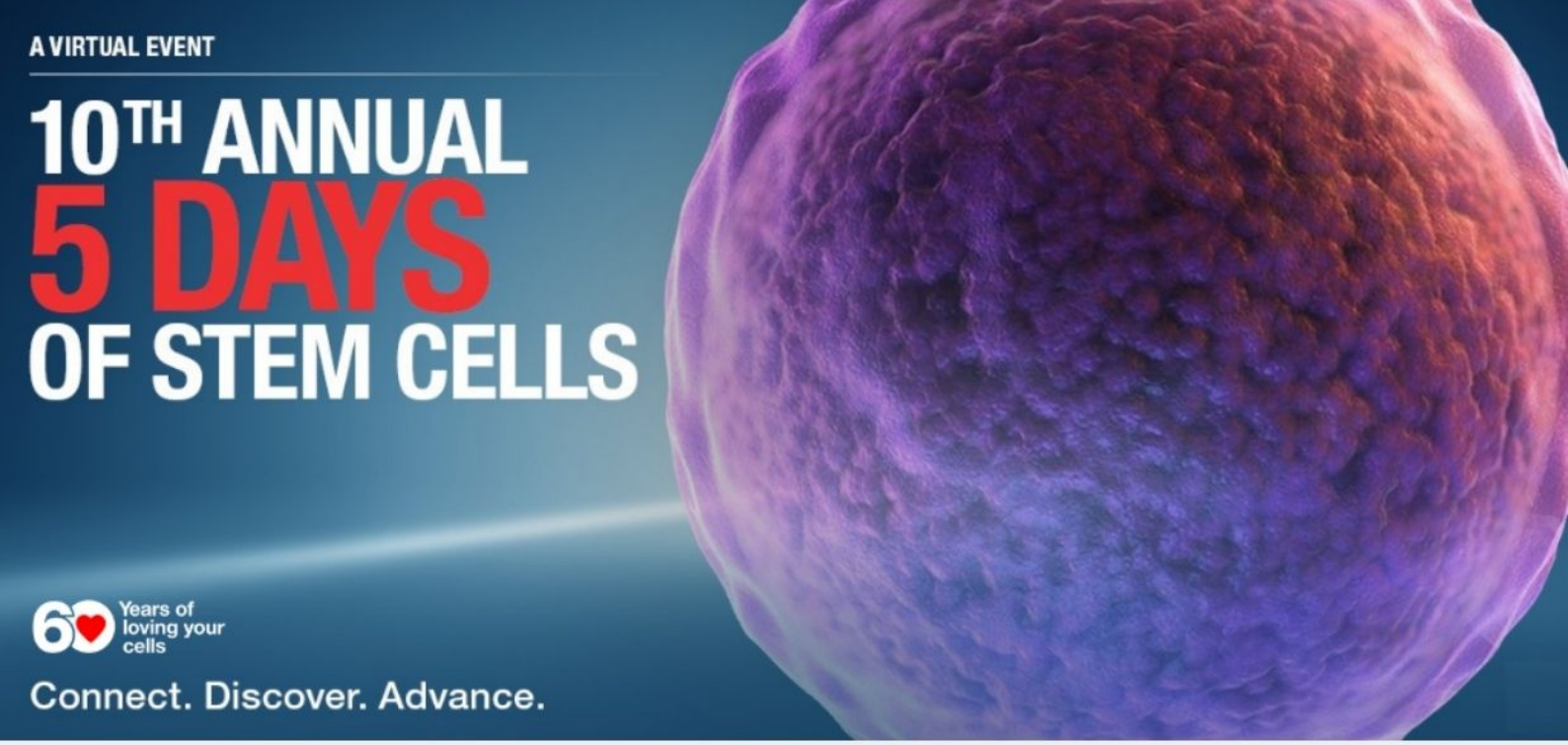

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SHORT COMMUNICATION

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Viral load decrease in SARS-CoV-2 BA.1 and BA.2 Omicron sublineages infection after treatment with monoclonal antibodies and direct antiviral agents

