SUPPLEMENTARY MATERIALS

SARS-COV-2 nasopharingeal viral load change in a multicenter randomized clinical trial on three different early therapies for COVID-19 in Non-hospitalized Adults with high risk of severe COVID-19 (MONET TRIAL).

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Study design and participants

The MONET trial (EudraCT: 2021-004188-28) is a multicentric, independent, phase-4, three-arm, superiority, open-label RCT conducted in seven Italian centers over March 2022-February 2023 and financed by the Italian Medicines Agency (Agenzia Italiana del Farmaco, AIFA). The study aimed to assess the efficacy and safety of 500 mg intravenous SOT (Arm 1), 300/300 mg intramuscular TIX/CIL (Arm 2) and oral 5-day course of NMV/r 300/100 mg (or 150/100 mg for those with a creatinine clearance of 30-60 mL/min) twice daily (Arm 3), randomly assigned in a 1:1:1 ratio, in non-hospitalized adults (aged >18 years) with early COVID-19 (diagnosed via direct antigen or nucleic acid tests within five days from the symptoms' onset) at high-risk of progression to severe disease as defined by AIFA criteria (*Determina AIFA Nella GU N.142 Del 16.06.2021. Available at: https://www. gazzettaufficiale.it/eli/gu/2021/06/16/142/sg/pdf, accessed 30 October 2024*): being aged 65 years or older or having at least one of the following risk factors: a body mass index (BMI) above 30, chronic kidney or liver disease, uncontrolled or complicated diabetes mellitus, any immunocompromising condition, cardio-cerebrovascular disease, oncologic and hematologic conditions, chronic obstructive pulmonary disease (COPD), hemoglobinopathies, and neurodevelopmental or neurodegenerative diseases.

Simple randomisation has been used to create the randomisation schedule which was managed through a centralized web-based system. At the screening, all participants signed an informed consent, and their data were entered anonymously into the system. To ensure concealment of allocation, the randomisation schedule was made available to authorized recruiters only after an individual was deemed eligible for the trial and had signed the informed consent. This was an open-label trial, so no blinding was in operation, although laboratory personnel measuring the viral load did not know which treatment the person had received. Participants were scheduled for three visits: visit 1 at enrolment and randomization, visit 2 between days 7 and 9, and visit 3 (at the clinic or by phone call) between days 28 and 30 after randomization.

Statistical analysis

Participants' characteristics at entry in the study have been described by trial arm (SOT, TIX/CIL and NMV/r) using medians and interquartile ranges (IQR) for continuous variables and frequencies and percentages for categorical variables, and any potential imbalance across treatment arms was assessed. The change in CT value over the time D1-D7 was evaluated both as a continuous change (in the log2 scale) and as a binary outcome using the threshold of 35 units (negative if above this threshold). For the continuous outcome, median and distribution of the CT values at D1 and median changes over D1-D7 were described by trial arm using boxplots. Also, mean values have been compared by trial arm using an unpaired t-test of the D1-D7 change and by means of linear regression. Because we detected a small imbalance in age, we also ran the linear regression after controlling for age. Participants were infected with sublineages of Omicron (mainly BA.2 and BA.4/5) and we treated these variants as potential effect measure modifiers. Variants have been obtained either from sequencing or inferred from public data of circulating variants in the Lazio region. Results of the treatment contrasts in the subsets of participants infected with these sublineages were shown in a forest plot and we formally tested the interaction in the age-adjusted linear regression model. For the binary outcome we used a chi-square test comparing the proportion of participants with a D7 CT value >35 by trial arm. Also, in this case we fitted a logistic regression model to account for residual confounding by age. All pairwise comparisons across the arms have been conducted and we used Dunnett correction for p-values accounting for the inflation of Type I error due to multiple comparisons.

