Supplementary Appendix

Supplement to: Launay O, Cachanado M, Luong Nguyen LB, et al. Immunogenicity and safety of beta-adjuvanted recombinant booster vaccine. N Engl J Med. DOI: 10.1056/NEJMc2206711

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

SUPPLEMENTARY APPENDIX

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METHODS

Study design and Patient population

We conducted a randomized, single-blinded, multicenter trial across 11 centers in France. Participants were recruited from December 8, 2021, to January 14, 2022.

The objective of the study was to evaluate the immunogenicity and safety of a homologous booster dose of a COVID-19 mRNA vaccine (BNT162b2) or adjuvanted recombinant vaccine with adjuvant (Sanofi/GSK MV(D614) formulation or MV B.1.351 (Beta) formulation) in recipients primed with two doses of BNT162b2 between 3 and 7 months earlier.

The protocol was conducted in accordance with the Declaration of Helsinki and French law for biomedical research. It was approved by the "CPP Ile de France III" Ethics Committee and the French Health Products Safety Agency (ANSM).

The study is registered with the ClinicalTrials.gov identifier NCT05124171 and with the EudraCT identifier 2021-004550-33.

Adults aged 18 years and older in good health or with stable health if there was a pre-existing medical history were eligible to participate if they previously received two doses of BNT162b2 with an interval of 3 to 6 weeks and the second dose administered between 3 and 7 months prior to the administration of the study booster dose. Main exclusion criteria were pregnancy or breastfeeding, acute febrile infection within the previous 72 h and/or presenting symptoms suggestive of COVID-19 within the previous 28 days or having been in contact with an infected individual for the last 14 days before the inclusion visit, virologically documented history of COVID-19 (PCR or serology), use of immunosuppressive medications or any immunosuppressive condition that may reduce the immune response, history of severe post-vaccination allergic manifestations or a history of allergic reaction at the time of the first vaccine injection, having received BCG (tuberculosis) vaccine within the previous year or another

vaccine within two weeks prior to the boost injection or scheduled to receive a licensed vaccine within 2 weeks after the boost injection.

Intervention

Following consent, participants were randomly assigned in a 1:1:1 ratio to receive BNT162b2, MV(D614) vaccine or MV(Beta) vaccine as a third dose. The Sanofi/GSK adjuvanted recombinant protein vaccines are based on pre-fusion S antigens with the transmembrane domain replaced with a trimerization domain with AS03, an oil-in-water emulsion that contains squalene and α-tocopherol based immunologic adjuvant manufactured by GSK.[1]

Randomization was stratified on center and age group (18–64 years or ≥ 65 years). A web-based randomization system was used (CleanWeb e-CRF, Telemedecine Technologies, S.A.S), with a centralized block randomization list with blocks of size 4 (not communicated to the investigating team). The randomization list was generated by an independent statistician from the trial clinical research unit (URC-EST). Participants were randomized by the investigator.

Vaccines were administered intramuscularly into the deltoid muscle on day 0 by trained personnel. The health care professional administering the vaccine was aware of the treatment group because of differences in the preparation of the vaccines. The injection was therefore performed by a person not involved in the study and the investigating physician did not know which vaccine the volunteer had received. The central laboratories performing the antibody analyses were also blinded to limit measurement bias. Blood samples were planned at D0, D3, D15, D28, D90 and D365 for serological analysis.

Outcomes: Antibody responses

Neutralizing antibodies against the Wuhan (D614) SARS-CoV-2 viral strains and B.1.351 (Beta), Delta and Omicron BA.1 variants were assessed with a microneutralization test as

previously described.[2] The test uses clinical strains of SARS-CoV-2 (100 TCID50/well), TMPRSS2-expressing VeroE6 cells and relies on cytopathic effect (CPE) identification at 5 days post-infection. It is a VNT100 (100% of wells lysed in duplicate format). The test is automated in a NSB3 laboratory for all dilution and dispensing steps and for CPE reading. Dilutions tested were 20, 40, 80, 160, 320, 640 and 1280. The range was extended if a titer of 1280 was observed in the first instance.

