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

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Conclusions

- Remdesivir and obeldesivir retained potent in vitro antiviral activity against the SARS-CoV-2 Omicron subvariants BA.2.86.1, JN.1.7, KP.2, KP.3.1.1, KP.3.3, LP.8.1, NB.1.8.1, XBB.2, XEC, and XFG, with potencies comparable with those of reference strains
- The Nsp12 substitution observed in the Omicron subvariant NB.1.8.1 remained susceptible to remdesivir and obeldesivir

Plain Language Summary

- Remdesivir and obeldesivir are antiviral drugs that are effective against SARS-CoV-2, the virus that causes COVID-19
- In previous laboratory experiments, both drugs have shown antiviral activity against different Omicron subvariants of SARS-CoV-2
- In this study, laboratory analyses showed that both remdesivir and obeldesivir remained effective against recent SARS-CoV-2 subvariants

References: **1.** National Center for Immunization and Respiratory Diseases (U.S.). Division of Viral Diseases. SARS-CoV-2 variant classifications and definitions. Centers for Disease Control and Prevention. Accessed July 14, 2025. https://stacks.cdc.gov/view/cdc/133705. **2.** World Health Organization. Updated working definitions and primary actions for SARS-CoV-2 variants. Accessed July 14, 2025. https://www.who.int/publications/m/item/updated-working-definitions-and-primary-actions-for--sars-cov-2-variants. **3.** World Health Organization. Statement on the update of WHO's working definitions and tracking system for SARS-CoV-2 variants of concern and variants of interest. Accessed July 14, 2025. https://www.who.int/news/item/16-03-2023-statement-on-the-update-of-who-s-working-definitions-and-tracking-system-for-sars-cov-2-variants-of-concern-and-variants-of-interest. **4.** World Health Organization. Tracking SARS-CoV-2 variants. Accessed July 14, 2025. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants. **5.** VEKLURY® (remdesivir) for injection, for intravenous use [package insert]. Gilead Sciences, Inc.; 2025. **6.** Mackman RL, et al. *J Med Chem*. 2023;66:11701-17. **7.** Martinez DR, et al. *Sci Transl Med*. 2024;16:eadj4504. **8.** Rodriguez L, et al. Presented at: Conference on Retroviruses and Opportunistic Infections (CROI); February 19-22, 2023; Seattle, WA, USA. Poster 562. **9.** Rodriguez L, et al. *Viruses*. 2025;17:168. **10.** Malone BF, et al. *Nature*. 2023;614:781-7.

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Introduction

- Since the onset of the COVID-19 pandemic, new variants of SARS-CoV-2 have continued to emerge, with the potential for increased transmission, enhanced antibody evasion, and reduced effectiveness of existing vaccines and antiviral therapeutics[1-3]
- Omicron and its subvariants account for all variants of interest and variants under monitoring circulating worldwide as of December 2024 and June 2025, respectively[4]
- Remdesivir (RDV), which is approved to treat COVID-19 in adult and pediatric patients, is an intravenous prodrug that is metabolized into an active nucleoside triphosphate (NTP)[5]
- Obeldesivir (ODV) is an oral mono-5′-isobutyryl ester prodrug that is metabolized into the same active NTP as RDV[6,7]
  - This NTP acts as an inhibitor of Nsp12, a highly conserved viral RNA-dependent RNA polymerase of SARS-CoV-2[5-7]
- RDV and ODV have maintained antiviral activity against previous Omicron subvariants (BA.1 through XBF) with respect to an ancestral strain[8,9]

Objectives

- To evaluate the in vitro antiviral activity of RDV and ODV against recent (May 2024–June 2025) SARS-CoV-2 Omicron subvariants using clinical isolates and site-directed mutants in a replicon system
- To conduct a structural analysis of Nsp12 substitutions observed in recent Omicron subvariants to assess their impact on RDV and ODV susceptibility