For the remaining secondary outcomes (inflammatory markers and antibody responses), we also used the D29 data. In more detail, we performed unadjusted parametric mixed linear models with random intercept and slopes at the 3 time points (D1, D7, D29) including the main effects (trial arm and time) as well as the interaction term. We fitted separate models for each of these other secondary outcome parameters and estimated at each time point the mean levels and difference by trial arm with the corresponding 95% confidence interval (CI). Interaction p-values have been reported and differences

by trial arm discussed only when there was evidence for interaction. Unadjusted estimates with 95% CI have been reported both in Tables and Figures. Secondary outcomes were not available for all participants as randomized. To verify the possibility of selection bias being introduced, for the main outcomes, we compared the characteristics of the study population with those of patients enrolled who were excluded because of missing values. Slightly smaller denominators were used in the other secondary outcomes' analyses (because additional participants had missing values for these) and exact numbers of persons included in these sub-analyses are described in the text. Most of the attrition was present for the D29 data. We did not specifically address this issue besides fitting a model which works for imbalanced designs.

All analyses were carried out using SAS version 9,4 (Cary, USA) and Stata Statistical Software: Release 18, produced by StataCorp LLC in College Station, TX.

Virological methods

Semi-quantitative estimation of the VL in NPS was assessed by Real-Time Polymerase Chain Reaction (RT-PCR), based on cycle threshold (CT) values, on the Abbott Alinity platform (Abbott Laboratories, Wiesbaden, Germany).

Whole-genome Sequencing was performed on NPS samples collected at baseline. Nucleic acid extraction was performed by QiaSymphony automatic extractor (QIAGEN, Hilden, Germany) with DSP Virus/Pathogen Kit (QIAGEN). Sequencing libraries were prepared by using Ion AmpliSeq SARS-CoV-2 Insight Research Assay, and Next Generation Sequencing (NGS) was carried out Ion Torrent Gene Studio S5 Prime (GSS5 Prime) platform, following the manufacturer's instructions (ThermoFisher, Waltham, MA, USA). SARS-CoV-2 genomes were assembled using the Easy-to-use SARS-CoV-2 Assembler pipeline (ESCA) (*Rueca M, Giombini E, Messina F, et al. The Easy-to-Use SARS-CoV-2 Assembler for Genome Sequencing: Development Study. JMIR Bioinform Biotechnol. 2022;3(1):e31536. Published 2022 Mar 14. doi:10.2196/31536*).

Serological methods

Detection of anti-nucleocapsid protein (anti-N) and anti-spike RBD (anti-S) SARS-CoV-2 IgG was performed by chemiluminescence techniques on the Abbott Alinity i platform, in accordance with the manufacturer's instructions (SARS-CoV-2 IgG and SARS-CoV-2 IgG II Quant, Abbott). Levels of anti-N IgG are reported as an index (sample/cut-off S/CO, cut-off to define a positive response ≥ 1.4), while quantification of anti-S IgG is indicated in Binding Antibody Units per mL (BAU/ml, cut-off to define a positive response ≥ 7.1), as recommended by the manufacturer.

Supplementary results

Figure S1. Love plot of comparison of sotrovimab (SOT) *versus* nirmatrelvir plus ritonavir (NMV/r)