Anti-SARS-CoV-2 IgG antibodies directed against the S1 domain of the virus Spike protein and the nucleocapsid protein were assessed using the QuantiVac ELISA kit from Euroimmun® (Lubeck, Germany) and expressed as BAU/ml.

Outcomes: T-Cell mediated immunity assessment

The cellular immune response was assessed *in vitro* by measuring production of IFNγ by CD4+ T-cells by ELISPOT (Diaclone) and IL-2 secreting $CD4^+$ T-cells by FLUOROSPOT (C.T.L) after stimulation with a pool of 15-mer overlapping peptides derived from the wild type (Wuhan) SARS-CoV-2 for both assays or from the Omicron variant of SARS-CoV-2 only for IFNγ Elispot, at concentration of 1 µg/ml. The samples tested were collected at baseline (D0) and D15 after the vaccine boost. CD4+ T-cells were obtained from defrost PBMC by a positive selection with a system MACS cell Separation using beads CD4 and LD columns (Miltenyi Biotec, Paris). $CD4^+$ T-cells were sensitized with the pool of spike peptides derived from the wild type SARS-CoV-2 Wuhan strain (JPT peptide technologies, Berlin, Germany) or the Omicron variant (peptides&elephants, Hennigsdorf, Germany) or a negative control (unstimulated cells in CTL-test medium, Bonn, Germany) or a positive control (cells stimulated with PMA-ionomycin (Sigma Saint-Quentin-Fallavier, France) for 20 h in a cell incubator at 37°C. After incubation, plates were revealed according manufacturer instructions, then scanned and analyzed on a C.T.L reader (S6 Ultimate). A response was considered positive if the number

of spots in the wells stimulated with the spike specific peptides was twofold higher than the number of spots in the negative control using a cutoff of 10 SFC/10^5 cells after background subtraction as previously detailed[3]. A positive response to vaccine was set up as a two-fold increase of the cytokine producing spike specific $CD4^+$ T-cell at D15 compared to D0.

Outcomes: Safety and adverse events

Injection-site and systemic solicited adverse events were collected for 7 days and unsolicited adverse events through 28 days after vaccination using diary cards provided to each participant. In the grading of adverse events, the FDA Toxicity Grading Scale (2007) was used (from grade 0, sign/symptom within normal limits to 4, life-threatening adverse event) for the following solicited adverse event: pain, arthralgia, asthenia or malaise, headache, fever, chills, swelling, lymphadenopathy, myalgia, nausea, edema, redness, vomiting; the WHO scale (mild, moderate or severe) was used for the following solicited adverse events, not present in FDA scale: itching, diarrhea, pain in extremities, insomnia.

Primary endpoint and sample size calculation

The primary endpoint was the proportion of subjects with an increase rate in neutralizing antibody titers of at least 10-fold, measured by a microneutralization technique, between day 0 and day 15 against D614 SARS-CoV-2 viral strain or B.1.351 variant.

The main other prespecified immunological endpoints were the rate of increase between day 0 and day 15 in neutralizing antibody titers against SARS-CoV-2 Wuhan (D614) and variants Beta, Delta and Omicron BA.1, geometric mean of anti-Spike IgG levels (expressed as BAU/mL).

As no data was available on the BNT162b2 vaccine, the sample size calculation was based on published data on the mRNA-1273 vaccine in which an increase rate of neutralizing antibody

titer of 23 against ancestral SARS-CoV-2 (D614G) and 32 for the B.1.351 variant after mRNA-1273 boost was described[4]. Using a conservative approach, we considered neutralizing activity to be sufficient if the increase rate was at least 10 at D15. We assumed a proportion of subjects with an increase rate of at least 10 between D0 and D15 of 80%. One hundred subjects per group allowed an estimation of this proportion with a 95% confidence interval (CI) of 7.8%. Thus, a total of 300 volunteers had to be randomized (100 per group).

Statistical analysis

The intent-to-treat (ITT) population included all randomized participants except those with positive nucleoprotein antibody serology at inclusion. Per protocol (PP) population included all randomized vaccinated participants without major protocol deviations, except participants presenting a positive nucleoprotein serology at day 0 or day 15, SARS CoV-2 infection after boost and lost to follow-up participants. The safety population included all randomized participants who received the vaccine booster dose. The immunogenicity analysis was performed on the per-protocol population.