Methods

- The prevalence of Nsp12 substitutions in Omicron subvariants was assessed using sequences from the Global Initiative on Sharing All Influenza Data EpiCoV™ database
- Structural analysis of the identified Nsp12 substitutions was conducted using a composite model of cryo-electron microscopy structures of the replication-transcription complex[10]
- The antiviral activity (half-maximal effective concentration [EC_{50}]) of RDV and ODV against clinical isolates (**Table 1**) of Omicron subvariants was assessed using nucleoprotein enzyme-linked immunosorbent assay in A549-hACE2:TMPRSS2 cells; a replicon system was used for Omicron subvariants that did not have available clinical isolates (LP.8.1, NB.1.8.1, and XFG)
- The antiviral activity of RDV and ODV against identified Nsp12 substitutions was also assessed using site-directed mutants in a replicon system
- EC_{50} values were calculated from curve fits using nonlinear regression
- All experiments were performed twice with technical triplicates

Table 1. SARS-CoV-2 Clinical Isolates

Variant	Isolate	Lineage-Defining Substitutions in the Replication Complex[a]	Source
WA1 (lineage A)	hCoV-19/USA-WA1/2020	–	BEI Resources (Catalog #NR-52281)
BA.2.86.1	hCoV-19/USA/CA-GS140831/2024	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	Gilead Sciences, Inc.
JN.1.7	hCoV-19/USA/MD-HP51675-PIDFZBZXSK/2024	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JHU, Pekosz Lab
KP.2	hCoV-19/USA/MD-HP51511-PIDEACTNPM/2024	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JHU, Pekosz Lab
KP.3.1.1	hCoV-19/USA/MD-HP51826-PIDNPNPNON/2024	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JHU, Pekosz Lab
KP.3.3	SARS-CoV-2/USA/MD-HP51771/2024	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JHU, Pekosz Lab
XBB.2	hCoV-19/USA/CA-GS136871/2024	Nsp12 P323L, G671S Nsp13 S36P, R392C Nsp14 I42V	Gilead Sciences, Inc.
XEC	HP50294	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	JHU, Pekosz Lab

[a]The characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in ≥75% of sequences within that lineage.
BEI Resources, Biodefense and Emerging Infections Research Resources Repository; JHU, Johns Hopkins University.

Results

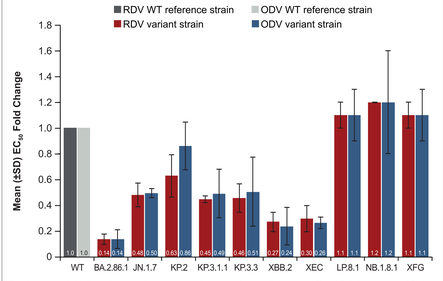
RDV and ODV Retain Potent Antiviral Activity Against Clinical Isolates of Omicron Subvariants

- Phenotyping of clinical isolates of BA.2.86.1, JN.1.7, KP.2, KP.3.1.1, KP.3.3, XBB.2, and XEC indicated no change of RDV or ODV in vitro antiviral activity (≤0.86-fold change) compared with the WA1 reference strain
  - Mean RDV EC_{50} values for all tested Omicron subvariants ranged from 21.8 to 87.3 nM, with fold changes ranging from 0.14 (BA.2.86.1) to 0.63 (KP.2) compared with WA1 (EC_{50} range, 72.4-190.9; mean EC_{50}, 126.9 nM; **Figures 1** and **2**; **Table 2**)
  - Mean ODV EC_{50} values for all tested Omicron subvariants ranged from 357.0 to 1923.5 nM, with fold changes ranging from 0.14 (BA.2.86.1) to 0.86 (KP.2) compared with WA1 (EC_{50} range, 1512.0-3061.0 nM; mean EC_{50}, 2293.8 nM; **Figures 1** and **2**; **Table 2**)

