SUPPLEMENTARY MATERIALS

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Mechanistic Modeling Projections of Antibody Persistence After Homologous Booster Regimens of COVID-19 Vaccine Ad26.COV2.S in Humans

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Supplementary Methods

Wild-type neutralization assay

As described previously,[1] six two-fold serial dilutions of the heat-inactivated human serum samples were prepared in 96-well transfer plate(s). The SARS-CoV-2 wild-type was subsequently added to the serum dilutions at a target working concentration (~125 foci/well) and incubated at 37°C for 60–90 minutes. The serum-virus mixture was then transferred onto assay plates, previously seeded overnight with Vero E6 African green monkey kidney cells, and incubated at 37°C for 60–90 minutes before the addition of carboxymethyl cellulose–containing overlay medium and further incubation for 24 hours. Following incubation, the cells were fixed with formalin and stained using an antibody pair specific for the SARS-CoV-2 S protein receptor binding domain (RBD), and immunostained infectious foci were visualized using TrueBlue™ substrate. Immunostained infectious foci were counted using the ImmunoSpot® Analyzer from CTL. The immunostained infectious foci counts were exported to SoftMax Pro (Molecular Devices) and the neutralizing titer of a serum sample was calculated as the reciprocal serum dilution corresponding to the 50% neutralization antibody titer (IC_{50}) for that sample.

Spike protein enzyme-linked immunosorbent assay

As described previously,[1] the ELISA was developed and qualified for human serum at Nexelis, Laval, Canada. In brief, purified SARS-CoV-2 pre-spike antigen was adsorbed to the wells of a microplate and diluted serum samples (test samples, standard, and quality controls) were added. Unbound sample was washed away, followed by incubation with an enzyme-conjugated anti-human IgG and colorimetric detection with 3,3′,5,5′-tetramethylbenzidine. A reference standard on each test plate was used to quantify the amount of antibodies against

SARS-CoV-2 pre-spike in the sample according to the unit assigned by the standard (EU/mL).

Interindividual variability model

As described previously,[1] interindividual variability in model parameters was assumed to follow a log-normal distribution:

$$P_i = \theta \cdot \exp(\eta_i)$$

where *i* represents the individual model parameter of the subject *i*, while θ is the population (typical) value of that model parameter, and η_i is a random realization obtained by sampling from a normal distribution with mean zero and variance ω^2 following the definition:

$$\eta_i \sim \mathcal{N}(0, \omega^2)$$

Additive error model

As described previously,[1] the residual variability in serum antibody titers was described with an additive model on the log_{10} scale of the observations:

$$\log_{10}(y_{i,j}) = \log_{10}(f_{i,j}) + \varepsilon_{i,j}$$

where $y_{i,j}$ is the observed value for the wtVNA or S-ELISA data point *j* in individual *i*, $f_{i,j}$ is the model-based predicted value for the wtVNA or S-ELISA data point *j* in individual *i*, and ε_{ij} is the residual error for the wtVNA or S-ELISA data point *j* in individual *i*, which was assumed to be a realization from a normal distribution with mean zero and variance σ:

$$\varepsilon \sim \mathcal{N}(0, \sigma^2)$$

Covariate analysis

As described previously,[1] covariate relationships were included multiplicatively as power models (for continuous covariates) or as conditional effects relative to the most common category (for categorical covariates).

Continuous: $P_{ij} = \theta_{j0} \cdot (\frac{X_{ki}}{M(x_k)})^{\theta_{jk}}$

Categorical: $P_{ij} = \theta_{j0} \cdot (1 + \theta_{jk} \cdot X_{ki})$

where P_{ij} is the typical value of the j-th parameter accounting for covariates of the i-th participant, X_{ki} is the baseline value for the k-th covariate in the i-th participant (for categorical covariates, X_{ki} is the covariate value for categories other than the reference one), θ_{jk} is the covariate coefficient for the j-th parameter and the k-th covariate (either a power coefficient for continuous covariates or a multiplication term for categorical covariates), $M(X_k)$ is the median value of the k-th covariate in the population, and θ_{j0} is the population value of the parameter P_{ij} (i.e., for corresponding to the median covariate value for continuous covariates or to the reference category for categorical covariates). The effect of age, sex, body weight, and race on k_{AbS}, k_{AbL} and intercept were evaluated using a forward-inclusion and backward-elimination approach, following a graphical and statistical analysis of the association between covariates and model parameters.

