Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

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SUPPLEMENTARY APPENDIX

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Methods

Objectives, Participants, and Oversight

This randomized, placebo-controlled, observer-blind study was part of an ongoing phase 1/2/3 trial assessing safety, immunogenicity, and efficacy of BNT162b2 in healthy individuals ≥12 years of age (NCT04368728). This report presents preliminary findings from a subset of 18–55- or 65–85-year-old participants from the phase 1 part of the study who completed an initial 2-dose series of 30 μg BNT162b2 approximately 3 weeks apart and who received a third 30-μg dose approximately 7–9 months after the second dose. Data were collected through the cut-off date (May 13, 2021). Participants were observed in the clinic for 30 minutes after intramuscular injection to evaluate any vaccine-associated immediate reactions.

Eligible participants for the third dose were healthy or had stable pre-existing disease (ie, disease not requiring a significant change in therapy or hospitalization for worsening disease during the 6 weeks before enrollment). Individuals who were immunocompromised or were receiving treatment with immunosuppressive therapy, or who had received any non-study coronavirus vaccination were excluded. Full inclusion and exclusion criteria applied at initial enrollment and additional study details are provided in the protocol, which is available at nejm.org. The dose 3 immunogenicity population consisted of randomized participants who received 2 doses of BNT162b2 as initially randomized, received a third BNT162b2 dose, and had at least 1 valid and determinate immunogenicity result after the third dose.

The study was conducted in accordance with the ethical principles of international guidelines including the International Council for Harmonisation Good Clinical Practice Guidelines, the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines, and applicable laws and regulations. Pfizer was responsible for the trial design and conduct, collection, analysis, and interpretation of the data, and writing of the manuscript. Both Pfizer and BioNTech manufactured BNT162b2. BioNTech was the regulatory sponsor of the trial and contributed to data interpretation and writing of the manuscript. All data were available to the authors, who vouch for its accuracy and completeness and for the adherence of the trial to the protocol.

Safety

Safety evaluations after the third BNT162b2 dose included reports of local reactions (injection site pain, redness, swelling) and systemic events (fever, vomiting, diarrhea, headache, fatigue, chills, muscle pain, joint pain) and use of antipyretic medications in the 7 days after BNT162b2 administration as reported by participants in electronic diaries. For comparison, reactogenicity data

after the first and second BNT162b2 doses are presented for participants who received a third dose. In addition, the occurrence of adverse events (AEs) and serious AEs was assessed up to 1 month after the third BNT162b2 dose.

Immunogenicity

A 50% plaque-reduction neutralization test (the highest serum dilution that prevented the formation of more than 50% of viral plaques) was used to determine geometric mean titers (GMTs) of serum-mediated virus suppression as described previously.[1,2] SARS-CoV-2 50% neutralization titers were assessed in sera drawn before BNT162b2 dose 1 (Day 1); 7 days, and 1 month after BNT162b2 dose 2; before dose 3; and 7 days and 1 month after dose 3. Neutralization titers were determined as described previously against the designated wild-type (recombinant USA-WA1/2020) and against the B.1.351 (recombinant USA-WA1/2020 with the full spike gene from the Beta variant) and the B.1.617.2 (recombinant USA-WA1/2020 with the full spike gene from the Delta variant) lineage target strains.[1,3] All samples from each of the time points were analyzed for this evaluation (ie, previously tested samples[3] were reanalyzed) to ensure the most accurate assessments of persistence of neutralizing antibodies and response to the third BNT162b2 dose.