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Abstract

The efficacy on the Omicron variant of the approved early coronavirus disease-2019 (COVID-19) therapies, especially monoclonal antibodies, has been challenged by in vitro neutralization data, while data on in vivo antiviral activity are lacking. We assessed potential decrease from Day 1 to Day 7 viral load (VL) in nasopharyngeal swabs of outpatients receiving Sotrovimab, Molnupiravir, Remdesivir, or Nirmatrelvir/ritonavir for mild-to-moderate COVID-19 due to sublineages BA.1 or BA.2, and average treatment effect by weighted marginal linear regression models. A total of 521 patients (378 BA.1 [73%], 143 [27%] BA.2) received treatments (Sotrovimab 202, Molnupiravir 117, Nirmatrelvir/ritonavir 84, and Remdesivir 118): median age 66 years, 90% vaccinated, median time from symptoms onset 3 days. Day 1 mean VL was 4.12 log2 (4.16 for BA.1 and 4.01 for BA.2). The adjusted analysis showed that Nirmatrelvir/ritonavir significantly reduced VL compared to all the other drugs, except versus Molnupiravir in BA.2. Molnupiravir was superior to Remdesivir in both BA.1 and BA.2, and to Sotrovimab in BA.2. Sotrovimab had better activity than

Remdesivir only against BA.1. Nirmatrelvir/ritonavir showed the greatest antiviral activity against Omicron variant, comparable to Molnupiravir only in the BA.2 subgroup. VL decrease could be a valuable surrogate of drug activity in the context of the high prevalence of vaccinated people and low probability of hospital admission.

KEYWORDS
antiviral agents, BA.1, BA.2, monoclonal antibodies, Omicron variant, virological efficacy

1 | INTRODUCTION

As of the end of 2021, the Omicron (B.1.1.529) variant of severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and its sublineages BA.1 and BA.2, have become the predominant variants responsible for coronavirus disease-2019 (COVID-19) circulating worldwide.[1] The large number of critical mutations in Spike protein of these subvariants raised concerns about the efficacy of therapies for the early phase of COVID-19, particularly of monoclonal antibodies (mAbs).[2]

Previously published in vitro data showed that mAbs combination Bamlanivimab/Etesevimab and Casirivimab/Imdevimab showed little neutralizing activity against BA.1 and BA.2[3,4]; conversely, Sotrovimab retained most of the activity against omicron/BA.1, but was escaped by omicron/BA.2, with a 16–37-fold-reduction in neutralizing activity[5,6]; finally, Tixagevimab/Cilgavimab retained most of the activity against BA.2, but it was not as effective against BA.1.[7,8] Differently from mAbs, antiviral agents, such as Remdesivir, Molnupiravir, or Nirmatrelvir/ritonavir, which target the highly conserved protein of SARS-CoV-2, consistently retained in vitro activity against both BA.1 and BA.2 sublineages.[9–11]

Analyses of in vivo data evaluating the clinical efficacy of these agents against the new variant are lacking. Primary endpoint in phase-3 randomized studies[12–17] in COVID-19 was typically the proportion of participants hospitalized or dead after randomization. Due to the lower risk of severe outcomes following SARS-CoV-2 Omicron infection,[18] and considering the high prevalence of vaccinated people[19] during the Omicron[20] wave, a clinical outcome is not suited to the current scenario. Viral load (VL) reduction from baseline through Day 7 was used as the endpoint of phase-2 studies of mAbs and may be a valuable surrogate marker of in vivo neutralizing or antiviral activity.[21,22]

We assessed the in vivo VL reduction in nasopharyngeal swab (NPS) collected on Day 1 and Day 7 from outpatients treated with Sotrovimab, Molnupiravir, Remdesivir, or Nirmatrelvir/ritonavir for mild-to-moderate COVID-19 due to sublineages BA.1 or BA.2.

2 | METHODS

This analysis uses the data of an observational study on the effectiveness of early treatment for outpatients with mild-to-moderate COVID-19. The study was approved by the Scientific Committee of the Italian Medicine Agency (AIFA) and by the Ethical Committee of the Lazzaro Spallanzani Institute, as National Review Board for the COVID-19 pandemic in Italy (approval number 380/2021).