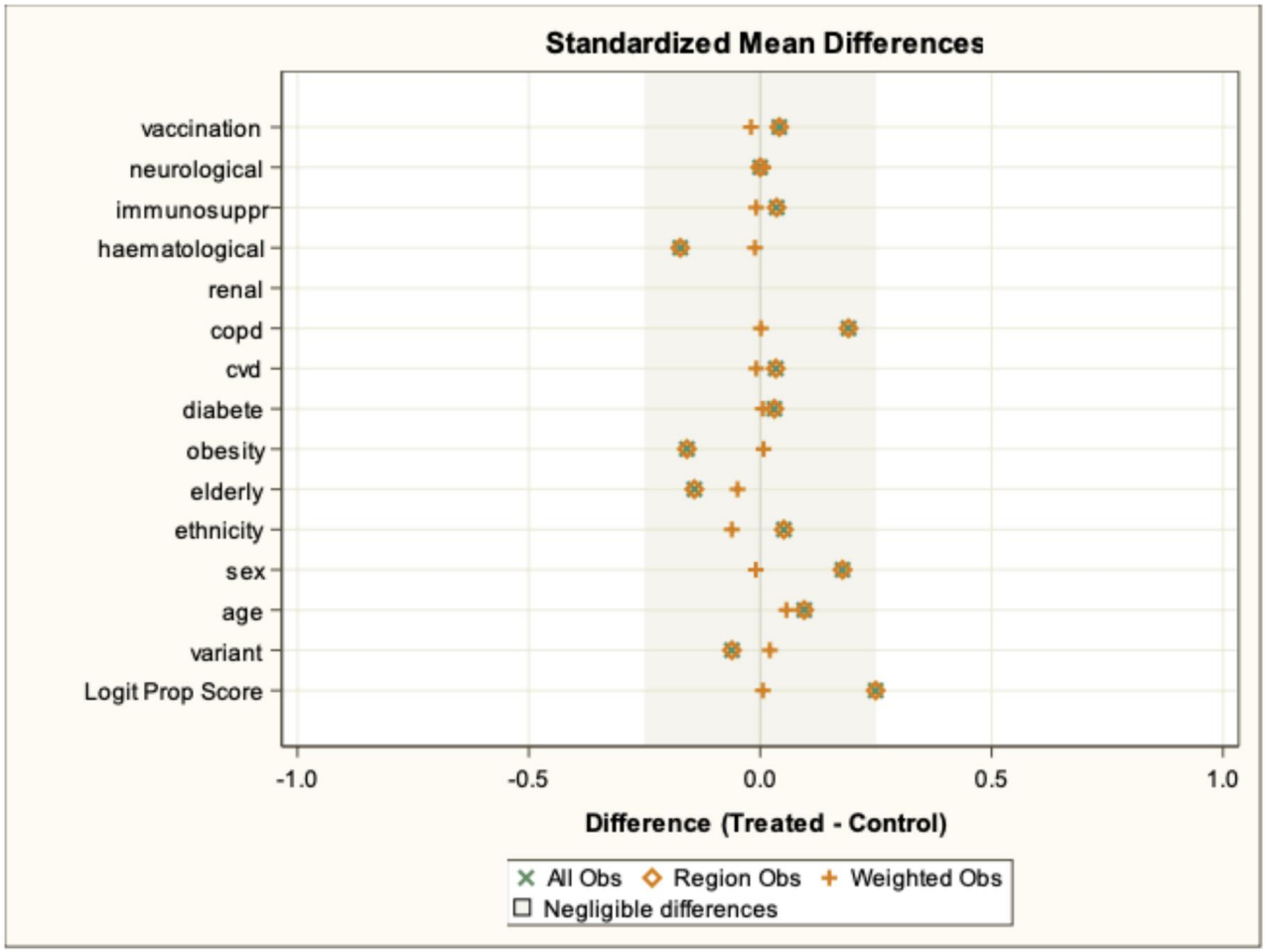

Figure S2. Love plot of comparison of tixagevimab plus cilgavimab (TIX/CIL) *versus* nirmatrelvir plus ritonavir (NMV/r)

