Supplemental Online Content

Portal-Celhay C, Forleo-Neto E, Eagan W, et al; COVID-19 Phase 2 Dose-Ranging Study Team. Virologic efficacy of casirivimab and imdevimab COVID-19 antibody combination in outpatients with SARS-CoV-2 infection: a phase 2 dose-ranging randomized clinical trial. *JAMA Netw Open.* 2022;5(8):e2225411. doi:10.1001/jamanetworkopen.2022.25411

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

Appendix 1: Study oversight

The trial was conducted in accordance with the principles of the Declaration of Helsinki, International Council for Harmonisation Good Clinical Practice guidelines, and applicable regulatory requirements. All patients provided written informed consent before participating in the trial. Ethics approval was obtained from the following ethics review boards: Western Institutional Review Board, Puyallup, WA, USA; Research Compliance Office, Stanford University, Palo Alto, CA, USA; Providence St. Joseph Health Institutional Review Board, Renton, WA, USA; and University of Maryland Baltimore Human Research Protection Office, Baltimore, MD, USA.

Appendix 2: Quantitative virology assay

Nasopharyngeal swabs were collected in 3 mL Viral Transport Medium (Catalog No. R99, Hardy Diagnostics, Santa Maria, CA, USA) at baseline (day 1) and study days 3, 5, 7, 15, and 22. Virologic testing for SARS-CoV-2 infection was performed at Viracor Eurofins (Lee's Summit, MO, USA) by quantitative real-time RT-PCR (RT-qPCR). RNA was extracted from clinical samples with the Applied Biosystems™ MagMAX™ Viral/Pathogen II (MVP II) Nucleic Acid Isolation Kit (Catalog No. A48383, ThermoFisher, Waltham, MA, USA) on the ThermoFisher KingFisher Flex. Viral nucleic acids were detected and quantified using a SARS-CoV-2 RT-qPCR swab assay (Viracor Eurofins) which is performed using oligonucleotide primers and TaqMan™ probes for the detection of 2 regions of the viral N protein gene region of SARS-CoV-2 and an internal extraction and amplification control target on ABI 7500 SDS Instruments (Applied Biosystems, Waltham, MA, USA). Assay data are presented in units of copies (genomic equivalents) of SARS-CoV-2 per mL of Viral Transport Medium (VTM) yielded from the collection of nasopharyngeal swab specimens (copies/mL). The limit of detection was 299 copies/mL (2.47 $\log_{10}$ copies/mL), the lower limit of quantification was 714 copies/mL (2.85 $\log_{10}$ copies/mL), and the upper limit of quantification (ULoQ) was 7.1×10^7 copies/mL (7.85 $\log_{10}$ copies/mL). Samples with results above the ULoQ were diluted and retested with the same assay. Analysis-positive RT-qPCR results (detected) below the lower limit of quantification were imputed as half of the lower limit of quantification (357 copies/mL) and negative RT-qPCR results (not detected) were imputed as 0 $\log_{10}$ copies/mL (1 copy/mL).

Appendix 3: SARS-CoV-2 serology testing

Serum was collected from patients at baseline. The EUROIMMUN Anti–SARS-CoV-2 ELISA (IgG) and the EUROIMMUN Anti–SARS-CoV-2 ELISA (IgA) assays were validated and run at ICON Laboratories (Farmingdale, NY) to detect endogenous anti-S1 protein antibodies. In addition, the Abbott Architect SARS-CoV-2 IgG assay was validated and run at ICON Laboratories to detect endogenous anti-nucleocapsid antibodies.

Appendix 4: Additional statistical analysis details

For between-group comparisons, the 95% CI half-width between any 2 treatment groups with this sample size would be 0.27 $\log_{10}$ copies/mL. The placebo IV and placebo SC arms were combined in the efficacy analyses of the viral load endpoints, as the route of administration does not alter the pharmacodynamic response of patients who received placebo. Active IV and SC CAS+IMD arms were not combined in any analysis, including for corresponding doses.