Statistical Analysis

This preliminary report includes descriptive results of safety and immunogenicity for the 23 eligible participants who received a third BNT162b2 dose. Safety endpoints are presented as counts, percentages, and associated Clopper-Pearson 2-sided 95% CIs, with AEs categorized by the *Medical Dictionary for Regulatory Activities* term (Version 23.1) for each group. SARS-CoV-2 serum neutralizing GMTs were calculated by exponentiating the mean of logarithmically transformed assay results; the associated 2-sided 95% CIs were obtained from the natural log scale of the results using the Student's *t* distribution and exponentiating the confidence limits. Geometric mean fold rises (GMFRs) were calculated by exponentiating the mean of the difference of logarithmically transformed assay results. Geometric mean ratios (GMRs) between strains were calculated as the mean of the difference of logarithmically transformed neutralization titers for each participant (ie, B.1.351 or B.1.617.2 strain minus wild-type strain) and exponentiating the mean. Associated 2-sided CIs for GMFRs and GMRs were obtained using the Student's *t* distribution for the mean difference on the logarithm scale and exponentiating the confidence limits.

Supplementary Discussion

Some SARS-CoV-2 variants have been associated with more rapid transmission, and potentially, greater pathogenicity,[4] leading to concerns about the potential for reduced vaccine-mediated protection. Studies of in vitro neutralization of a number of SARS-CoV-2 variants have found that BNT162b2-immune sera neutralize all SARS-CoV-2 variants tested to date, including B.1.351 (Beta variant) and B.1.617.2 (Delta variant).[1,5-10] Although the neutralization activity of BNT162b2-immune sera against recombinant SARS-CoV-2 with the B.1.351 lineage spike was lower, the efficacy and effectiveness of BNT162b2 against the B.1.351 variant has remained very high, particularly for severe outcomes.[1,11,12] In the phase 2/3 trial, there was 100% observed vaccine efficacy of BNT162b2 against COVID-19 in the subgroup of participants from South Africa, with 8 of the 9 cases (all occurring in placebo recipients) confirmed to be caused by the B.1.351 variant.[11] Real-world data also indicate that 2 doses of BNT162b2 are 75%, 88%, and 90% effective against B.1.351 (Beta), B.1.617.2 (Delta), and B.1.1.7 (Alpha) variants, respectively.[12,13]

It is possible that protection against variants that show reduced neutralization by BNT162b2-immune sera could wane more quickly than protection against more readily neutralized strains. The high neutralizing titers against the B.1.351 strain after a third dose, exceeding those after 2 doses, and the more comparable titers between the wild-type and B.1.351 strains after dose 3 is encouraging. These data suggest that a third dose could prolong protection and further increase the breadth of protection.

Correlates of protection have not been established for COVID-19; therefore, the durability of protection from vaccination and the required frequency of booster doses are unknown at this time. To date, results obtained from the global phase 1/2/3 study of BNT162b2 indicate robust protection lasting at least 6 months, with 97% vaccine efficacy against severe COVID-19 during the interval from 7 days to approximately 6 months after dose 2, despite waning of efficacy against COVID-19 of any severity from 96.2% during the interval from 7 days to <2 months after dose 2 to 83.7% during the interval from 4 months to approximately 6 months after dose 2.[11,14] Booster doses have the potential to keep protection high if immunity continues to decline over time.

Limitations of the data include the small sample size of the study, which precludes hypothesis testing or meaningful subgroup analysis, and the administration of the third dose at a relatively narrow time interval after the second BNT162b2 dose. In addition, this study did not examine the effect of boosting on other types of immunity, such as $CD4^+$ and $CD8^+$ T-cell immunity and did not evaluate protection. In contrast to neutralizing titers for some variants, T-cell responses appear refractory to sequence changes in the SARS-CoV-2 variants evaluated to date.[15]

Further studies of BNT162b2 booster dosing and boosting with vaccine candidates that use the same nucleoside-modified mRNA technology but encode spike glycoproteins from variants of concern, such as B.1.351, are ongoing or planned, including a study with a larger number of participants and randomization of participants to booster or placebo.