All consecutive patients presenting from the 21st of December 2021 to the 15th of March 2022 to the National Institute for Infectious Diseases "L. Spallanzani" with a confirmed SARS-CoV-2 Omicron (BA.1 or BA.2) diagnosis and a mild-to-moderate COVID-19, who met AIFA criteria for eligibility for early treatment by mAbs or antiviral agents were enrolled. Treatment allocation was subject to drug availability, time from symptoms onset, and presence of comorbidities as defined by AIFA criteria.

Outpatients visits, with a medical evaluation, vital signs recording, and laboratory tests, were scheduled at baseline (day of treatment, Day 1) and after 7 days (Day 7). Patients were followed-up for the occurrence of clinical events through Day 30 after starting treatment through a telephone visit.

SARS-CoV-2 load in NPS was assessed using Abbott Alinity m RealTime System (Abbott Laboratories) on Day 1 and Day 7, and expressed as log2 of cycle threshold (CT) values.[23] Identification of SARS-CoV-2 variants was performed by Sanger sequencing of the Spike coding gene on samples collected on Day 1 using the ABI 3500 analyzer (Applied Biosystem).[24] SARS-COV-2 serology was performed by two chemiluminescence microparticle assays (CMIA) detecting antiNucleoprotein and anti-Spike/RBD Immunoglobulins G (IgG) (ARCHITECT SARS-CoV-2 IgG, and ARCHITECT SARS-CoV-2 IgG II Quantitative; Abbott Laboratories, respectively).[25,26] According to the manufacturer's instructions, for the two CMIA, Index > 1.4 and Binding Antibody Units (BAU)/ml ≥ 7.1 are considered positive for anti-N and anti-Spike/RBD IgG, respectively.

Primary endpoint was log2 VL variation from Day 1 to Day 7. We adopted the log transformation because the distribution of the VL change in the raw scale was positively skewed and significantly deviating from the normal distribution. Secondary endpoints were the proportion of negative NPS at Day 7 and the proportion of patients who experienced COVID-related clinical failure, defined as hospitalization due to development of severe COVID-19 or death from any cause over Days 0–30.

Because of the observed large between-patients variability in Day 1 value, we have also performed a sensitivity analysis using the percentage variation at Day 7 as an alternative endpoint. This was calculated as the difference between the value at Day 7 minus the

value at Day 1 divided by the value at Day 1 (all values in the log2 scale).

Main characteristics of the participants, assessed on Day 1, were compared by treatment strategy using χ^2 (categorical variables) and Kruskal–Wallis (continuous variable) tests. We estimated potential outcomes and the average treatment effect (ATE) of treatment on VL change on Day 7. Because we had 4 drugs to compare this led to 6 possible 2-by-2 comparisons in separate parallel trials. We controlled for confounding by modeling the treatment assignment (via inverse probability of weighting) or the outcome (via regression adjustment) or both (doubly robust methods). The latter provides unbiased estimates for the treatment effect even if one of the models is misspecified. According to our assumptions, we identified the following key confounding factors: calendar month of infusion, immunodeficiency at time of infusion, and duration of symptoms. All analyses were controlled for these factors.

Proportion of participants who experienced the secondary endpoints was shown by treatment group and compared using a χ^2 test. All analyses were stratified by type of Omicron variant detected (BA.1 vs. BA.2).

3 | RESULTS

Of 568 participants enrolled, 521 had a VL measured at Day 7: 202 received Sotrovimab, 117 Molnupiravir, 84 Nirmatrelvir/ritonavir, and 118 Remdesivir. Overall, 250 (48%) were female, 469 (90%) were vaccinated and 81 (15%) had negative baseline serology. Median age was 66 years (interquartile range 55–76) and median time from symptoms onset to Day 1 was 3 days (2–4). BA.1 and BA.2 were detected in 378 (73%) and 143 (27%), respectively. A higher proportion of chronic respiratory disease (χ^2, $p < 0.001$), liver disease ($p < 0.001$), and immunodeficiency ($p = 0.01$) was observed on Day 1 among participants receiving Sotrovimab. The baseline mean VL was 4.12 (standatd deviation; [SD] 0.27) $\log_2$ CT (4.16 for BA.1 and 4.01 for BA.2). Detailed characteristics according to treatment groups are reported in Table 1. Linear regression analysis calculating the ATE of therapies when compared to each other in separately emulated parallel trials showed that Nirmatrelvir/ritonavir significantly reduced VL compared to other drugs both in the BA.1 and BA.2 subgroups. In contrast, there was no difference in activity between Molnupiravir and Nirmatrelvir/ritonavir against BA.2.