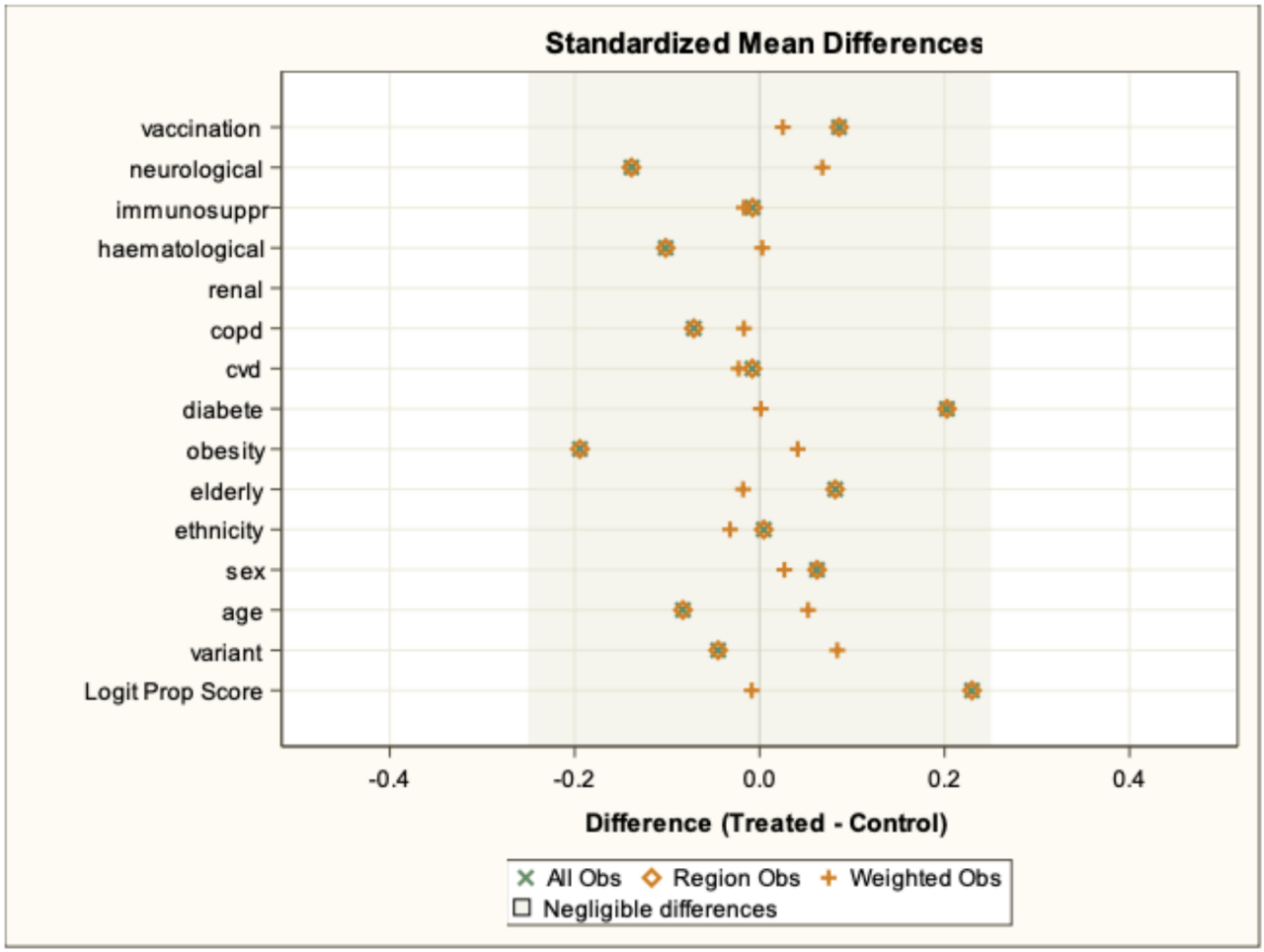

Figure S3. Love plot of comparison of sotrovimab (SOT) *versus* tixagevimab plus cilgavimab (TIX/CIL)