For each viral strain (D614 and B.1.351), the primary endpoint was described using frequencies, percentages and 95% CIs calculated using the exact method. In a post hoc analysis, groups were compared using Chi-2 test.

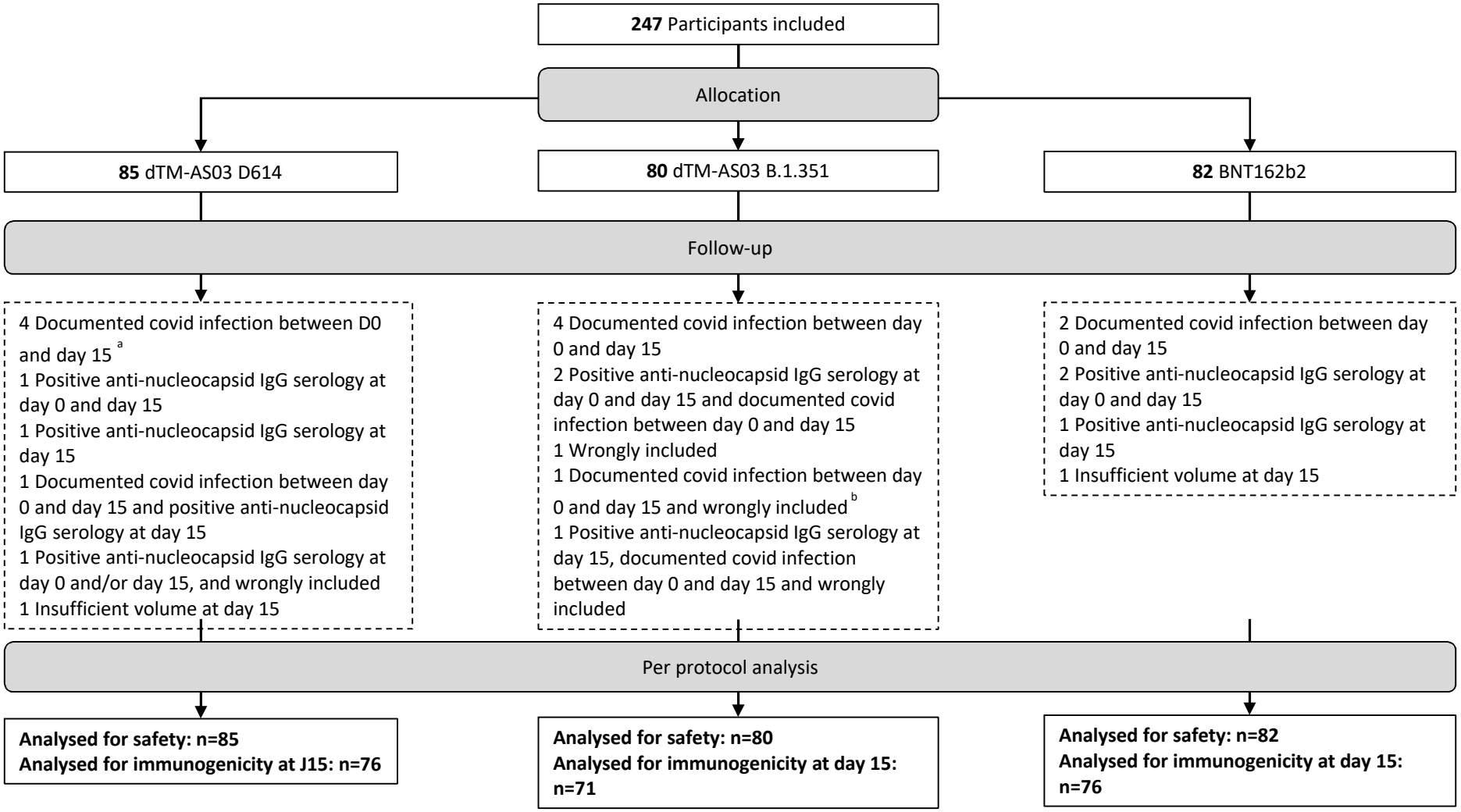
Baseline patient characteristics were described overall and for each group using the number (percentage) for categorical variables and the mean (SD) or median [interquartile range], according to distribution, and range for quantitative variables. For each group, the anti-SARS-CoV-2 IgG antibody titers directed against the S1 domain of the spike protein and the neutralizing antibody titers measured by a microneutralization technique against Wuhan strain (D614) and variants (B.1.351, Delta, Omicron BA.1) measured at day 0, day 3 and day 15 were