No evidence for a difference was also found against BA.1 between Sotrovimab and Molnupiravir.

Sotrovimab had better activity than Remdesivir only against BA.1 (Figures 1A,B).

Detailed results of potential decrease in VL and ATE for all possible 2-by-2 treatment comparisons separately for BA.1 and BA.2 are also shown in Supporting Information: Tables 1 and 2.

All variations of SARS-CoV-2 RNA levels from Day 1 to Day 7 according to treatment groups are reported in Supporting Information: Figure 1.

Results were similar when we used the alternative endpoint of percentage variation at Day 7 (Supporting Information: Table 3).

Proportion of participants with CT ≤ 40 at Day 7 was 6·7% (35/521, 31 infected with BA.1 and 4 with BA.2). See details in Table 2.

COVID-19-related hospitalization or death from any cause through Day 30 was assessed in 568 patients: 9 patients (7/226 [3.1%] Sotrovimab [5 BA.1] and 2/87 [2.3%] Nirmatrelvir/ritonavir [2 BA.1]) experienced clinical failure.

4 | DISCUSSION

Our study showed that considering the reduction of VL as a marker of antiviral activity in vivo, Nirmatrelvir/ritonavir had the strongest activity in all face-to-face treatment comparisons in patients infected with BA.1 and BA.2, with the only exception of no evidence for a difference versus Molnupiravir for BA.2 infected. Molnupiravir had better activity against Remdesivir in both BA.1 and BA.2, comparable activity against BA.1, and better activity in BA.2 than Sotrovimab. Sotrovimab had better activity than Remdesivir against BA.1 but there was no significant difference between Sotrovimab and Remdesivir for BA.2.

We evaluated the decrease in VL in the NPS as a surrogate for drug activity that could reflect the clinical response to treatment. Due to the low rate of hospitalization and death in persons infected with Omicron variants, it has become increasingly difficult to design clinical studies with adequate statistical power. Therefore, in the absence of clinical events, the change in VL could be a candidate surrogate endpoint for clinical response.

More studies are needed to test whether early VL decrease is a strong and consistent surrogate or whether it might be subject to what is known as the "surrogate paradox."[27,28]

Anyway, our results showed concordance of VL decrease from Day 1 to Day 7 with known data on early COVID-19 therapies and reflected previously in vitro published data: the virologic efficacy of Nirmatrelvir/ritonavir was the counterpart to the high clinical efficacy demonstrated in the registrative trials[15] and real-life data.[29] The lower change in VL in patients with BA.2 compared with BA.1 during Sotrovimab therapy was also in agreement with the lower neutralizing activity observed in vitro for this monoclonal antibody.[9] Likewise, the poor activity on VL reduction of Remdesivir with both BA.1 and BA.2 subvariants agreed with the data from the Pinetree study.[16] Molnupiravir activity toward both variants, with a better profile on BA.2 also seemed to agree with recent in vitro data.[10]

The main limitations of our analysis are the observational nature of the study and the lack of a randomized design, which does not allow to rule out confounding bias. These limitations are partially mitigated by the use of weighted marginal linear regression models and appropriate control of measured confounding factors. Our results are however important as, to the best of our knowledge, this is the first analysis to evaluate the in vivo efficacy of currently available treatments against the Omicron BA.1 and BA.2 variants.