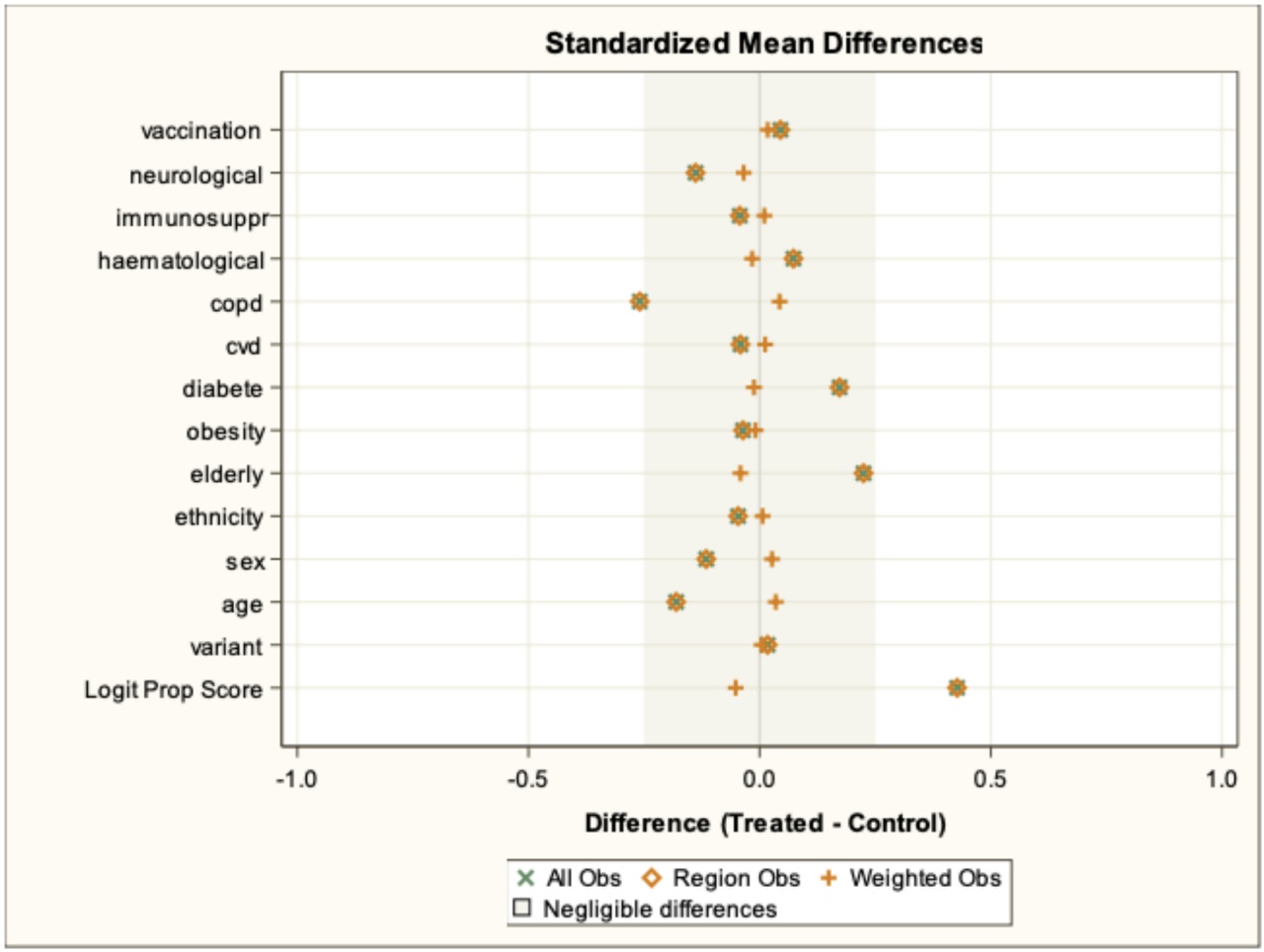

Figure S4. Difference in mean cycle threshold (CT) values change seven days after the enrollment (D1-D7) from fitting linear regression models, adjusted for age, among participants infected with Omicron BA.2 and BA.4/5. Abbreviations: SOT, sotrovimab; TIX/CIL, tixagevimab plus cilgavimab; NMV/r, nirmatrelvir plus ritonavir; vs., *versus*.