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Table S1. Additional study details, demographics, study flow and interval to dose 3

	Initial age group	
	18–55 years old (N=11)*	**65–85 years old (N=12)**
Demographics		
Male, n (%)	2 (18)	6 (50)
Age at dose 3, years		
Mean (SD)	38.8 (10.00)	69.3 (2.96)
Median (range)	39.0 (24–55)	69.0 (65–75)
Disposition, n (%)		
Received dose 3	11 (100)	12 (100)
Withdrawn	0	0
Time between doses 2 and 3, months		
Mean (SD)	8.2 (0.27)	8.4 (0.12)
Median (range)	8.2 (7.9–8.8)	8.4 (8.2–8.5)

The study was conducted at 2 sites in the United States. As of the cut-off date (May 13, 2021), 23 of the 24 original phase 1 participants who received 2 doses of 30 µg BNT162b2 received a third 30 µg BNT162b2 dose. Additional demographic characteristics of these healthy participants have been reported previously.[3]

*One of the original phase 1 participants in the 18–55-year-old group reported previously[3] did not receive a third BNT162b2 dose and was not included in these dose 3 analyses.

Figure S1. Local reactions reported within 7 days after administration of BNT162b2 according to dose and age group

Results are for the safety population, which included all 23 participants who received the third BNT162b2 dose. Data for dose 1 and dose 2 are from the time of original dosing as reported previously[3] and are for the 11/12 participants 18–55 years of age and 12/12 participants 65–85 years of age who received a third BNT162b2 dose. 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 (leads to an emergency department visit or hospitalization). Redness and swelling were graded as mild (>2.0–5.0 cm in diameter), moderate (>5.0–10.0 cm in diameter), severe (>10.0 cm in diameter), or grade 4 (necrosis or exfoliative dermatitis for redness and necrosis for swelling). The numbers above the bars show the overall percentage of the participants who reported the specified local reaction. Error bars are the 95% CI.