The efficacy analyses were based on observed data, with no imputation for missing data. Viral load values less than the LLoQ (714 copies/mL, equivalent to 2.85 $\log_{10}$ copies/mL) of the RT-qPCR assay, but with positive qualitative results, were set to half of the LLoQ of the RT-qPCR assay. Values with nondetectable RNA were set to 0 $\log_{10}$ copies/mL if the reason for the negative value was not a failed test. Viral load values above the upper limit of quantification were retested using the reflex test with diluted sample.

To enable bridging with other studies in the CAS+IMD clinical study program, the primary efficacy variable, time-weighted average daily change from baseline (TWACB), was the same as in other studies (eg, COV-2067; Weinreich DM et al, *N Engl J Med*; 2021). TWACB is a composite variable where multiple measurements (baseline through day 7) are combined into a single variable for analysis. As recommended in ICH E9 “Statistical Principles in Clinical Trials” the advantage of combining multiple measurements into a single variable, it is avoids adjusting the type I error rate for incorporating multiple measurements. TWACB was calculated using the linear trapezoidal rule to calculate area under the curve (AUC) and then

TWACB was determined using AUC. The calculation for time-weighted average (TWA) is the following:

$$TWA = \frac{AUC}{t_k - t_1} = [\sum_{i=2}^{k}(t_i - t_{i-1}) * (V_i + V_{i-1})/2]/(t_k - t_1)$$

where V_i is the result of change in viral load (log_{10} copies/mL) at time point t_i from baseline. As baseline (day 1) is time point t_1, then $V_1 = 0$.

The primary efficacy analysis was conducted using 803 randomized and treated patients enrolled in the study up to day 7, in order to evaluate approximately 400 patients who were seronegative at baseline. Active treatment arms were compared to the combined placebo group in descending order, as depicted below:

Hierarchy Number	Description
1	2400 mg IV vs pooled placebo
2	1200 mg IV vs pooled placebo
3	1200 mg SC vs pooled placebo
4	600 mg IV vs pooled placebo
5	600 mg SC vs pooled placebo
6	300 mg IV vs pooled placebo

For the secondary efficacy analysis, a mixed effects model for repeated measures (MMRM) assessed the time course of the change from baseline in viral load (log_{10} copies/mL) at each visit through day 7 for both the Seronegative mFAS and the Overall mFAS. The MMRM has terms for baseline viral load, treatment, visit, and all two-way interactions as fixed effects and subject as a random effect.

Appendix 5: Pharmacokinetic analysis methods

Bioanalytical methods

The concentrations of casirivimab (REGN10933) and imdevimab (REGN10987) in human serum were measured using validated immunoassays which employ streptavidin microplates from Meso Scale Discovery (MSD, Gaithersburg, MD, USA). The methods utilized 2 anti-idiotypic, monoclonal antibodies, each specific for either casirivimab or imdevimab as the capture antibodies. Captured total casirivimab and total imdevimab were detected using 2 different, non-competing, anti-idiotypic monoclonal antibodies, each also specific for either casirivimab or imdevimab. In these total assays, concentrations of total casirivimab or total imdevimab were measured without regard to binding site occupancy. The assays detect antibody with either 1 or 2 unoccupied binding sites, as well as antibody with both binding sites occupied. The bioanalytical methods specifically quantified the total concentrations of each anti–SARS-CoV-2 spike monoclonal antibody separately, with no interference from the other antibody. The assays have a lower limit of quantification (LLoQ) of 0.156 mg/L for each analyte in the undiluted serum sample.

Pharmacokinetic methods

The pharmacokinetics (PK) analysis population included all participants in the modified full analysis set (mFAS) who received active study drug and who had at least 1 non-missing total analyte (total casirivimab or total imdevimab) concentration result following dosing with study drug (modified concentration-response analysis set, or mCRAS). Participants were analyzed based on actual treatment received. Overall, 429 patients were included in the PK analysis set.

Blood samples for measurement of casirivimab and imdevimab concentrations in serum were collected from patients at predose, within 60 minutes after the end of infusion (intravenous [IV] only) or at least 1 hour after study drug administration (subcutaneous [SC] only) and on days 3, 5, and 7.