described as geometric means with two-sided 95% confidence intervals (95% CI). Adverse reactions and events were described using frequencies and percentages.

The statistical analysis was conducted using SAS software version 9.4 (SAS Institute, Inc., Cary, North Carolina, USA). R freeware (version 3.6.3) and GraphPad Prism software (version 9.2.0, San Diego, California USA) were used for the graphs.

RESULTS

Figure S1. Study flow chart.



[a] Day 15 visit and blood sample not performed

[b] Day 15 visit performed by phone and blood sample not performed

Table S1. Patient characteristics at enrolment (per-protocol population).

	Total population (n=223)	**Sanofi/GSK-D614 (n=76)**	**Sanofi/GSK-B.1.351 (n=71)**	**Pfizer BNT162b2 (n=76)**
Age, y				
Mean (SD)	40.6 (13.0)	40.2 (13.5)	41.4 (11.3)	40.4 (13.9)
Range	18–73	18–73	22–68	20–69
Female gender, n (%)	90 (40.4)	29 (38.2)	23 (35.2)	36 (47.4)
Body mass index, kg/m^2				
Mean (SD)	25.0 (4.5)	25.2 (4.5)	25.4 (4.5)	24.4 (4.6)
Range	15.2–40.8	16.8–35.6	18.5–40.8	15.2–40.4
Current smoker, n (%)	49 (22.1)	16 (21.3)	17 (23.9)	16 (21.1)
Comorbidity, n (%)				
Obesity [a]	27 (12.1)	13 (17.1)	8 (11.3)	6 (7.9)
Hypertension	11 (4.9)	6 (7.9)	2 (2.8)	3 (3.9)
Dyslipidemia)	6 (2.7)	4 (5.3)	1 (1.4)	1 (1.3)
Diabetes	2 (0.9)	2 (2.6)	0	0
Time between 1st and 2nd dose, days				
Median (IQR)	39 (31; 42)	39 (30: 42)	39 (33; 42)	38 (32; 40)
Range	21–49	21–49	21–44	21–42
Time between 2nd and 3rd dose, days				
Median (IQR)	174 (164; 187)	176 (167.5; 188)	171 (164; 184)	174.5 (160; 188)
Range	121–223	121–211	148–223	141–212

[a] Body mass index > 30 kg/m^2

Table S2. Neutralizing antibodies against D614 (wild-type; Wuhan) SARS-CoV-2 and Beta, Delta and Omicron BA.1 variants in the three randomized groups (per-protocol population).

	Sanofi/GSK-D614 (N=76)		**Sanofi/GSK-B.1.351 (N=71)**		**Pfizer BNT162b2 (N=76)**	
	N[a]	**GMT (95% CI)**	**N[a]**	**GMT (95% CI)**	**N[a]**	**GMT (95% CI)**
Wild-type (D614; Wuhan)						
D0	76	86.8 (66.0; 114.3)	71	84.0 (67.5; 104.6)	76	96.0 (71.7; 128.6)
D15	76	1168.4 (928.0; 1471.2)	71	1801.4 (1414.8; 2293.6)	76	1364.4 (1091.9; 1704.8)
Beta variant						
D0	76	38.6 (29.4; 50.5)	71	33.2 (27.5; 40.2)	76	38.6 (31.2; 47.7)
D15	76	416.9 (334.2; 520.0)	71	1053.0 (840.3; 1319.5)	76	428.4 (346.6; 529.7)
Delta variant						
D0	76	45.9 (34.7; 60.6)	71	39.2 (32.0; 48.1)	76	41.5 (33.1; 52.0)
D15	76	491.3 (393.3; 613.7)	71	894.4 (678.6; 1063.3)	76	553.1 (443.2; 690.2)
Omicron BA.1 variant						
D0	76	22.5 (18.8; 27.0)	71	17.4 (15.3; 19.9)	76	19.1 (16.3; 22.4)
D15	76	123.9 (101.5; 151.4)	71	253.2 (201.8; 317.6)	76	139.5 (115.0; 169.3)

Table S3. Anti-S1 antibodies from day 0 to day 15 (per-protocol population).