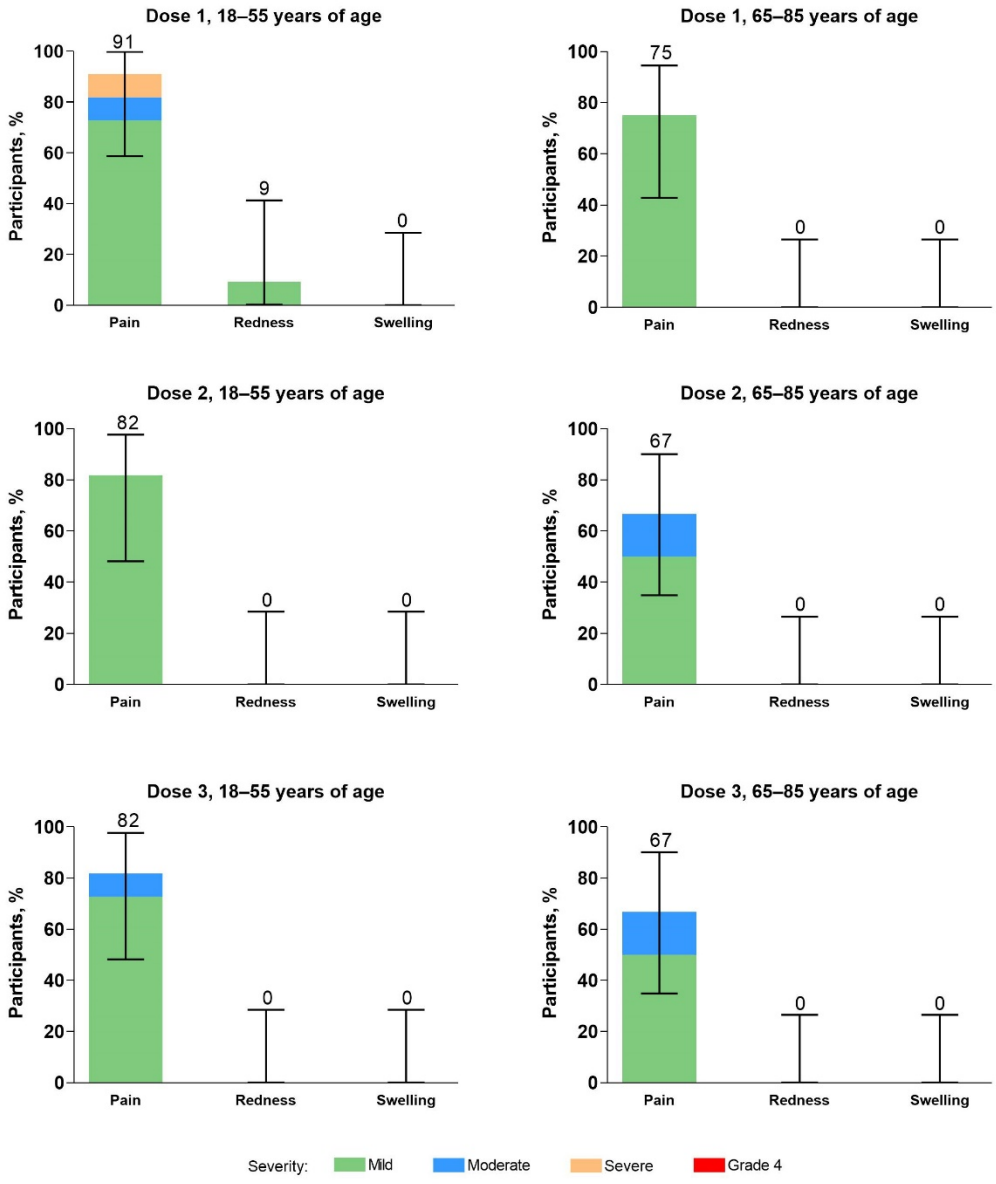
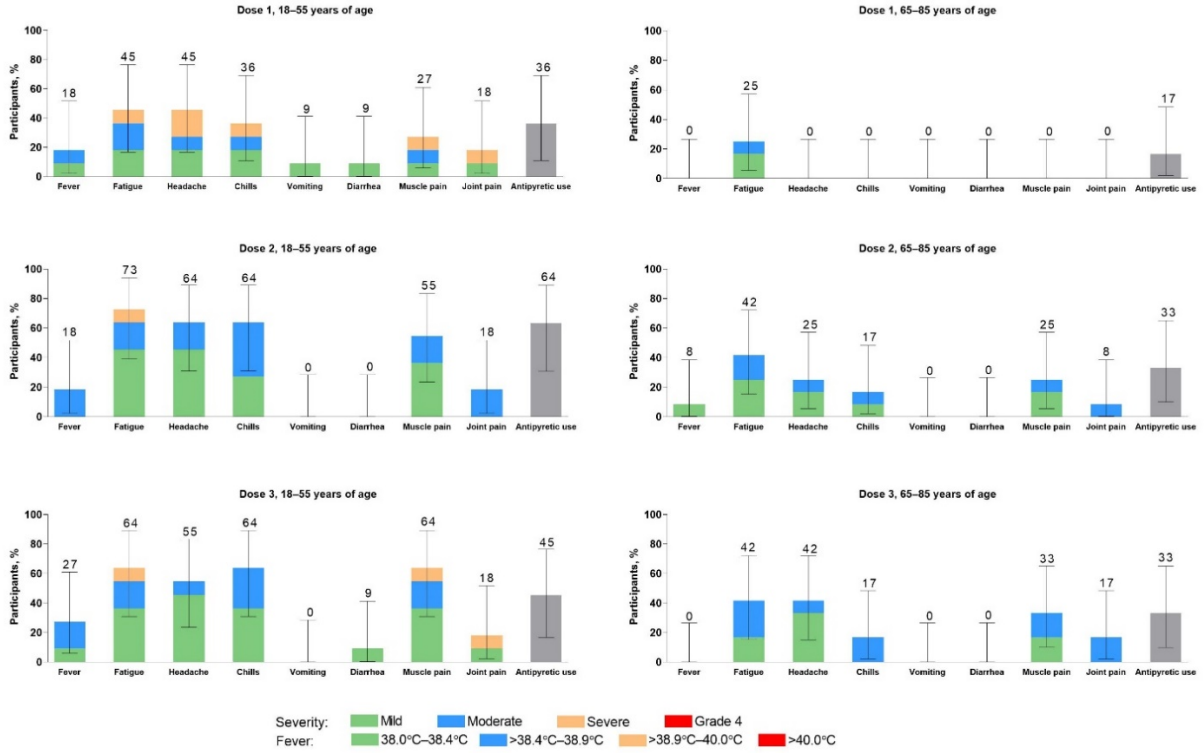