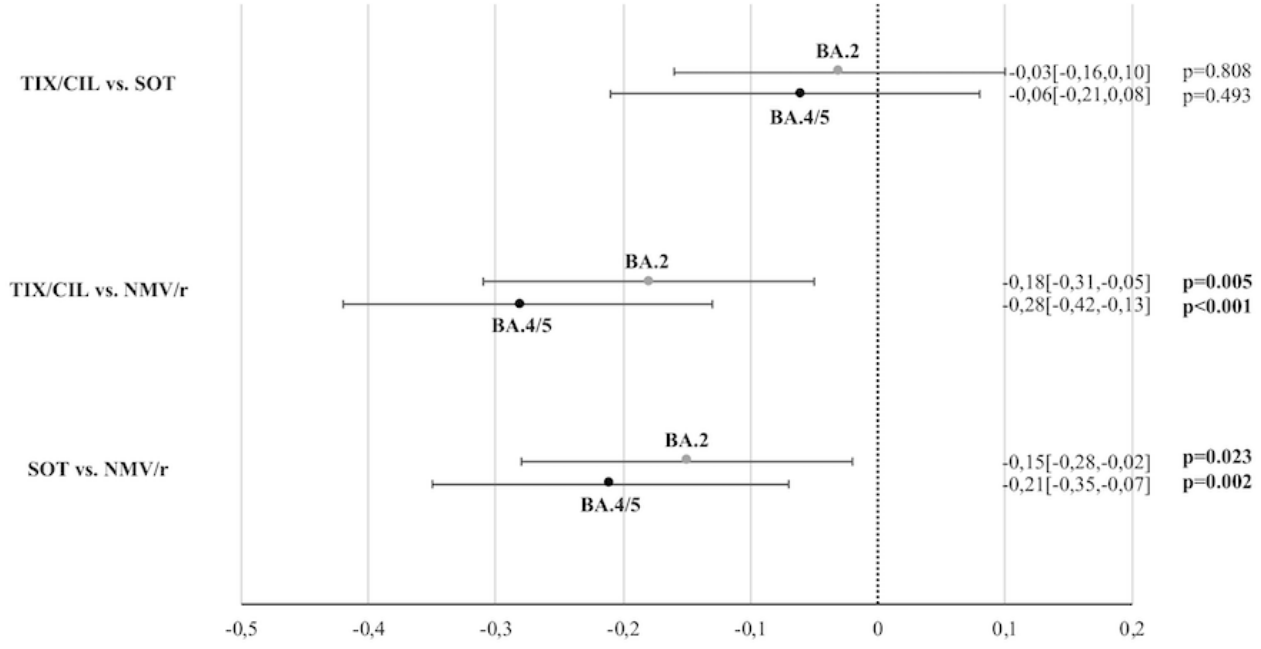

Figure S5. Kinetics over D1-D29 of inflammatory markers (CRP, C-reactive protein; d-dimer; NLR, neutrophils-to-lymphocytes ratio) by trial arm (SOT, sotrovimab; TIX/CIL, tixagevimab plus cilgavimab; NMV/r, nirmatrelvir plus ritonavir).