	Sanofi/GSK-D614 (N=76)		Sanofi/GSK-B.1.351 (N=71)		Pfizer BNT162b2 (N=76)	
Anti-S1 antibodies, BAU/mL, GMT (95% CI)	**N[a]**	**GMT (95% CI)**	**N[a]**	**GMT (95% CI)**	**N[a]**	**GMT (95% CI)**
Day 0	76	277.1 (229.7; à 334.3)	71	206.8 (134.5; 318.0)	76	253.6 (214.4; 300.0)
Day 15	76	1875.1 (1628.0; 2159.6)	71	2240.8 (1931.3; 2600.0)	76	2405.4 (2130.8; 2715.4)

Figure S2. Induction of TH1-CD4$^+$ T-cell response after a boost with BNT162b2 mRNA or Sanofi/GSK D614 or Sanofi/GSK B.1.351 vaccines.

CD4$^+$ T-cells were purified from PBMC collected before or 15 days after boosting by the various vaccines. IFNγ Elispot and IL-2 Fluorospot assays were performed by incubating CD4$^+$ T-cells with a pool of 15-mer overlapping peptides derived from the wild type (Wuhan) SARS-CoV-2 for both assays or from the Omicron variant of SARS-CoV-2 for the detection of IFNg producing cells. The number of spots was enumerated on an Elispot/Fluorospot reader with a positivity threshold set up at 10 spots per 10^5 cells. The increase between the medians at D15 and D0 for the various CD4$^+$ T-cell population is shown.