References

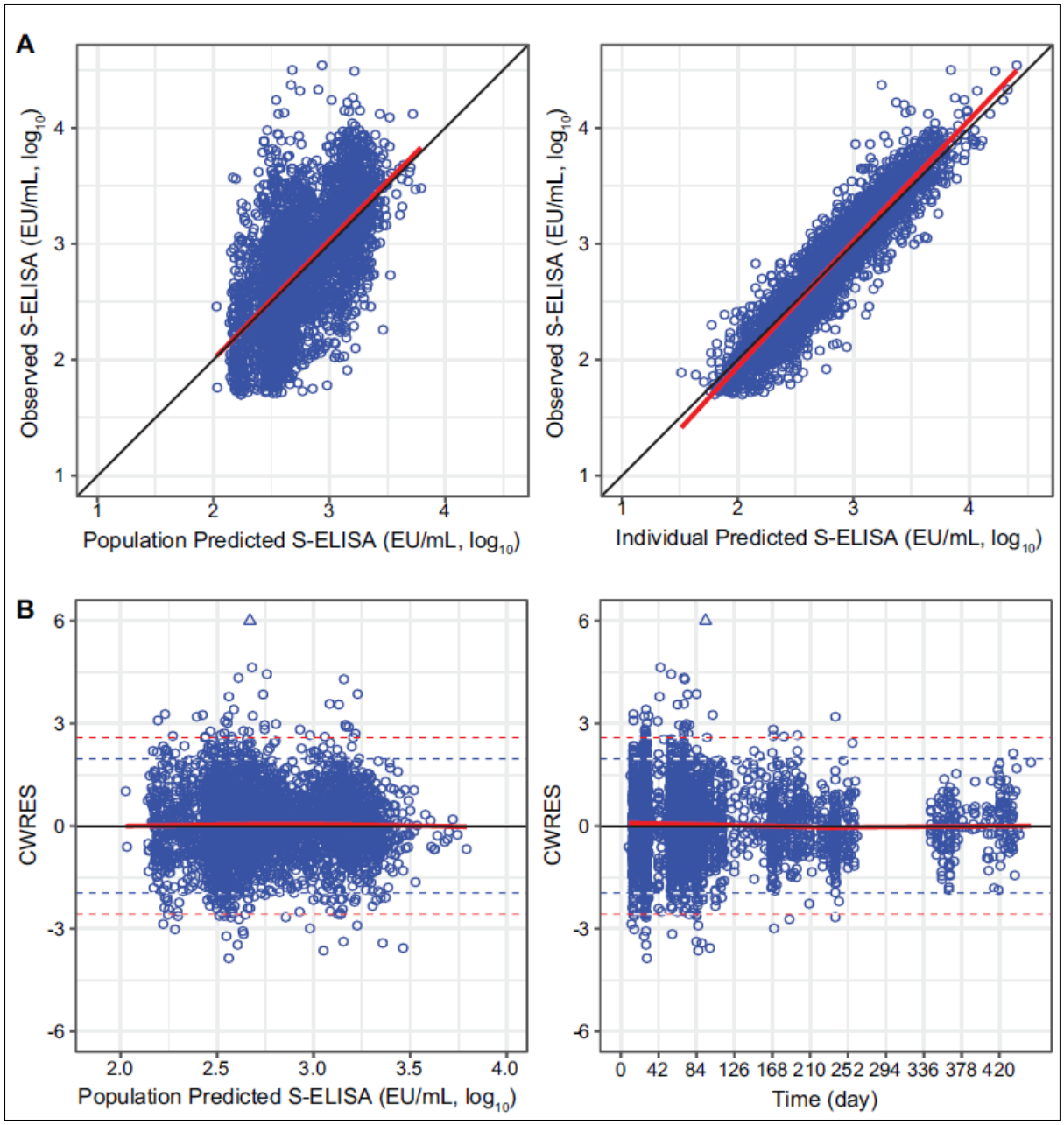
1. Dari, A. *et al.* Quantifying antibody persistence after a single dose of COVID-19 vaccine Ad26.COV2.S in humans using a mechanistic modeling and simulation approach. *Clin. Pharmacol. Ther.* **113**, 380-389 (2022).

