# Remdesivir and Obeldesivir Retain Potent Activity Against SARS-CoV-2 Omicron Variants

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### Conclusions

- Permension of the Remdesivir and obeldesivir retained potent in vitro antiviral activity against the SARS-CoV-2 Omicron subvariants BA.2.86, EG.1.2, EG.5.1, EG.5.1.4, HK.3, HV.1, JN.1, JN.1.7, JN.1.18, KP.2, KP.3, LB.1, XBB.1.5.72, XBB.2.3.2, and XBC.1.6, with potencies comparable to those of reference strains
- Nsp12 polymorphisms observed in Omicron subvariants remained susceptible to remdesivir and obeldesivir

## Plain Language Summary

- Remdesivir and obeldesivir are antiviral drugs that inhibit SARS-CoV-2, the virus that causes COVID-19
- Both remdesivir and obeldesivir have previously shown antiviral activity in laboratory experiments against different Omicron subvariants of SARS-CoV-2<sup>1,2</sup>
- Here, laboratory analyses showed that remdesivir and obeldesivir were effective against recent SARS-CoV-2 subvariants

#### Introduction

- New variants of SARS-CoV-2 continue to emerge, including variants of concern (VOCs), variants of interest (VOIs), and variants under monitoring (VUMs), with the potential to cause new waves of infection with increased circulation, enhanced antibody evasion, and reduced effectiveness of existing vaccines and antiviral therapeutics<sup>3-5</sup>
- As of August 2024, Omicron and its subvariants account for all VOCs, VOIs, and VUMs currently circulating worldwide<sup>5,6</sup>
- Remdesivir (RDV) is an intravenous prodrug approved to treat COVID-19 in adult and pediatric patients that is metabolized into an active nucleoside triphosphate (NTP) capable of targeting Nsp12, a highly conserved viral RNA-dependent RNA polymerase of SARS-CoV-2<sup>7</sup>
- Obeldesivir (ODV) is an oral mono-5'-isobutyryl ester prodrug that is metabolized into the same active NTP as RDV<sup>8,9</sup>
- The antiviral activity of RDV and ODV against previous Omicron subvariants (BA.1 through XBB.1.9.1) was maintained with respect to the ancestral WA1 strain<sup>1,2</sup>

## Objectives

- To characterize the in vitro antiviral activity of RDV and ODV against recent SARS-CoV-2 Omicron subvariants using clinical isolates and site-directed mutants (SDMs) in a replicon system bearing observed Nsp12 polymorphisms
- To conduct a structural analysis of Nsp12 polymorphisms observed in recent Omicron subvariants with respect to RDV and ODV susceptibility

#### Methods

- Omicron subvariant sequences from the Global Initiative on Sharing All Influenza Data (GISAID) EpiCoV<sup>™</sup> database were analyzed to identify the prevalence of Nsp12 polymorphisms
- Structural analysis of identified polymorphisms was performed using a previously developed cryo-electron microscopy—based model of the replication-transcription complex<sup>10</sup>
- The antiviral activity of RDV and ODV against Omicron subvariants was assessed in clinical isolates (Table 1) using nucleoprotein enzyme-linked immunosorbent assay (ELISA) in A549-hACE2-TMPRSS2 cells; for Omicron subvariants that did not have available clinical isolates (JN.1.7, JN.1.18, KP.2, KP.3, and LB.1), a replicon system was used

Lineage-defining

 The antiviral activity of RDV and ODV against identified Nsp12 polymorphisms was also assessed using SDMs in a replicon system