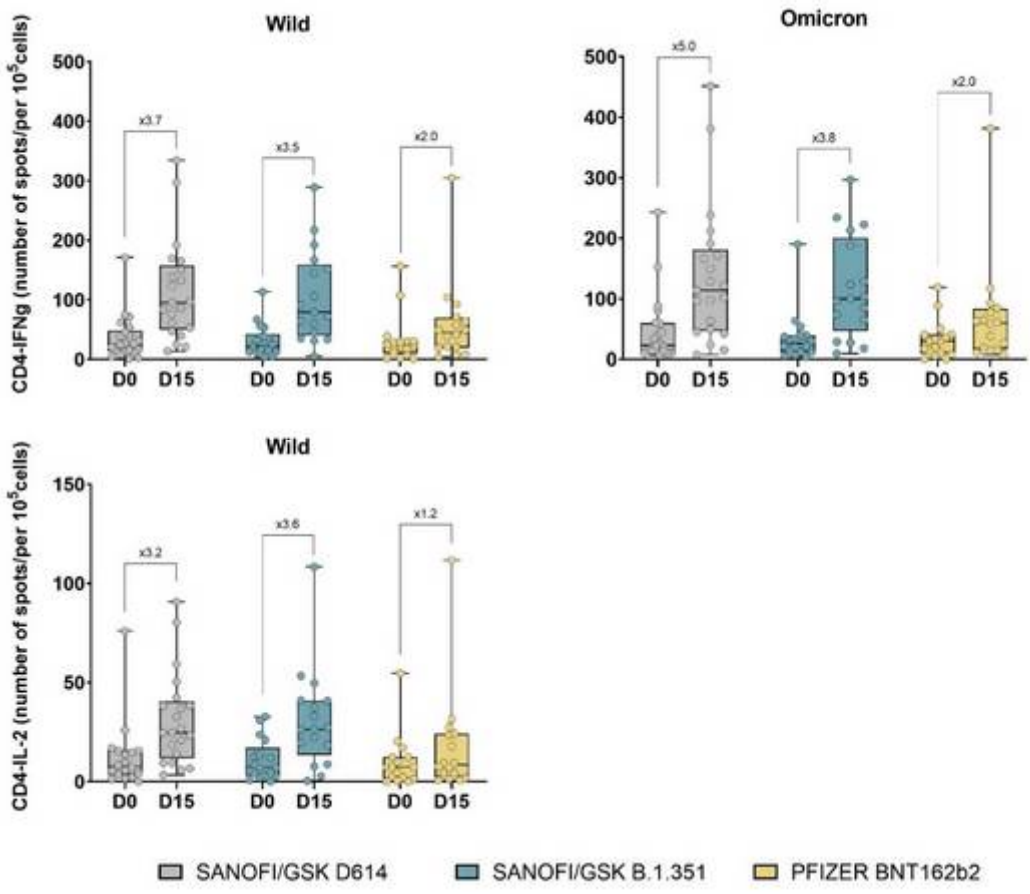


Figure S3. Frequency of induction of various TH1 $CD4^+$ T-cell populations after a boost with BNT162b2 mRNA or Sanofi/GSK D614 or Sanofi/GSK B.1.351 vaccines.

After a boost with the various vaccines, a vaccine response for the different anti-SARS-CoV-2 TH1 CD4 subpopulations was considered significant if the ratio between D15 and D0 was >2 with a number of spots at D15 > 10 (after background subtraction).

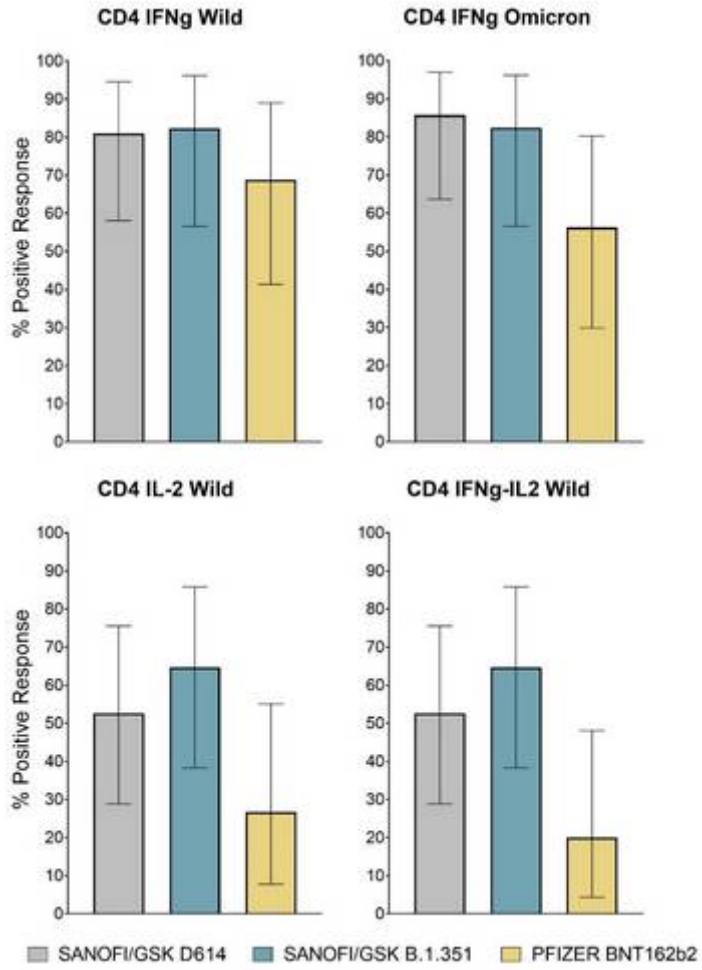


Figure S4. Rates and grades of severity of solicited adverse events reported from day 0 to day 7 by participants from the three randomized groups of the safety population (G1, Sanofi/GSK D614; G2, Sanofi/GSK-B.1.351; G3, BNT162b2) according to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (Modified FDA scale/September 2007).