Supplementary Results

wtVNA geometric mean levels

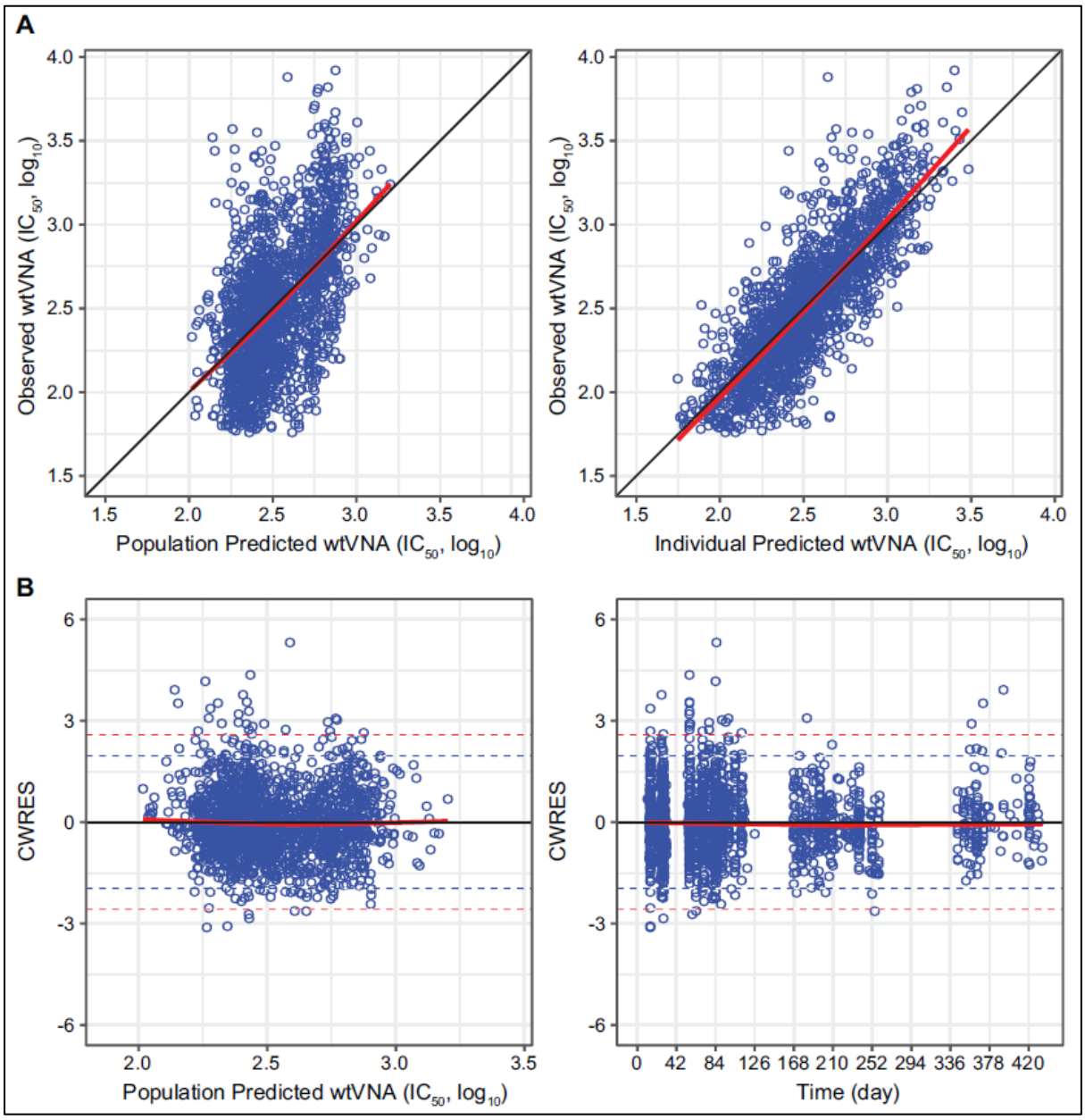
The geometric mean peak levels of neutralizing antibodies were 271 for a single dose of Ad26.COV2.S, and increased to 584, 854, and 944 after homologous boosting at 2, 3, and 6 months post prime, respectively. At day 155 after second peak, wtVNA geometric mean levels were 221 after single-dose Ad26.COV2.S, and increased to 238, 245, and 582 after homologous boosting at 2, 3, and 6 months post prime, respectively. The relative reduction between the peak and the antibody level at 155 days from second peak was 18.5% after single-dose Ad26.COV2.S, and increased to 59.2%, 71.3%, and 38.3% after boosting at 2, 3, and 6 months post prime, respectively.