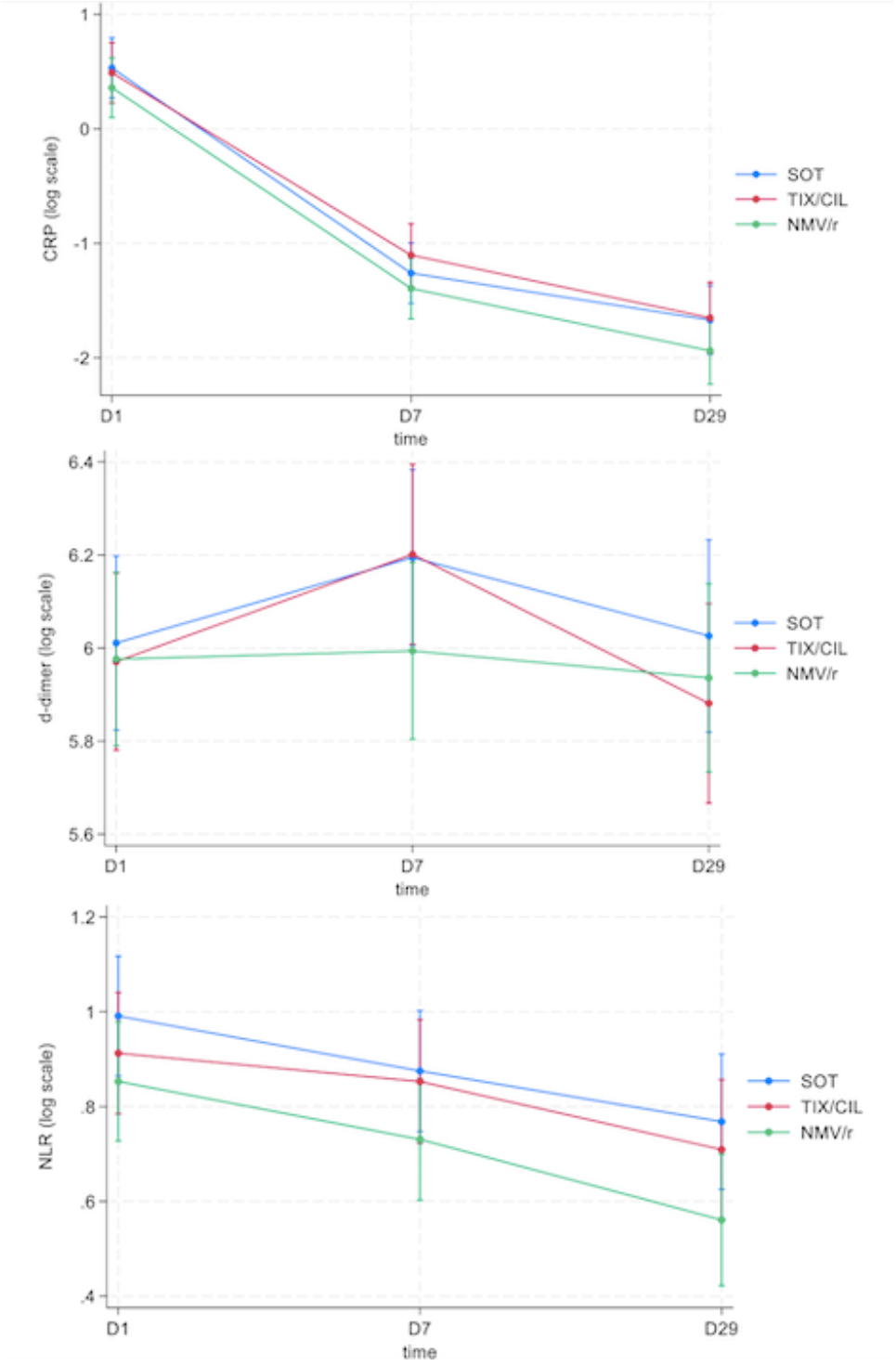

Table S1. Estimated means (95% confidence interval, CI) from fitting a mixed model of the inflammatory markers (CRP, C-reactive protein; d-dimer; NLR, neutrophils-to-lymphocytes ratio) change over D1-D29, by trial arm (SOT, sotrovimab; TIX/CIL, tixagevimab plus cilgavimab; NMV/r, nirmatrelvir plus ritonavir).

	Times Predicted means			
CRP (log scale)				
	D1 95% CI	**D7 95% CI**	**D29 95% CI**	**p-value***
Trial arm				0.605
SOT	0.51 (0.25, 0.76)	-1.30 (-1.55, -1.04)	-1.68 (-1.97, -1.38)	
TIX/CIL	0.46 (0.20, 0.72)	-1.13 (-1.39, -0.86)	-1.70 (-2.01, -1.39)	
NMV/r	0.38 (0.12, 0.64)	-1.40 (-1.66, -1.14)	-1.95 (-2.24, -1.66)	
*F-test type 3 interaction p-value				
d-dimer (log scale)				
	D1 95% CI	**D7 95% CI**	**D29 95% CI**	**p-value***
Trial arm				0.131
SOT	6.01 (5.83, 6.19)	6.19 (6.01, 6.37)	6.01 (5.81, 6.21)	
TIX/CIL	5.97 (5.78, 6.16)	6.21 (6.02, 6.39)	5.90 (5.69, 6.11)	
NMV/r	5.97 (5.79, 6.15)	5.99 (5.81, 6.18)	5.94 (5.74, 6.14)	
*F-test type 3 interaction p-value				
NLR (log scale)				
	D1 95% CI	**D7 95% CI**	**D29 95% CI**	**p-value***
Trial arm				0.932
SOT	0.96 (0.83, 1.09)	0.84 (0.72, 0.97)	0.68 (0.54, 0.83)	
TIX/CIL	0.92 (0.79, 1.05)	0.86 (0.73, 0.99)	0.67 (0.52, 0.82)	
NMV/r	0.85 (0.72, 0.98)	0.73 (0.60, 0.86)	0.56 (0.42, 0.71)	
*F-test type 3 interaction p-value				

Figure S6. Kinetics over D1-D29 of antibody level (serum anti-S IgG and anti-N IgG) by trial arm (SOT, sotrovimab; TIX/CIL, tixagevimab plus cilgavimab; NMV/r, nirmatrelvir plus ritonavir).