#### Table 1. SARS-CoV-2 Clinical Isolates

		Substitutions in the	
Variant	Isolate	Replication Complex <sup>a</sup>	Source
WA1 (lineage A)	hCoV-19/USA-	—	BEI Resources Cat# NR- 52281
BA.2.86	hCoV-19/USA/MI-UM- 10052670540/2023	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	BEI Resources Cat# NR- 59638
EG.1.2	hCoV-19/USA/ MD-HP46933- PIDKWGVVMR/2023	Nsp9 T35I Nsp12 P323L G671S Nsp13 S36P R392C Nsp14 I42V	JHU, The Pekosz Lab
EG.5.1	hCoV-19/USA/MD- HP47946	Nsp9 T35I Nsp12 P323L G671S Nsp13 S36P R392C Nsp14 I42V	JHU, The Pekosz Lab
EG.5.1.4	hCoV-19/USA/CA- GS138000/2024	Nsp9 T35I Nsp12 P323L G671S Nsp13 S36P R392C Nsp14 I42V	Gilead Sciences, Inc.
HK.3	hCoV-19/USA/ MD-HP49292- PIDWKNSGGH/2023	Nsp9 T35I Nsp12 D63N P323L G671S Nsp13 S36P R392C Nsp14 I42V	JHU, The Pekosz Lab
HV.1	hCoV-19/USA/CA- GS137943/2024	Nsp9 T35I Nsp12 P323L G671S Nsp13 R392C Nsp14 I42V V182I	Gilead Sciences, Inc.
JN.1	hCoV-19/USA/New York/PV96109/2023	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	BEI Resources Cat# NR- 59693
XBB.1.5.72	hCoV-19/USA/CA- GS136867/2024	Nsp12 P323L G671S Nsp13 S36P R392C Nsp14 I42V	Gilead Sciences, Inc.
XBB.2.3.2	hCoV-19/Japan/ TY42-006/2023	Nsp12 P323L G671S Nsp13 R392C Nsp14 I42V	NIID, Japan
XBC.1.6	hCoV-19/Japan/ TY42-004/2023	Nsp12 P323L G671S G823insD Nsp13 P77L T127I I33V	NIID, Japan

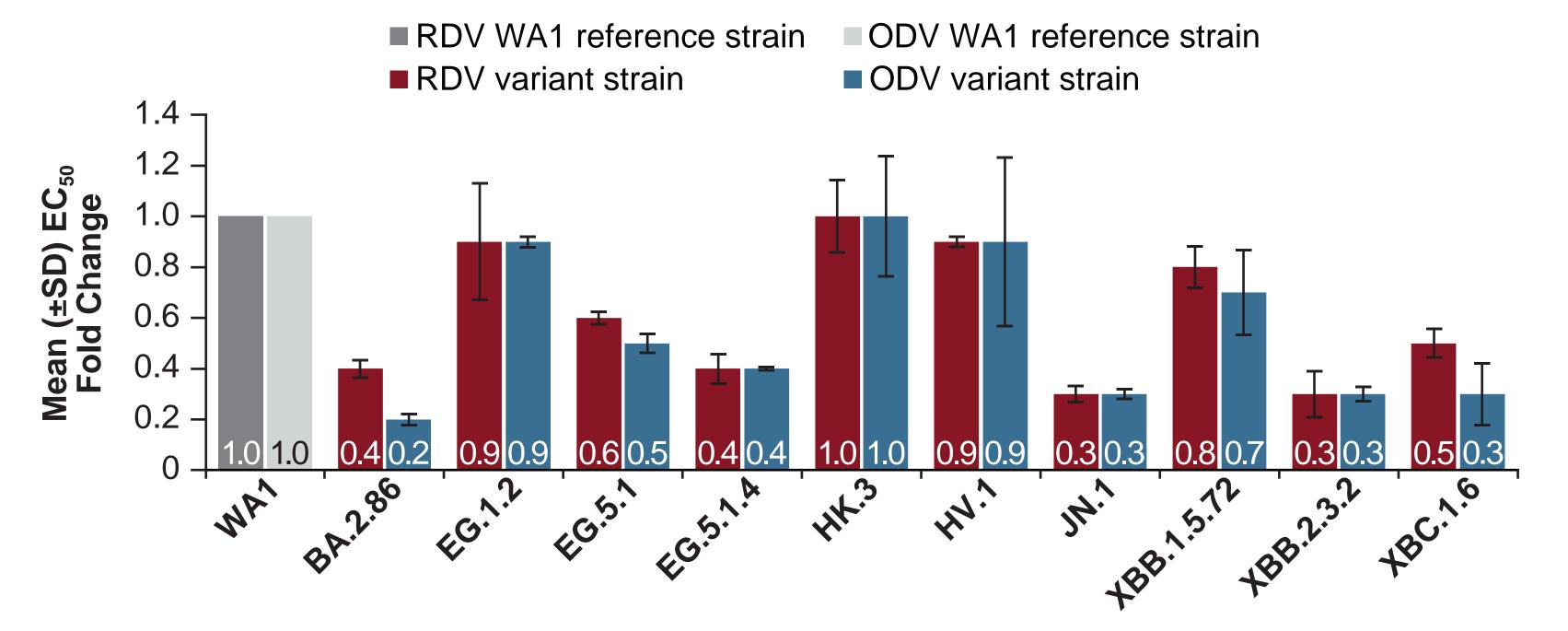
BEI Resources, Biodefense and Emerging Infections Research Resources Repository; JHU, Johns Hopkins University; NIID, National Institute of Infectious Diseases.