TABLE 1 Main characteristics at enrollment by intervention

Characteristics	Regimen started					
	Sotrovimab N = 202	Molnupiravir N = 117	Remdesivir N = 118	Nirmatrelvir/ritonavir N = 84	*p* Value[a]	Total N = 521
Gender, *n* (%)					0.454	
Female	105 (52.0%)	52 (44.4%)	52 (44.1%)	41 (48.8%)		250 (48.0%)
Age, years					0.092	
Median (IQR)	63 (52–75)	68 (57–75)	70 (57–78)	63 (55–76)		66 (55–76)
Older than 65, *n* (%)	93 (46.0%)	65 (55.6%)	71 (60.2%)	36 (42.9%)	0.028	265 (50.9%)
Days from sypmtoms onset to MAbs infusion					0.003	
Median (IQR)	3 (2–5)	3 (2–4)	4 (2–5)	3 (2–4)		3 (2–4)
Comorbidities/risk factors, *n* (%)						
Diabetes	28 (13.9%)	24 (20.5%)	23 (19.5%)	19 (22.6%)	0.239	94 (18.0%)
Obesity (BMI > 30)	93 (46.0%)	64 (54.7%)	73 (61.9%)	53 (63.1%)	0.012	283 (54.3%)
CVD	31 (15.3%)	27 (23.1%)	24 (20.3%)	17 (20.2%)	0.357	99 (19.0%)
Chronic respiratory disease	22 (10.9%)	2 (1.7%)	1 (0.8%)	1 (1.2%)	<0.001	26 (5.0%)
Renal impairment	7 (3.5%)	1 (0.9%)	2 (1.7%)	0 (0.0%)	0.174	10 (1.9%)
Hepatic disease	61 (30.2%)	20 (17.1%)	21 (17.8%)	9 (10.7%)	<0.001	111 (21.3%)
Cancer	32 (15.8%)	15 (12.8%)	16 (13.6%)	17 (20.2%)	0.485	80 (15.4%)
Primary/secondary immunodeficiency	52 (25.7%)	17 (14.5%)	18 (15.3%)	10 (11.9%)	0.010	97 (18.6%)
Neurologic disease	16 (7.9%)	6 (5.1%)	3 (2.5%)	4 (4.8%)	0.229	29 (5.6%)
Vital signs at baseline						
SpO_2, median (IQR)	98 (97–99)	98 (97–99)	98 (97–98)	98 (97–99)	0.173	98 (97–99)
Fever (>37.5°C), *n* (%)	8 (4.2%)	0 (0.0%)	2 (1.8%)	0 (0.0%)	0.036	10 (2.0%)
BMI, median (IQR)	24.65 (22.20–28.57)	25.26 (23.23–29.40)	26.19 (23.23–29.02)	26.06 (23.44–29.48)	0.093	25.31 (22.99–29.05)
Laboratory values, median (IQR)						
Ferritin, ng/ml	99.00 (39.00–181.0)	89.00 (48.00–173.0)	84.00 (44.00–155.0)	94.00 (55.00–145.0)	0.885	91.00 (47.00–171.0)
C-reactive protein, mg/dl	0.95 (0.32–2.48)	0.93 (0.29–1.91)	0.88 (0.34–1.67)	0.99 (0.37–1.98)	0.793	0.93 (0.32–2.09)
Lymphocytes,/uL	1270 (870.0–1670)	1460 (1145–2045)	1470 (1100–1790)	1500 (1120–1940)	<0.001	1400 (1010–1840)

TABLE 1 (Continued)