Figure S1 Goodness-of-fit plots of the mechanistic model for binding antibody concentrations (S-ELISA). Observed concentrations plotted against the population-predicted and individual-predicted concentrations **(A)**. CWRES plotted against population-predicted concentrations and versus time **(B)**.



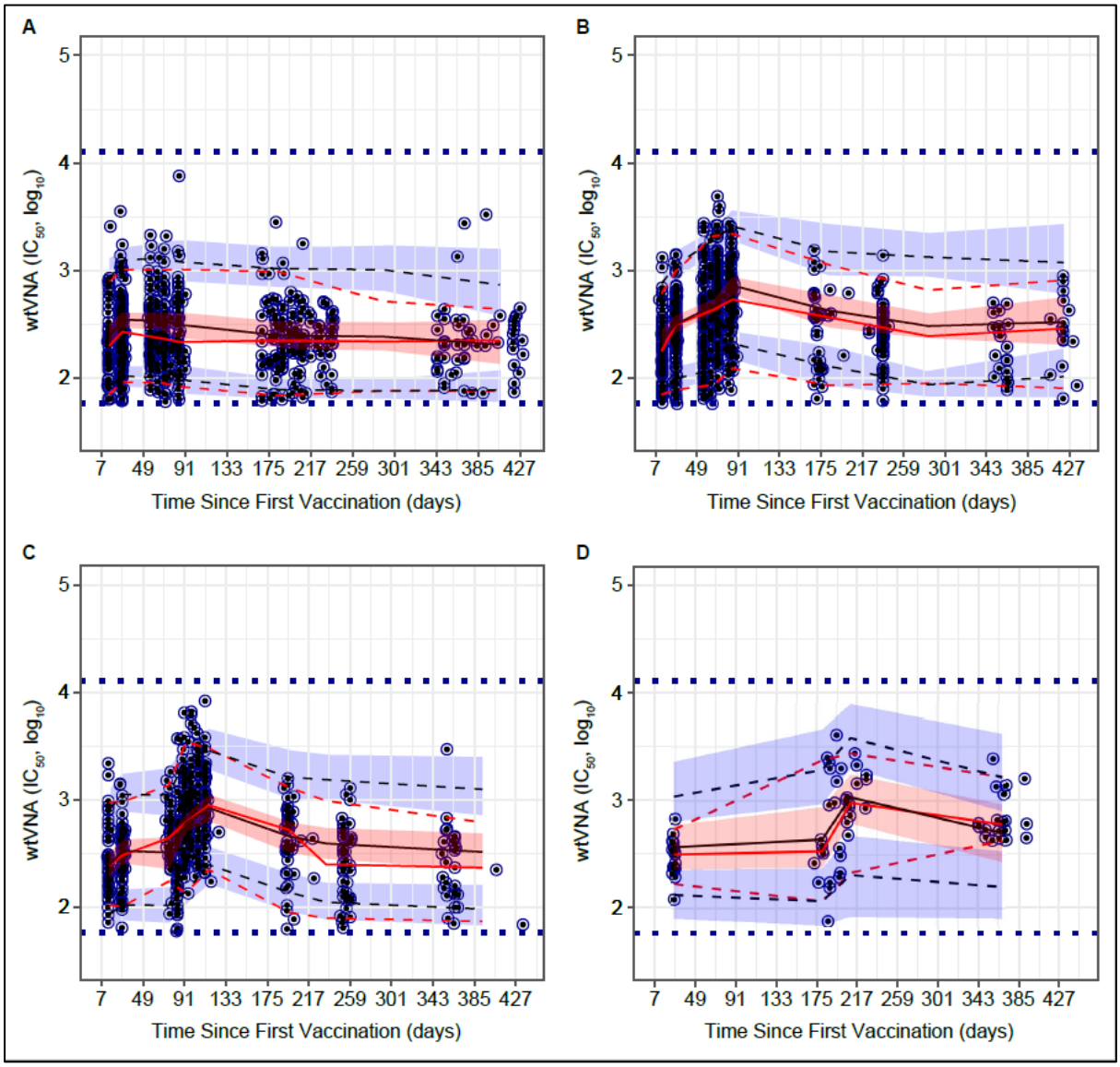
Blue open circles represent the observations and predictions (upper panels), CWRES (lower panels). Solid black lines represent identity lines. Red lines represent the trend line (locally weighted scatterplot smoothing [LOWESS]). Blue and red dashed lines represent the 95% and 99% CI, respectively. CI, confidence interval; CWRES, conditional weighted residuals; S-ELISA, spike protein enzyme-linked immunosorbent assay.

Figure S2 Goodness-of-fit plots of the mechanistic model for the neutralizing antibody titer (wtVNA). Observed titers plotted against the population-predicted and individual-predicted titers **(A)**. CWRES plotted against the population-predicted titers and versus time **(B)**.