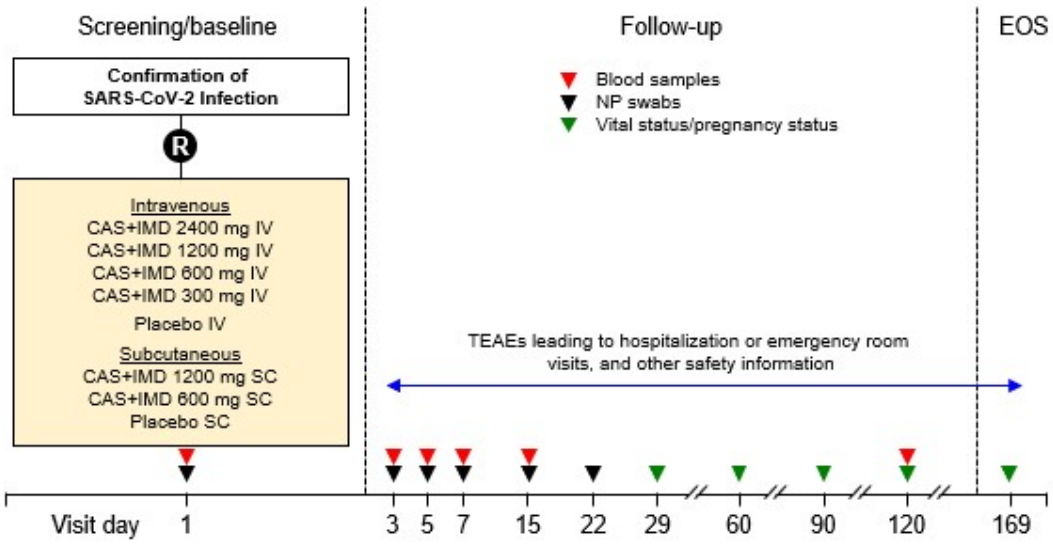
Concentrations of total CAS+IMD (sum of casirivimab + imdevimab) in serum were used for all PK and concentration-response analyses.

eTable 1. TWACB in Viral Load from Day 1 to Day 7 in Seronegative mFAS

		IV				SC	
	Pooled placebo (n = 77)	**CAS+IMD 300 mg IV (n = 80)**	**CAS+IMD 600 mg IV (n = 68)**	**CAS+IMD 1200 mg IV (n = 72)**	**CAS+IMD 2400 mg IV (n = 62)**	**CAS+IMD 600 mg SC (n = 75)**	**CAS+IMD 1200 mg SC (n = 73)**
TWACB (log_{10} copies/mL)							
N	74	76	66	67	61	71	71
Mean (SD)	-1.64 (1.11)	-2.24 (1.17)	-2.40 (1.09)	-2.24 (1.12)	-2.40 (1.07)	-2.29 (1.09)	-2.23 (1.12)
Q1:Q3	-2.40:-0.91	-2.87:-1.70	-3.13:-1.70	-2.94:-1.35	-3.10:-1.66	-3.15:-1.74	-2.86:-1.51
Min:max	-5.2:2.9	-5.1:2.3	-5.6:0.1	-5.7:-0.3	-4.6:0.6	-4.1:1.5	-5.5:0.6
Difference vs placebo (log_{10} copies/mL)							
LS mean (SE)		-0.57 (0.16)	-0.66 (0.17)	-0.56 (0.16)	-0.71 (0.17)	-0.56 (0.16)	-0.56 (0.16)
95% CI		-0.88, -0.25	-0.99, -0.34	-0.89, -0.24	-1.05, -0.38	-0.88, -0.24	-0.87, -0.24
P value		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Abbreviations: CI, confidence interval; IV=intravenous; LS, least square; mFAS, modified full analysis set; SC=subcutaneous; SD, standard deviation; TWACB=time-weighted average change from baseline.