Characteristics	Regimen started Sotrovimab *N* = 202	Molnupiravir *N* = 117	Remdesivir *N* = 118	Nirmatrelvir/ritonavir *N* = 84	*p* Value[a]	Total *N* = 521
Baseline SARS-COV-2						
Serology, *n* (%)					0.006	
Anti-N positive	4 (2.0%)	3 (2.6%)	3 (2.5%)	3 (3.6%)		13 (2.5%)
Anti-S positive	138 (68.3%)	90 (76.9%)	97 (82.2%)	72 (85.7%)		397 (76.2%)
Negative	43 (21.3%)	16 (13.7%)	15 (12.7%)	7 (8.3%)		81 (15.5%)
Unknown	17 (8.4%)	8 (6.8%)	3 (2.5%)	2 (2.4%)		30 (5.8%)
Vaccination, *n* (%)					0.185	
Yes (partly or fully)	182 (91.0%)	108 (93.1%)	101 (85.6%)	78 (92.9%)		469 (90.5%)
Vaccine type, *n* (%)					0.463	
BNT162b2	87 (73.7%)	50 (75.8%)	38 (74.5%)	39 (75.0%)		214 (74.6%)
mRNA-1273	22 (18.6%)	10 (15.2%)	10 (19.6%)	6 (11.5%)		48 (16.7%)
ChAdOx1	9 (7.6%)	6 (9.1%)	3 (5.9%)	7 (13.5%)		25 (8.7%)
Ad26.COV2.S	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		0 (0.0%)
Other/unknown	64 (35.2%)	42 (38.9%)	50 (49.5%)	26 (33.3%)		182 (38.8%)
SARS-COV-2 variant, *n* (%)						
Omicron BA1	146 (72.3%)	99 (84.6%)	84 (71.2%)	49 (58.3%)	<0.001	378 (72.6%)
Omicron BA2	56 (27.7%)	18 (15.4%)	34 (28.8%)	35 (41.7%)	<0.001	143 (27.4%)
Baseline CT						
Mean (SD)	17.57 ± 3.39	17.75 ± 3.72	17.99 ± 3.31	17.23 ± 3.49	0.283	17.65 ± 3.46
log2 scale Mean (SD)	4.11 ± 0.27	4.12 ± 0.28	4.15 ± 0.26	4.08 ± 0.28	0.283	4.12 ± 0.27
Less than 25, *n* (%)	195 (96.5%)	114 (97.4%)	113 (95.8%)	81 (96.4%)	0.919	503 (96.5%)
MASS score, median (IQR)	3 (0–5)	3 (2–5)	3 (2–5)	3 (1–4)	0.444	3 (1–5)
Baseline symptoms score, median (IQR)	11 (6–14)	11 (7–16)	8 (4–15)	12 (8–16)	0.172	10 (6–15)

Abbreviations: BMI, body mass index; IQR, interquartile range; MASS score, Monoclonal Antibody Screening Score; SD, standatd deviation; SpO_2, peripheral oxygen saturation.

[a]Chi-square or Kruskal–Wallis test as appropriate.