Figure S2. Systemic events reported within 7 days after administration of BNT162b2 according to dose and age group

Results are for the safety population, which included all 23 participants who received the third BNT162b2 dose. Data for dose 1 and dose 2 are from the time of original dosing as reported previously[1] and are for the 11/12 participants 18–55 years of age and 12/12 participants 65–85 years of age who received a third BNT162b2 dose. Fever categories are designated in the legend key. Fatigue, headache, chills, new or worsened muscle pain, and new or worsened joint pain were graded as mild (does not interfere with activity), moderate (some interference with activity), or severe (prevents daily routine activity). Vomiting was graded as mild (1–2 times in 24 hours), moderate (>2 times in 24 hours), or severe (requires intravenous hydration) and diarrhea as mild (2–3 loose stools in 24 hours), moderate (4–5 loose stools in 24 hours) or severe (≥6 loose stools in 24 hours). Grade 4 for all systemic events indicated an emergency department visit or hospitalization. The numbers above the bars show the overall percentage of the participants who reported the specified systemic event. Error bars are the 95% CI.



References

1. Liu Y, Liu J, Xia H, et al. Neutralizing Activity of BNT162b2-Elicited Serum. N Engl J Med 2021;384:1466-8.
2. Muruato AE, Fontes-Garfias CR, Ren P, et al. A high-throughput neutralizing antibody assay for COVID-19 diagnosis and vaccine evaluation. Nat Commun 2020;11:4059.
3. 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;383:2439-50.
4. US Centers for Disease Control and Protection. SARS-CoV-2 Variant Classifications and Definitions. 2021. (Accessed July 29, 2021, at https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/variant-surveillance/variant-info.html#print.)
5. 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. Nat Med 2021;27:620-1.
6. Zou J, Xie X, Fontes-Garfias CR, et al. The effect of SARS-CoV-2 D614G mutation on BNT162b2 vaccine-elicited neutralization. NPJ Vaccines 2021;6:44.
7. Muik A, Wallisch AK, Sänger B, et al. Neutralization of SARS-CoV-2 lineage B.1.1.7 pseudovirus by BNT162b2 vaccine-elicited human sera. Science 2021;371:1152-3.
8. Liu J, Liu Y, Xia H, et al. BNT162b2-elicited neutralization of B.1.617 and other SARS-CoV-2 variants. Nature 2021 (Epub ahead of print).
9. Liu Y, Liu J, Xia H, et al. BNT162b2-Elicited Neutralization against New SARS-CoV-2 Spike Variants. N Engl J Med 2021;85:472-4.
10. World Health Organization. Tracking SARS-CoV-2 variants. 2021. (Accessed July 29, 2021, at https://www.who.int/en/activities/tracking-SARS-CoV-2-variants/.)
11. Thomas SJ, Moreira ED, Kitchin N, et al. Six Month Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine. N Engl J Med 2021 [Submitted].
12. Abu-Raddad LJ, Chemaitelly H, Butt AA, National Study Group for C-V. Effectiveness of the BNT162b2 Covid-19 Vaccine against the B.1.1.7 and B.1.351 Variants. N Engl J Med 2021;385:187-9.
13. Bernal JL, Andrews N, Gower C, et al. Effectiveness of COVID-19 vaccines against the B.1.617.2 variant. medRxiv 2021:2021.05.22.21257658.
14. Polack FP, Thomas SJ, Kitchin N, et al. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. N Engl J Med 2020;383:2603-15.
15. Tarke A, Sidney J, Methot N, et al. Negligible impact of SARS-CoV-2 variants on CD4 (+) and CD8 (+) T cell reactivity in COVID-19 exposed donors and vaccinees. bioRxiv 2021. preprint.