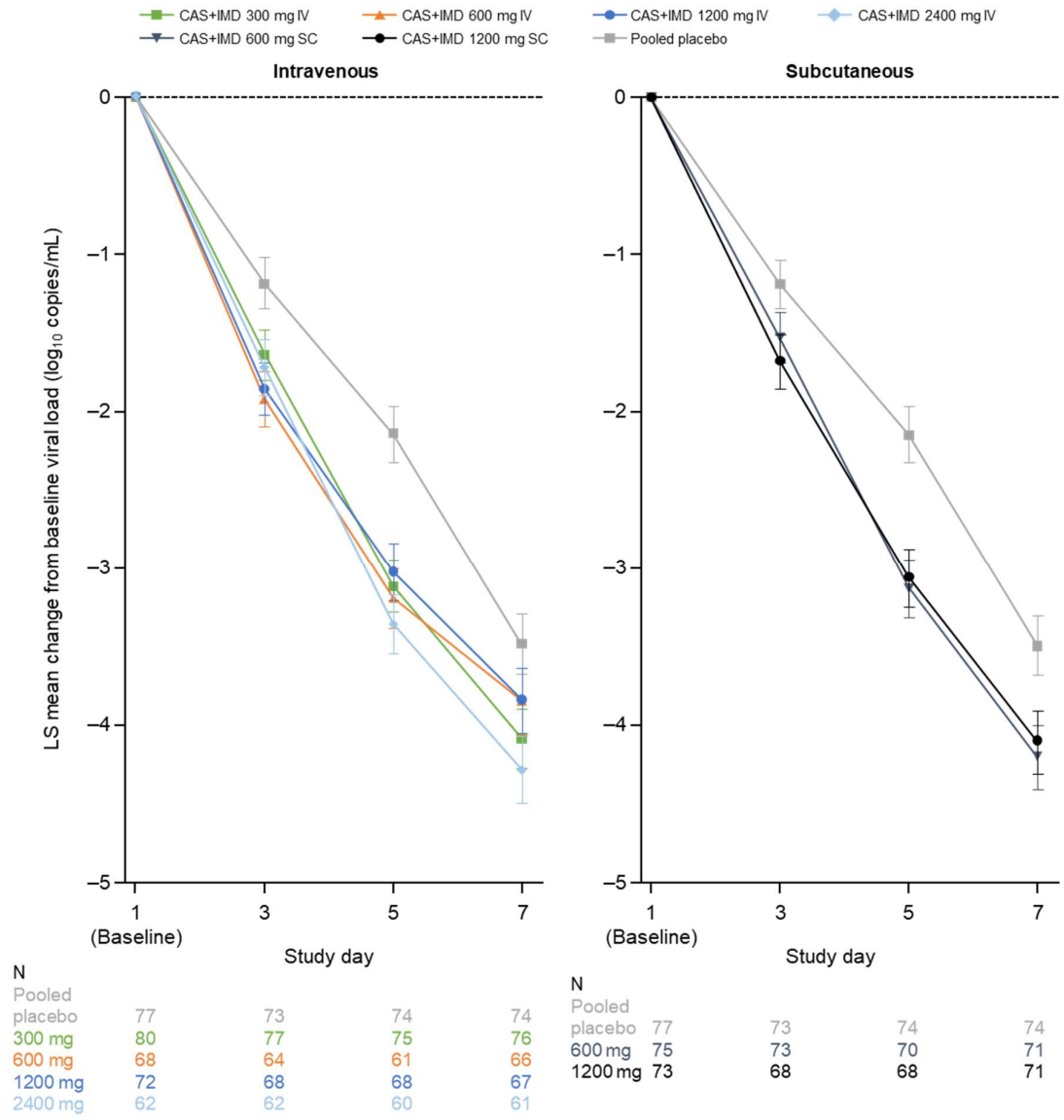
eFigure 1. Study Design



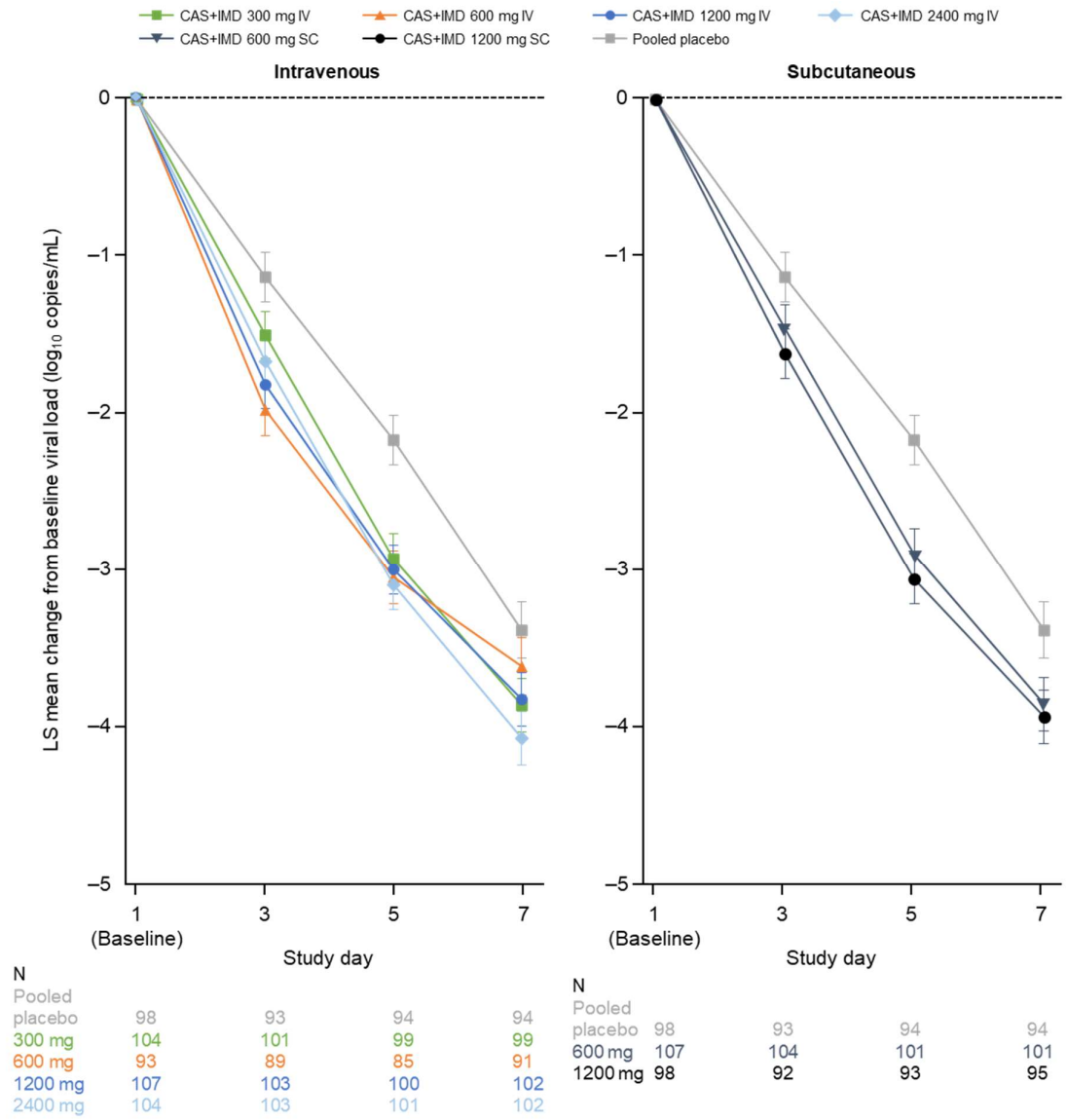
Abbreviations: EOS=end of study; IV=intravenous; NP=nasopharyngeal; R=randomization; SARS-CoV-2=severe acute respiratory syndrome coronavirus 2; SC=subcutaneous; TEAE=treatment-emergent adverse event.

eFigure 2. LS Mean Change From Baseline in Viral Load of IV and SC Doses at Each Visit in the Seronegative mFAS Population and Overall mFAS Population

(A)