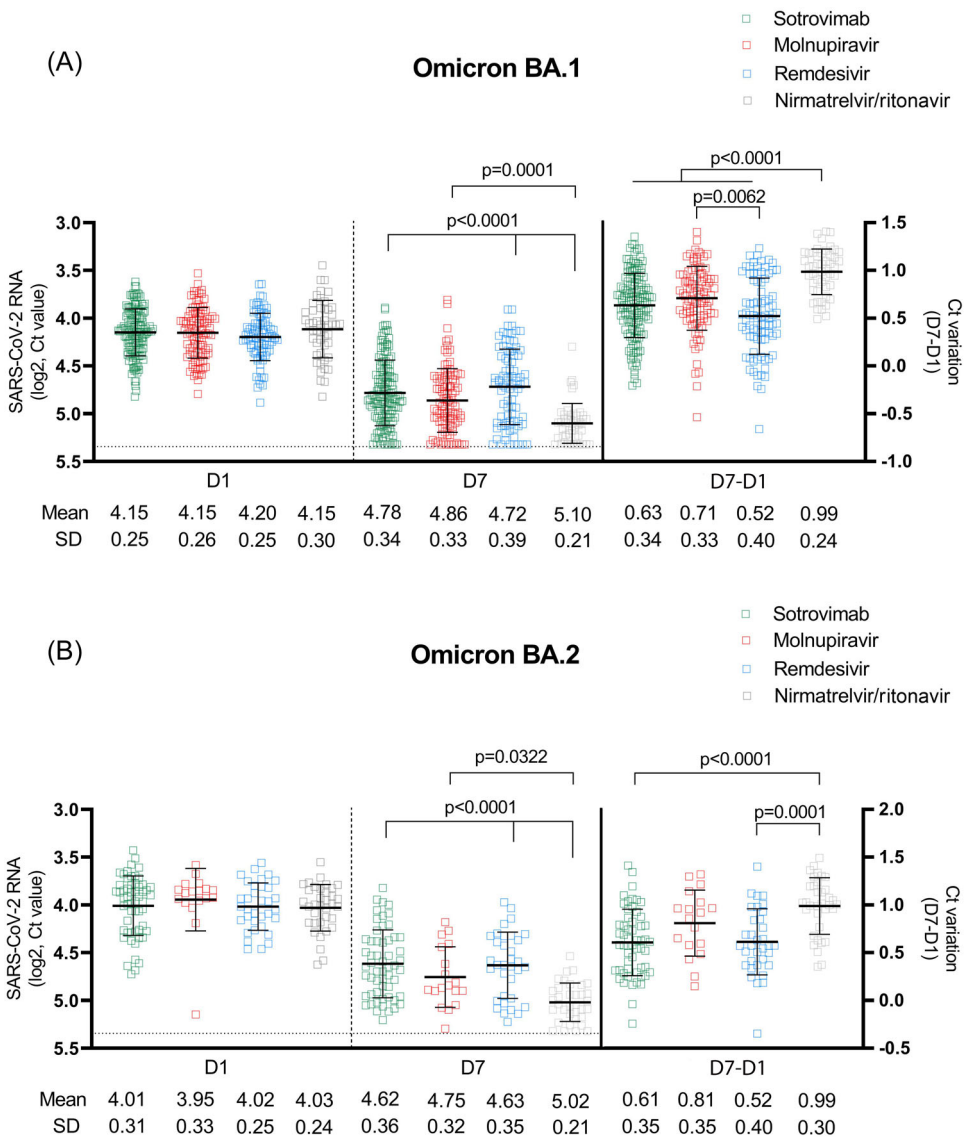

FIGURE 1 SARS-CoV-2 RNA levels at D1 and D7 in patients treated with Sotrovimab, Molnupiravir, Remdesivir, and Nirmatrelvir/ritonavir. Dot-plots showing the comparison of viral loads detected at D1 and D7 and the variation of RNA levels observed between the two time-points by intervention in (A) patients with Omicron BA.1 infection treated with Sotrovimab (*n* = 146), or Molnupiravir (*n* = 99), or Remdesivir (*n* = 84), or Nirmatrelvir/r (*n* = 49); (B) patients with Omicron BA.2 infection treated with Sotrovimab (*n* = 56), or Molnupiravir (*n* = 18), or Remdesivir (*n* = 34), or Nirmatrelvir/r (*n* = 35). Viral RNA levels are expressed as log2 CT values. Mean of log2 CT values and SD are shown. Statistical analysis of the comparisons between treatment groups was performed by Kruskal-Wallis test, adjusted with Dunn's multiple comparisons test. Horizontal dashed line represents the limit of detection (CT: 40.0), values ≥40 are considered negative

Even if the evolution of Sars-Cov-2 variants is faster than the generation of data on drug efficacy and the current epidemiological scenario is dominated by new sublineages, data such as ours can still contribute to the classification of the disease, especially in light of the direct correlation with BA.2 of some sublineages (e.g., BA.2.75[30] in India) and the resulting similar susceptibility. Furthermore, we do not know whether future variants will reoccur with similar mutations to previous ones (as has already happened, e.g., with the reappearance in BA.4 and BA.5 of the mutation at position 425, already seen in the Delta variant).

In conclusion, according to our VL change dynamic model and assumptions, in outpatients with mild-to-moderate COVID-19, Nirmatrelvir/ritonavir appears to be the option with the strongest in vivo antiviral activity against the Omicron variant among all other treatment options examined. Only for Molnupiravir and limited to the BA.2 sublineage, the antiviral effect appeared to be comparable to that observed with Nirmatrelvir/ritonavir. Because of the low incidence of hospital admissions in the Omicron era, the emulation of trials with surrogate endpoints such as in vivo neutralizing activity can provide useful information for treatment decisions of early COVID-19.