#### Results

RDV and ODV Retain Potent Antiviral Activity Against Clinical Isolates of Omicron Subvariants

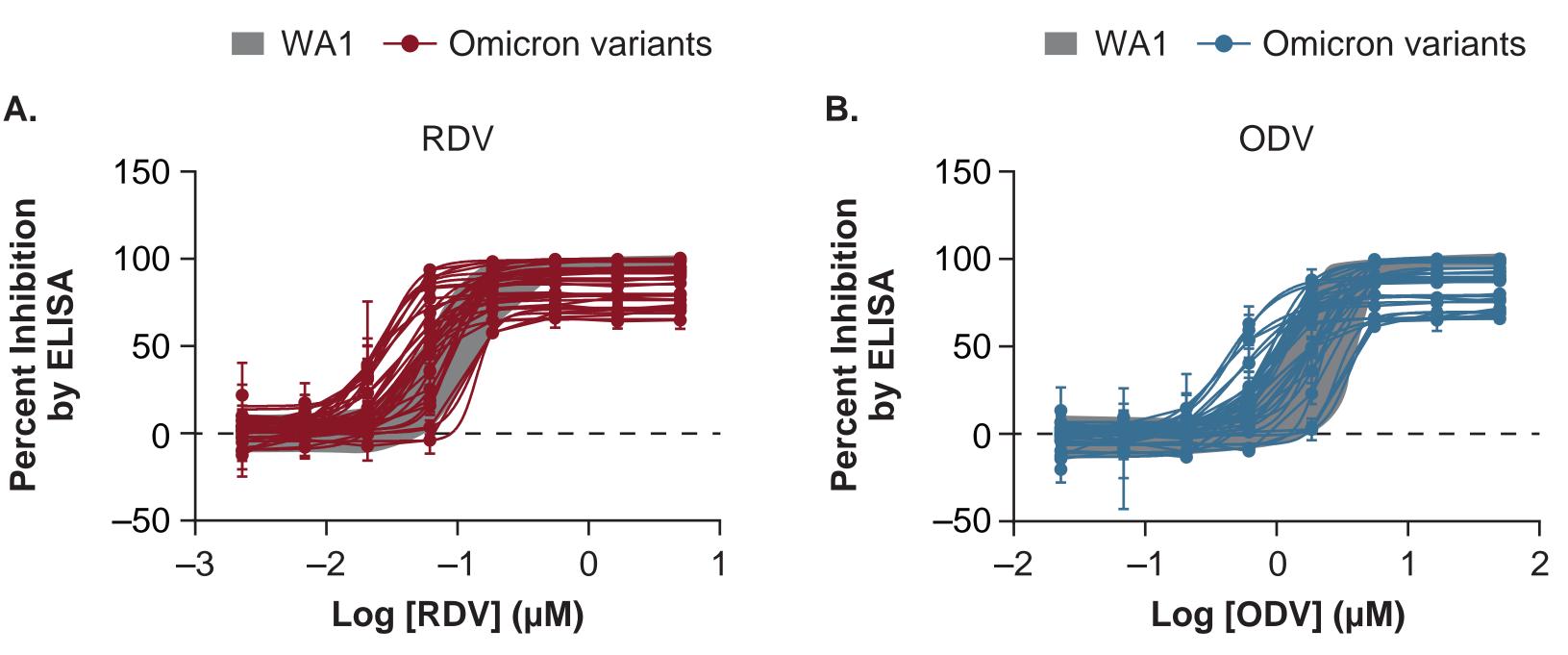
- RDV and ODV maintained activity against Omicron subvariants compared with the WA1 reference strain
- Mean RDV half-maximal effective concentration (EC<sub>50</sub>) values for all tested Omicron subvariants ranged from 21.8 to 135.5 nM, with a 0.3-fold (JN.1 and XBB.2.3.2) to 1.0-fold (HK.3) change compared with the reference ancestral strain, WA1 (EC<sub>50</sub> range, 57.8-154.0 nM; mean EC<sub>50</sub>, 91.7 nM; Figures 1 and 2; Table 2)
- Mean ODV EC<sub>50</sub> values for all tested Omicron subvariants ranged from 438.2 to 3192.5 nM, with a 0.2-fold (BA.2.86) to 1.0-fold (HK.3) change compared with WA1 (EC<sub>50</sub> range, 1240.0-4125.0 nM; mean EC<sub>50</sub>, 2310.5 nM; Figures 1 and 2; Table 2)