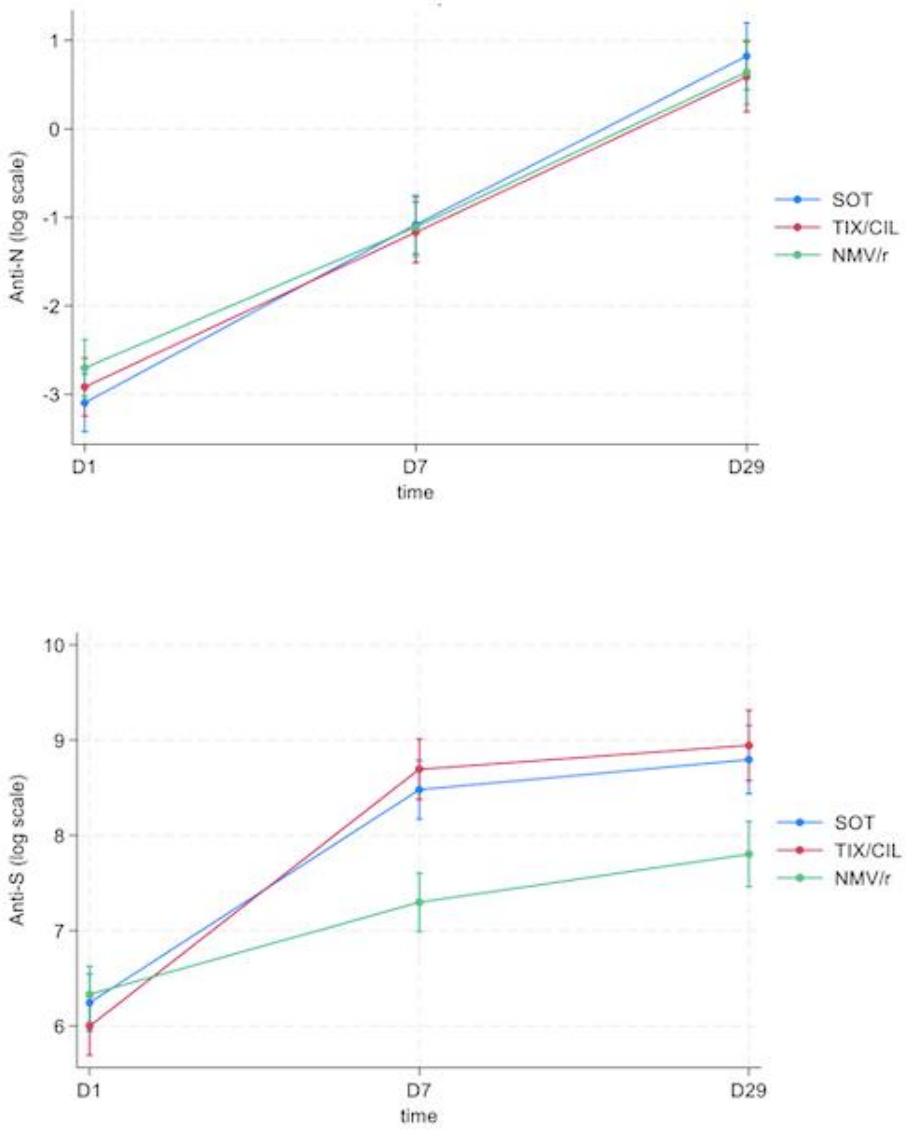

Table S2. Estimated means (95% confidence interval, CI) from fitting a mixed model of serum anti-S IgG level change over D1-D29, by trial arm (SOT, sotrovimab; TIX/CIL, tixagevimab plus cilgavimab; NMV/r, nirmatrelvir plus ritonavir).

	Times Predicted means			
Anti-S (log scale)				
	D1 **95% CI**	**D7** **95% CI**	**D29** **95% CI**	**p-value***
Trial arm				<0.001
SOT	6.27 (5.96, 6.58)	8.50 (8.19, 8.81)	8.75 (8.38, 9.12)	
TIX/CIL	5.92 (5.61, 6.23)	8.67 (8.35, 8.99)	8.93 (8.55, 9.31)	
NMV/r	6.38 (6.08, 6.68)	7.32 (7.01, 7.63)	7.84 (7.49, 8.19)	

*F-test type 3 interaction p-value