RDV and ODV Retain Potent Antiviral Activity Against Omicron Subvariants in the Replicon System

- Phenotyping of replicons of LP.8.1, NB.1.8.1, and XFG showed no loss of RDV or ODV susceptibility (≤1.2-fold change) compared with the SH01 reference strain
  - Mean RDV EC_{50} values were 12.6 and 16.6 nM, representing a 1.1- and 1.2-fold change compared with the SH01 reference strain, respectively (**Figures 1** and **3**; **Table 3**)
  - Mean ODV EC_{50} values were 465.0 and 761.9 nM, representing a 1.1- and 1.2-fold change compared with the SH01 reference strain, respectively (**Figures 1** and **3**; **Table 3**)

Figure 1. RDV and ODV EC_{50} Fold Changes[a] From WT Reference Strain[b] Against Clinical Isolates and Replicons of SARS-CoV-2 Omicron Subvariants



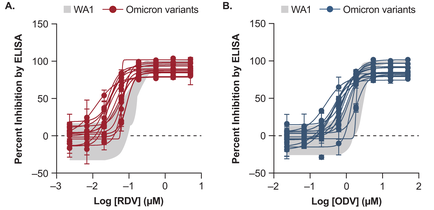
[a]Fold change was calculated for each experiment, and a mean fold change was calculated with these values. The cutoffs for the assays were 2.8-fold for clinical isolates tested with RDV, 2.9-fold for clinical isolates tested with ODV, 2.5-fold for replicons tested with RDV, and 2.3-fold for replicons tested with ODV.
[b]The WT reference strain for variants BA.2.86.1 through XEC was WA1, and the WT reference strain for variants LP.8.1 through XFG was SH01.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; RDV, remdesivir; WT, wildtype.

Table 2. RDV and ODV EC_{50} Values[a] Against Clinical Isolates of SARS-CoV-2 Omicron Subvariants

Variant Lineage (Clinical Isolate)	RDV EC_{50} (nM)	ODV EC_{50} (nM)
WA1 (lineage A)	Range: 72.4-190.9	Range: 1512.0-3061.0
BA.2.86.1	21.8	357.0
JN.1.7	57.2	1107.0
KP.2	87.3	1923.5
KP.3.1.1	40.2	903.5
KP.3.3	63.8	1099.0
XBB.2	43.4	605.4
XEC	25.5	503.6

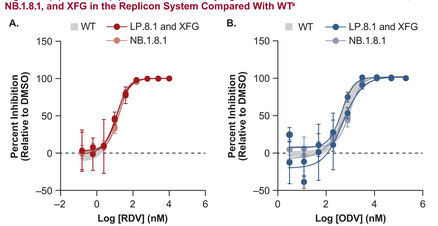
[a]EC_{50} values are the mean of ≥2 independent experiments.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; RDV, remdesivir.

Figure 2. (A) RDV and (B) ODV Retain Potency Against the Omicron Subvariants BA.2.86.1, JN.1.7, KP.2, KP.3.1.1, KP.3.3, XBB.2, and XEC Compared With the WA1 Reference Strain[a]



[a]Each variant was tested 2 times with technical triplicates. The range of RDV or ODV activity against the WA1 reference strain (8 replicates) is shaded in gray.
ELISA, enzyme-linked immunosorbent assay; ODV, obeldesivir; RDV, remdesivir.

Figure 3. (A) RDV and (B) ODV Retain Potency Against the Omicron Subvariants LP.8.1, NB.1.8.1, and XFG in the Replicon System Compared With WT[a]



[a]Each variant was tested 2 times with technical triplicates. The range of RDV or ODV activity against the WT reference strain (4 replicates) is shaded in gray.
DMSO, dimethyl sulfoxide; ODV, obeldesivir; RDV, remdesivir; WT, wildtype.

