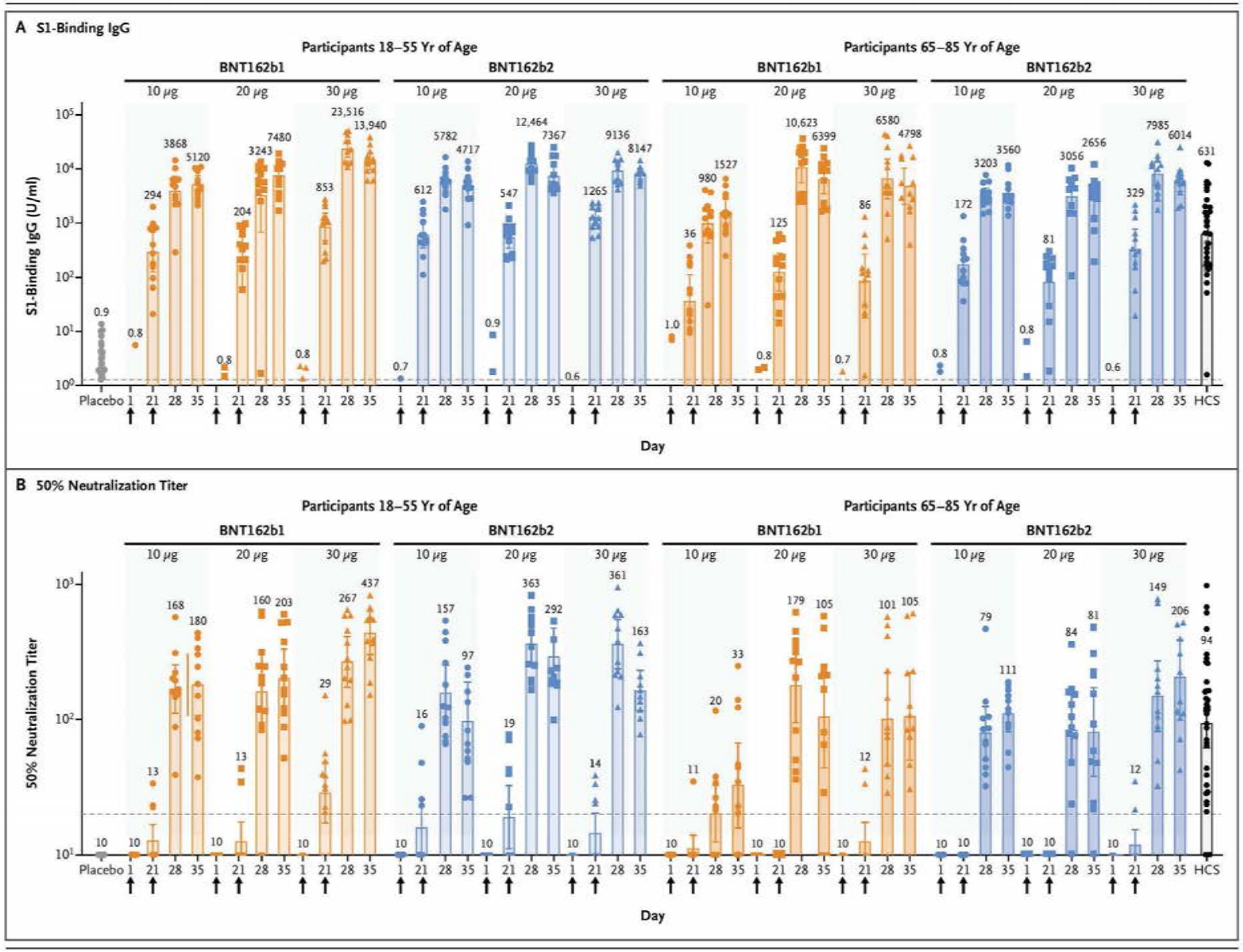

Figure 4 (facing page). Immunogenicity of BNT162b1 and BNT162b2.

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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