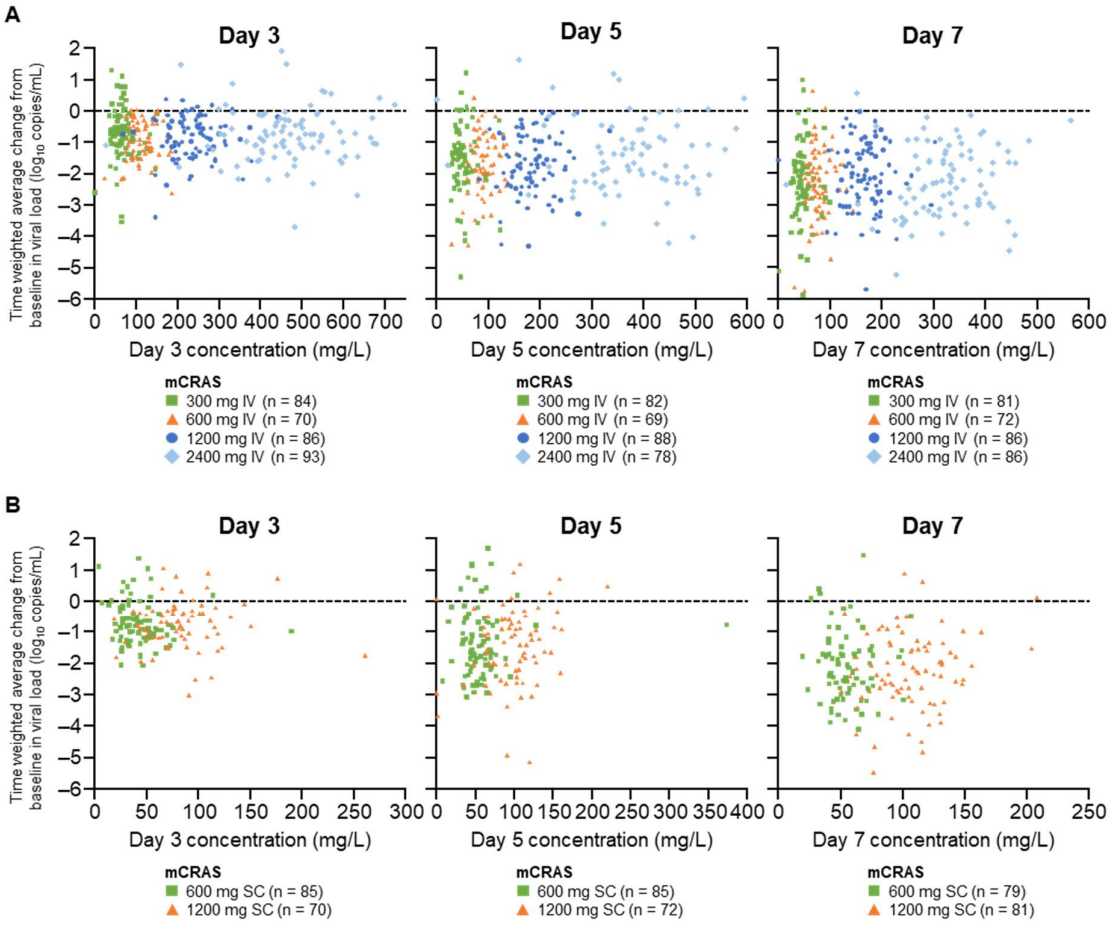


(B)



Abbreviations: IV=intravenous; LSM=least-squares mean; mFAS=modified full analysis set; SC=subcutaneous; SE=standard error.

eFigure 3. TWACB in Viral Load vs Total Casirivimab and Imdevimab Concentrations[a] in Serum in Individual Patients at Days 3, 5, and 7 for IV and SC Treatment Groups



Abbreviations: IV=intravenous; mCRAS=modified concentration-response analysis set; SC=subcutaneous; TWACB=time-weighted average change from baseline.
[a] Total CAS+IMD concentration is the sum of total casirivimab and total imdevimab concentrations in serum.