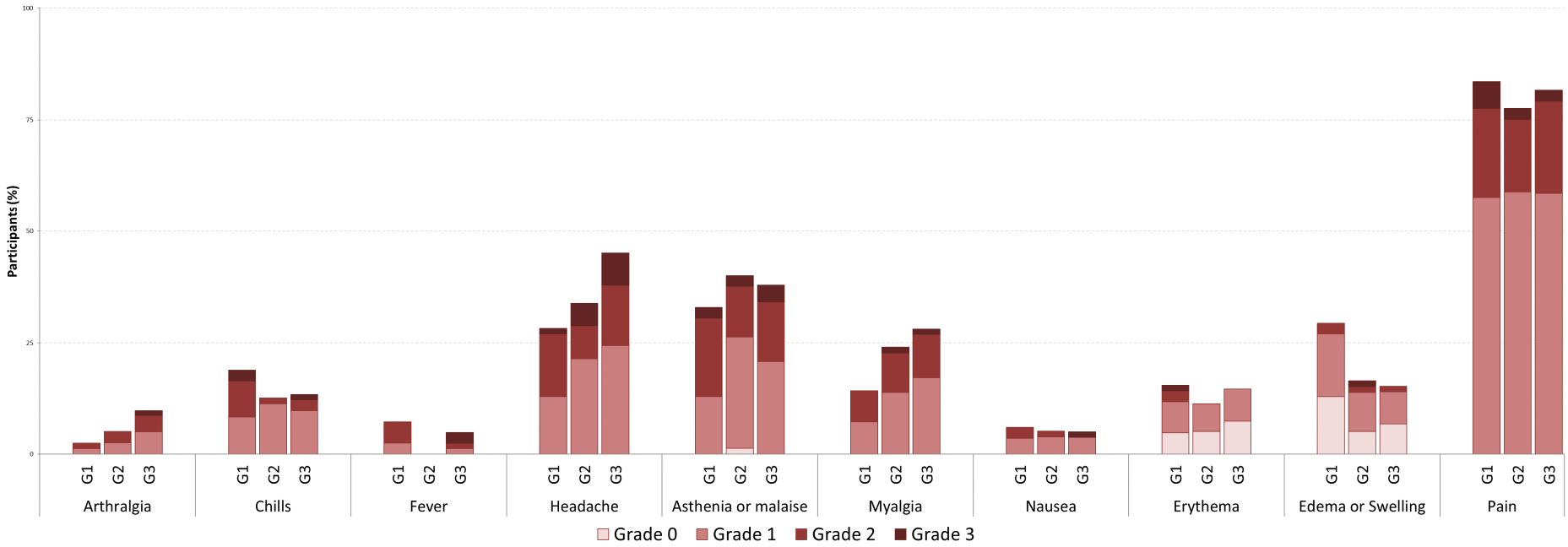


Table S4. Solicited injection-site and systemic adverse events (AE) in safety population.

	SANOFI/GSK-D614 n=85	SANOFI/GSK-B.1.351 n=80	PFIZER BNT162b2 n=82
		n (%)	
At least one solicited AE between D0 and D7	77 (90.6)	71 (88.8)	76 (92.7)
At least one injection-site AE between D0 and D7	71 (83.5)	64 (80.0)	69 (84.1)
Pain			
No	14 (16.5)	18 (22.5)	15 (18.3)
Grade 1	49 (57.6)	47 (58.8)	48 (58.5)
Grade 2	17 (20.0)	13 (16.3)	17 (20.7)
Grade 3	5 (5.9)	2 (2.5)	2 (2.4)
Redness			
No	72 (84.7)	71 (88.8)	70 (85.4)
Grade 0	4 (4.7)	4 (5.0)	6 (7.3)
Grade 1	6 (7.1)	5 (6.3)	6 (7.3)
Grade 2	2 (2.4)	0 (0)	0 (0)
Grade 3	1 (1.2)	0 (0)	0 (0)
Edema or swelling			
No	60 (70.6)	67 (83.8)	69 (84.1)
Grade 0	11 (12.9)	4 (5.0)	6 (7.3)
Grade 1	12 (14.1)	7 (8.8)	6 (7.3)
Grade 2	2 (2.4)	1 (1.3)	1 (1.2)
Grade 3	0 (0)	1 (1.3)	0 (0)
Itching			
No	77 (90.6)	75 (93.8)	78 (95.1)
Mild	8 (9.4)	5 (6.3)	3 (3.7)
Moderate	0 (0)	0 (0)	1 (1.2)