Table 3. RDV and ODV EC_{50} Values From the SH01 Reference Strain for Omicron Subvariants in the Replicon System

System	Lineage-Defining Substitutions in the Replication Complex[a]	Variant Lineages in Which Defining Substitutions Were Found	RDV EC_{50} (nM)[b]	ODV EC_{50} (nM)[b]
SH01 reference strain	–	–	12.5	552.7
Replicon	Nsp9 T35I Nsp12 P323L Nsp13 R392C Nsp14 I42V	LP.8.1 XFG	12.6	465.0
Replicon	Nsp9 P57S, P80L Nsp12 D284Y, P323L, G671S Nsp13 S36P, R392C Nsp14 I42V	NB.1.8.1	16.6	761.9

[a]The characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in ≥75% of sequences within that lineage.
[b]The EC_{50} values are the mean of ≥2 independent experiments.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; RDV, remdesivir.

Characterization of Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants

- Genomic analysis of >17 million SARS-CoV-2 sequences revealed unique substitutions in Nsp12 compared with WA1
- One Nsp12 substitution (D284Y) was observed in ≥75% of Nsp12 sequences of the Omicron subvariant NB.1.8.1, which was designated as a variant under monitoring by the World Health Organization in May 2025[9] (**Table 4**)
- D284Y is the only new lineage-defining substitution in Nsp12 identified in BA.2.86.1, JN.1.7, KP.2, KP.3.3, KP.3.1.1, LP.8.1, NB.1.8.1, XBB.2, XEC, or XFG compared with earlier Omicron subvariants
- When this Nsp12 substitution was introduced in the replicon system, the mean RDV and ODV EC_{50} values were 11.8 and 569.6, with fold changes of 0.8 and 0.9, respectively, compared with the reference strain (**Table 4**)

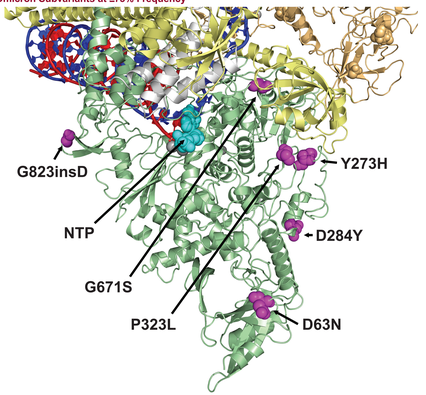
Table 4. Genotypic and Phenotypic Characterization of Nsp12 Substitutions Observed in Omicron Subvariants at ≥75% Frequency

Nsp12 Lineage-Defining Substitution	Omicron Lineage	Frequency, %[a]	RDV		ODV	
			RDV EC_{50} (nM)[b]	Fold Change From SH01 Reference Strain[c]	ODV EC_{50} (nM)[b]	Fold Change From SH01 Reference Strain[c]
SH01 reference strain	B	–	13.9	1.0	654.3	1.0
D284Y	NB.1.8.1	99.8	11.8	0.8	569.6	0.9

[a]Frequency in the GISAID EpiCoV™ database as of September 4, 2025.
[b]EC_{50} values are the mean of ≥2 independent experiments.
[c]Fold change was calculated for each experiment, and a mean fold change was calculated with these values.
EC_{50}, half-maximal effective concentration; GISAID, Global Initiative on Sharing All Influenza Data; ODV, obeldesivir; RDV, remdesivir.

- Structural analysis showed that the Nsp12 lineage-defining substitution D284Y, along with previously identified Nsp12 lineage-defining substitutions (ie, G823insD, G671S, P323L, D63N, and Y273H), did not have direct interaction with the incoming active NTP metabolite of RDV and ODV or the viral RNA (**Figure 4**)

Figure 4. Structural Analysis of Identified Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants at ≥75% Frequency[a]



[a]Nsp12 is shown in green, Nsp7 is shown in white, Nsp8 is shown in yellow, and Nsp13 is shown in orange.
NTP, nucleoside triphosphate.