Figure 1. RDV and ODV EC<sub>50</sub> Fold Changes<sup>a</sup> From WA1 Reference Strain Against Clinical Isolates of SARS-CoV-2 Omicron Subvariants



<sup>a</sup>Fold change was calculated for each experiment, and a mean fold change was calculated with these values EC<sub>50</sub>, half-maximal effective concentration; ODV, obeldesivir; RDV, remdesivir.

Figure 2. RDV (A) and ODV (B) Retain Potency Against the Omicron Subvariants BA.2.86, EG.1.2, EG.5.1, EG.5.1.4, HK.3, HV.1, JN.1, XBB.1.5.72, XBB.2.3.2, and XBC.1.6 When Compared With the WA1 Reference Strain



ach variant was tested 2 times with technical triplicates. The range of RDV or ODV activity against the WA1 reference strain (16 or 18 replicates, respectively) is shaded in gray.

LISA, enzyme-linked immunosorbent assay; ODV, obeldesivir; RDV, remdesivir.

Table 2. RDV and ODV EC<sub>50</sub> Values<sup>a</sup> Against Clinical Isolates of SARS-CoV-2 Omicron Subvariants

Variant Lineage (Clinical Isolate)	RDV EC <sub>50</sub> (nM)	ODV EC <sub>50</sub> (nM)
WA1 (lineage A)	Range: 57.8-154.0	Range: 1240.0-4125.0
BA.2.86	26.0	438.2
EG.1.2	59.9	1090.0
EG.5.1	44.4	905.0
EG.5.1.4	44.3	1473.0
HK.3	93.3	1921.0
HV.1	135.5	3192.5
JN.1	25.0	912.0
XBB.1.5.72	82.8	2270.5
XBB.2.3.2	21.8	675.0
XBC.1.6	40.9	638.0

<sup>a</sup>EC<sub>50</sub> values are the mean of ≥2 independent experiments. EC<sub>50</sub>, half-maximal effective concentration; ODV, obeldesivir; RDV, remdesivi RDV and ODV Retain Potent Antiviral Activity Against Omicron Subvariants in the Replicon System

• When lineage-defining substitutions for subvariants for which clinical isolates were not available were introduced in the replicon system, mean RDV and ODV EC<sub>50</sub> values were 12.6 and 465.0 nM, respectively, representing a 1.1-fold change for both RDV and ODV compared with the reference ancestral strain (Table 3)

Table 3. RDV and ODV EC<sub>50</sub> Values and Fold Changes From SH01 Reference Strain for Omicron Subvariants in the Replicon System

	Substitutions in the	Variant Lineages in Which Defining Substitutions Were Found	RDV		ODV	
System			RDV EC <sub>50</sub> (nM) <sup>b</sup>	Fold Change From SH01 Reference Strain	ODV EC <sub>50</sub> (nM) <sup>b</sup>	Fold Change From SH01 Reference Strain
SH01 reference strain	_	<del>_</del>	11.0	1	451.1	1
Replicon	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JN.1.7 JN.1.18 KP.2 KP.3 LB.1	12.6	1.1	465.0	1.1

<sup>a</sup>The characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in ≥75% of sequences within that lineage. The lineage-defining substitutions were the same for all variants tested in these analys <sup>b</sup>The EC<sub>50</sub> values are the mean of ≥2 independent experiments.