Blue open circles represent the observations and predictions (upper panels), CWRES (lower panels). Solid black lines represent identity lines. Red lines represent the trend line (locally weighted scatterplot smoothing [LOWESS]). Blue and red dashed lines represent the 95% and 99% CIs, respectively. CI, confidence interval; CWRES, conditional weighted residuals; IC_{50}, 50% neutralization antibody titer; wtVNA, wild-type virus neutralization assay.

Figure S3 Visual predictive check of the mechanistic model for the neutralizing antibodies following a single dose of Ad26.COV2.S (5×10^{10} vp) **(A)**, a homologous booster dose at 2 months **(B)**, a homologous booster dose at 3 months **(C)**, and a homologous booster dose at 6 months **(D)**.



Blue dots represent observed data (log_{10} transformed). Continuous red lines represent the median of observed data; continuous red dashed lines represent the 5th and 95th percentiles of observed data. Black continuous line and red shaded area represent the median of simulated data and 95% CIs, respectively. Blue dashed lines and blue shaded area represent the 5th and 95th percentiles of simulated data and 95% CIs, respectively. Horizontal blue dashed lines show the assay-specific LLOQ and ULOQ values. CI, confidence interval; LLOQ, lower limit of quantification; ULOQ, upper limit of quantification; wtVNA, wild-type virus neutralization assay.