Dot-plots showing the comparison of VLs detected at D1 and D7 and the variation of RNA levels observed between the two time-points by intervention in (A) patients with Omicron BA.1 infection

TABLE 2 Proportion of participants with undetectable Sars-CoV-2 in nasopharyngeal swab collected at Day 7 (defined as cycle threshold, CT ≤ 40) by intervention

	Regimen started					
	Sotrovimab *n* (%)	Molnupiravir *n* (%)	Remdesivir *n* (%)	Nirmatrelvir/ritonavir *n* (%)	Total *n* (%)	*p* Value[a]
Omicron (BA.1 + BA.2)						
Day 7 CT > 40	8 (3.96)	9 (7.69)	7 (5.93)	11 (13.10)	35 (6.72)	0.0001
Day 7 CT ≤ 40	194 (96.04)	108 (92.31)	111 (94.07)	73 (86.90)	486 (93.28)	
Omicron BA.1						
Day 7 CT > 40	8 (5.48)	9 (9.09)	7 (8.33)	7 (14.29)	31 (8.20)	0.0010
Day 7 CT ≤ 40	138 (94.52)	90 (90.91)	77 (91.67)	42 (85.71)	347 (91.80)	
Omicron BA.2						
Day 7 CT > 40	0 (0)	0 (0)	0 (0)	4 (11.43)	4 (2.80)	0.0031
Day 7 CT ≤ 40	56 (100)	18 (100)	34 (100)	31 (88.57)	139 (97.20)	

[a]Fisher's exact test.

treated with Sotrovimab (*n* = 146), or Molnupiravir (*n* = 99), or Remdesivir (*n* = 84), or Nirmatrelvir/ritonavir (*n* = 49); (B) patients with Omicron BA.2 infection treated with Sotrovimab (*n* = 56), or Molnupiravir (*n* = 18), or Remdesivir (*n* = 34), or Nirmatrelvir/ritonavir (*n* = 35). Viral RNA levels are expressed as log2 CT values. Mean of log2 CT values and SD are shown. Statistical analysis of the comparisons between treatment groups was performed by Kruskal–Wallis test, adjusted with Dunn's multiple comparisons test. Horizontal dashed line represents the limit of detection (CT: 40.0), values ≥ 40 are considered negative.

5 | INMI COVID-19 Outpatient Treatment Study Group

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AUTHOR CONTRIBUTIONS

Andrea Antinori and Valentina Mazzotta conceptualized and designed the study. Valentina Mazzotta, Francesca Colavita, and Alessandro Cozzi Lepri wrote the first draft of the manuscript and referred to appropriate literature. Alessandro Cozzi Lepri was also the main responsible person for formal data analysis. Andrea Antinori, Alessandro Cozzi Lepri, Valentina Mazzotta, Francesca Colavita, Fabrizio Maggi, and Emanuele Nicastri conceived, supervised the study and contributed to data interpretation. Jessica Paulicelli, Claudia Cimaglia, and Pierluca Piselli were responsible for data collection and curation. Francesco Vaia, Enrico Girardi, Pierluca Piselli, AnnaRosa Garbuglia, Fabrizio Maggi, Ilaria Mastrorosa, Alessandra Vergori revised the manuscript content, reviewed and edited the manuscript. Francesca Colavita, Lavinia Fabeni, Eleonora Lalle, AnnaRosa Garbuglia, Francesca Colavita, and Fabrizio Maggi performed all virological test. Valentina Mazzotta, Ilaria Mastrorosa, Alessandra Vergori, Serena Vita, Emanuela Caraffa, Eugenia Milozzi, Raffaella Libertone, Gaetano Maffongelli, and Silvia Rosati enrolled participants. All authors agreed with and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

A. A. declares consultancy fees from Gilead Sciences, Merck, GSK, Pfizer, Astra Zeneca, and research institutional grants from Gilead Sciences and the Italian Medicine Agency (AIFA).

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DATA AVAILABILITY STATEMENT

Anonymized participant data will be made available upon reasonable requests directed to the corresponding author. Proposals will be reviewed and approved by investigator, and collaborators on the basis of scientific merit. After approval of a proposal, data can be shared through a secure online platform after signing a data access agreement.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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