EC<sub>50</sub> half-maximal effective concentration: ODV, obeldesivir: RDV, remdesivir.

#### Characterization of Nsp12 Amino Acid Polymorphisms Observed in Omicron Subvariants

- SARS-CoV-2 genomic analysis of >2.5 million Omicron subvariant sequences revealed unique polymorphisms in Nsp12 compared with the Wuhan strain
- [HK.3] and G823insD [XBC.1.6]) were observed in ≥75% of Nsp12 sequences
   Less prevalent polymorphisms were observed with frequencies ranging

When compared with earlier Omicron subvariants, 2 new defining mutations (D63N)

- from 1.0% to 15.9% (T26I, D40G, T85I, I171V, Y175H, I223M, T225I, D258N, Y289H, D303N, T394M, P461S, V476A, M666I, T803I, and V848I; **Table 4**)
- When these Nsp12 polymorphisms were introduced in the replicon system:
   Mean RDV EC<sub>50</sub> values ranged from 3.9 to 18.0 nM, with a fold change
- range of 0.6 to 1.6 compared with the reference ancestral strain (**Table 4**)

   Mean ODV EC<sub>50</sub> values ranged from 329.0 to 1021.7 nM, with a fold change range of 0.5 to 1.5 compared with the reference ancestral strain (**Table 4**)
- Structural analysis showed that none of the polymorphisms had direct interaction with the incoming active NTP metabolite of RDV and ODV or the viral RNA (Figure 3)

Figure 3. Structural Analysis of Identified Nsp12 Amino Acid Polymorphisms Observed in Omicron Subvariants at ≥1% Frequency