	SANOFI/GSK-D614 n=85	SANOFI/GSK-B.1.351 n=80	PFIZER BNT162b2 n=82
	n (%)		
At least one systemic AE between D0 and D7	42 (49.4)	50 (62.5)	53 (64.6)
Asthenia or malaise			
No	57 (67.1)	48 (60.0)	51 (62.2)
Grade 0	0 (0)	1 (1.3)	0 (0)
Grade 1	11 (12.9)	20 (25.0)	17 (20.7)
Grade 2	15 (17.6)	9 (11.3)	11 (13.4)
Grade 3	2 (2.4)	2 (2.5)	3 (3.7)
Arthralgia			
No	83 (97.6)	76 (95.0)	74 (90.2)
Grade 1	1 (1.2)	2 (2.5)	4 (4.9)
Grade 2	1 (1.2)	2 (2.5)	3 (3.7)
Grade 3	0 (0)	0 (0)	1 (1.2)
Headache			
No	61 (71.8)	53 (66.3)	45 (54.9)
Grade 1	11 (12.9)	17 (21.3)	20 (24.4)
Grade 2	12 (14.1)	6 (7.5)	11 (13.4)
Grade 3	1 (1.2)	4 (5.0)	6 (7.3)
Fever			
No	79 (92.9)	80 (100)	78 (95.1)
Grade 1	2 (2.4)	0 (0)	1 (1.2)
Grade 2	4 (4.7)	0 (0)	1 (1.2)
Grade 3	0 (0)	0 (0)	2 (2.4)
Chills			
No	69 (81.2)	70 (87.5)	71 (86.6)
Grade 1	7 (8.2)	9 (11.3)	8 (9.8)
Grade 2	7 (8.2)	1 (1.3)	2 (2.4)
Grade 3	2 (2.4)	0 (0)	1 (1.2)
Lymphadenopathy			

	SANOFI/GSK-D614 n=85	SANOFI/GSK-B.1.351 n=80	PFIZER BNT162b2 n=82
	n (%)		
No	82 (96.5)	77 (96.3)	76 (92.7)
Grade 1	3 (3.5)	2 (2.5)	2 (2.4)
Grade 2	0 (0)	0 (0)	4 (4.9)
Grade 3	0 (0)	1 (1.3)	0 (0)
Myalgia			
No	73 (85.9)	61 (76.3)	59 (72.0)
Grade 1	6 (7.1)	11 (13.8)	14 (17.1)
Grade 2	6 (7.1)	7 (8.8)	8 (9.8)
Grade 3	0 (0)	1 (1.3)	1 (1.2)
Nausea			
No	80 (94.1)	76 (95.0)	78 (95.1)
Grade 1	3 (3.5)	3 (3.8)	3 (3.7)
Grade 2	2 (2.4)	1 (1.3)	0 (0)
Grade 3	0 (0)	0 (0)	1 (1.2)
Vomiting			
No	84 (98.8)	80 (100)	82 (100)
Grade 2	1 (1.2)	0 (0)	0 (0)
Diarrhea			
No	81 (95.3)	75 (93.8)	75 (91.5)
Mild	4 (4.7)	4 (5.0)	4 (4.9)
Moderate	0 (0)	1 (1.3)	2 (2.4)
Severe	0 (0)	0 (0)	1 (1.2)
Pain in the extremities			
No	84 (98.8)	77 (96.3)	82 (100)
Mild	1 (1.2)	2 (2.5)	0 (0)
Moderate	0 (0)	1 (1.3)	0 (0)
Insomnia			
No	82 (96.5)	74 (92.5)	79 (96.3)

	SANOFI/GSK-D614 n=85	SANOFI/GSK-B.1.351 n=80	PFIZER BNT162b2 n=82
	n (%)		
Mild	1 (1.2)	5 (6.3)	3 (3.7)
Moderate	2 (2.4)	0 (0)	0 (0)
Severe	0 (0)	1 (1.3)	0 (0)

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