Table S1 Summary demographics for the continuous and categorical variables

	Total S-ELISA N=978	**Total wtVNA N=375**
Sex, n (%)		
Male	503 (51.4)	203 (54.1)
Female	475 (48.6)	172 (45.9)
Age (years)		
Mean (SD)	52.2 (17.0)	48.6 (17.8)
Median	55.0	47.0
Range	(18.0; 84.0)	(18.0; 80.0)
Age (dichotomic), n (%)		
18–59	541 (55.3)	243 (64.8)
≥60	437 (44.7)	132 (35.2)
Weight (kg)		
Mean (SD)	76.0 (17.4)	70.1 (13.3)
Median	73.8	69.8
Range	(40.8; 160)	(41.8; 105)
Height (cm)		
Mean (SD)	170 (10.3)	169 (10.7)
Median	170	169
Range	(142; 204)	(142; 196)
Body mass index (kg/m^2)		
Mean (SD)	26.1 (5.05)	24.2 (3.03)
Median	25.4	24.3
Range	(16.6; 59.7)	(17.4; 32.5)
Race, n (%)		
White, non-Hispanic, or Latino	712 (72.8)	217 (57.9)
Asian	120 (12.3)	104 (27.7)
Other	146 (14.9)	54 (14.4)

Table S2 Projected percentages of SARS-CoV-2–seronegative participants with measurable wtVNA titers (persistence of neutralizing antibody responses) up to 24 months after administration of a single-dose regimen and multiple homologous booster regimens of Ad26.COV2.S (5×10^{10} vp), and observed antibody persistence at 8 and 12 months post primary vaccination with Ad26.COV2.S.

Treatment regimen	Predicted		Observed	
	Time	Predicted % above LLOQ for the overall population	Actual time,[a] Bin range	Observed % above LLOQ, (95% CI),[b] N
Single dose	8 months	87.5%	Day 236 (230, 245]	94.7% (75.3%, 99.7%), 19
Single dose	12 months	78.4%	Day 370 (350, 390]	90.0% (74.4%, 96.6%), 30
Single dose	18 months	66.8%		
Single dose	24 months	55.2%		
Booster dose (D1, D57)	8 months	99.3%	Day 238 (230, 245]	91.2% (81.1%, 96.2%), 57
Booster dose (D1, D57)	12 months	94.5%	Day 358 (350, 390]	83.3% (64.1%, 93.3%), 24
Booster dose (D1, D57)	18 months	87.9%		
Booster dose (D1, D57)	24 months	80.3%		
Booster dose (D1, D85)	8 months	99.4%	Day 254 (245, 260]	100% (91%, 100%), 39
Booster dose (D1, D85)	12 months	95.3%	Day 360 (350, 390]	93.1% (78%, 98.1%), 29
Booster dose (D1, D85)	18 months	88.4%		
Booster dose (D1, D85)	24 months	81.0%		
Booster dose (D1, D183)[c]	8 months	89.0%	Day 205 (190, 217]	100% (81.6%, 100%), 17
Booster dose (D1, D183)[c]	12 months	89.0%	Day 361 (350, 390]	100% (78.5%, 100%), 14
Booster dose (D1, D183)	18 months	85.3%		
Booster dose (D1, D183)	24 months	80.0%		

[a]"Actual time" is chosen as the closest to the nominal simulated time (Time). "Bin range" refers to the lowest excluded value up to the largest included value of the range. Manual binning was used instead of actual visits to account for the heterogeneity of planned visits across studies. The following bins were defined: [0,21], (21,35], (35,67], (67,85], (85,113], (113,176], (176,190], (190,217], (217, 230], (230, 245], (245, 260], (260, 300], (300, 350], (350, 390], (390, 440]. [b]95% CI for binomial probabilities based on Wilson's method. [c]Given the large time range for binning, additional samples of the same participant were included in the computations of sample size for the 6-month booster arms. N=number of participants in the final analysis dataset (8-month and 12-month timepoints only). CI, confidence interval; LLOQ, lower limit of quantification; wtVNA, wild-type virus neutralization assay.