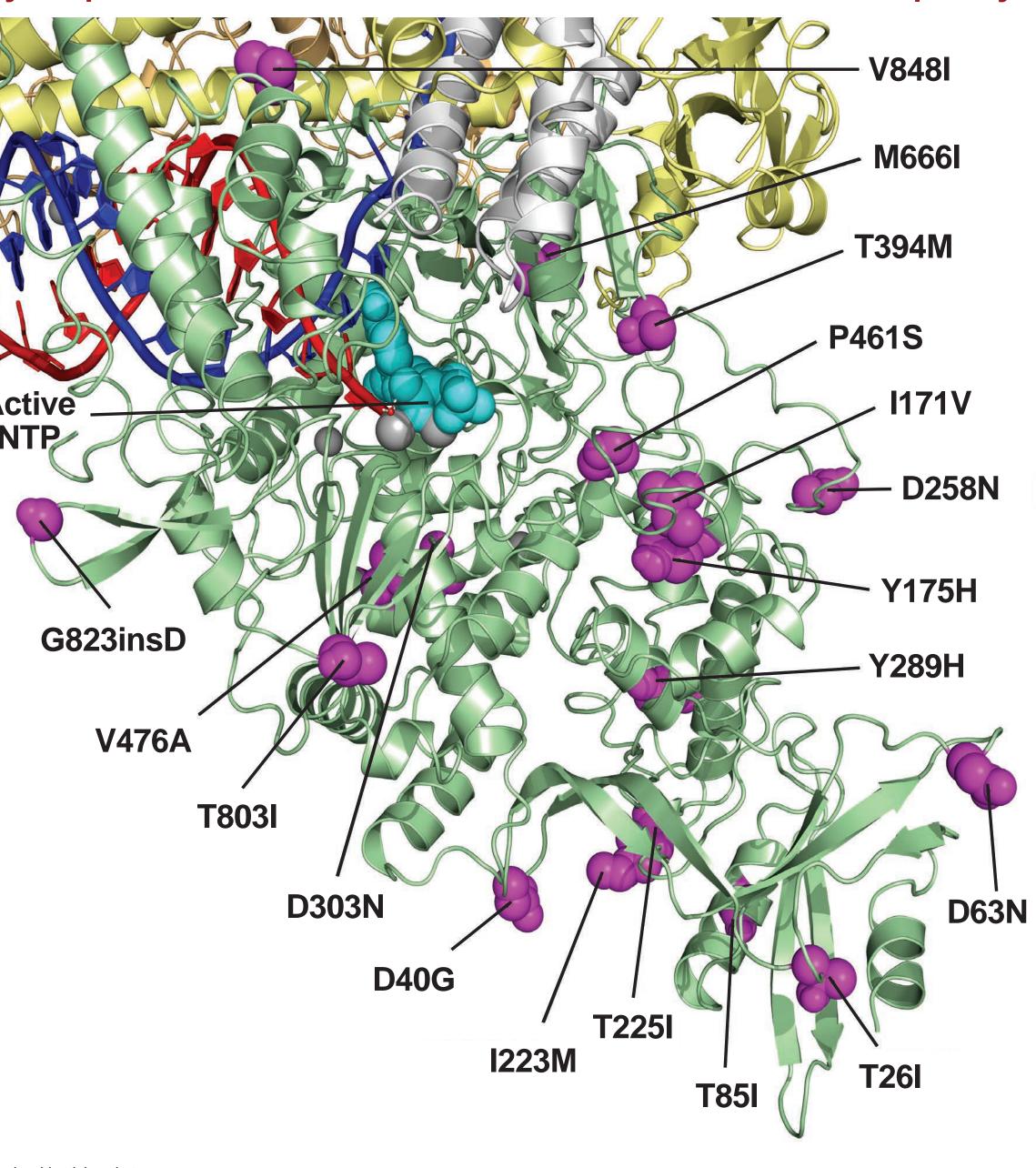


Table 4. Genotypic and Phenotypic Characterization of Nsp12 Amino Acid Polymorphisms Observed in Omicron Subvariants at ≥1% Frequency

			RDV		ODV	
Nsp12 Polymorphism	Omicron Lineage	Frequency, <sup>a</sup> %	RDV EC <sub>50</sub> (nM) <sup>b</sup>	Fold Change From SH01 Reference Strain	ODV EC <sub>50</sub> (nM) <sup>b</sup>	Fold Change From SH01 Reference Strain
SH01 reference strain	В	_	7.4	1	644.0	1
T26I	BA.2.86	1.2	6.4	1.2	856.4	1.2
D40G	BQ.1	1.2	13.3	1.2	690.3	1.2
DOON	EG.5.1	2.5	15.1	1.3	790.7	1.2
D63N	HK.3	99.8				
TOFI	BA.2.86	1.2	3.9	0.9	838.7	1.2
T85I	EG.5.1	1.1				
I171V	EG.5	1.0	6.0	1.2	632.9	1.1
Y175H	CH.1.1	1.2	5.6	1.1	690.0	1.2
I223M	XBB.1.9.1	2.0	17.2	1.5	767.0	1.3
T225I	DV.7	10.6	8.0	1.5	1021.7	1.4
D258N	XBB.1.9.2	1.0	12.0	1.0	816.5	1.3
Y289H	XBB.2.3	1.4	15.8	1.3	986.7	1.5
D303N	BA.2.86	15.9	15.9	1.4	563.5	1.0
T394M	BA.2.86	1.8	5.4	1.0	574.3	1.1
P461S	XBB.1.9.1	1.7	18.0	1.6	640.9	1.1
V476A	EG.5	1.0	6.7	0.6	329.0	0.5
M666I	XBB.1.9.1	1.4	9.8	0.8	603.0	0.9
T803I	XBB.2.3	1.5	6.3	0.6	369.7	0.6
G823insD	XBC.1.6	89.7	6.3	0.8	739.5	1.0
V848I	XBB.1.16	2.5	13.4	1.1	847.3	1.3
<sup>a</sup> Frequency in the GISAID database a <sup>b</sup> EC <sub>50</sub> values are the mean of ≥2 indep EC <sub>50</sub> , half-maximal effective concentration	as of March 4, 2024. Dendent experiments.					<u> </u>

<sup>b</sup>EC<sub>50</sub> values are the mean of ≥2 independent experiments. EC<sub>50</sub>, half-maximal effective concentration; GISAID, Global Initiative on Sharing All Influenza Data; ODV, obeldesivir; RDV, remdesiv

NTP, nucleoside triphosphate

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