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

545

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Key Findings

- Remdesivir (RDV) and obeldesivir (ODV) retained potent in vitro antiviral activity against current SARS-CoV-2 variants of concern/interest/under monitoring (VOCs/VOIs/VUMs) with potencies comparable to the WA1 reference strain
- Nsp12 substitutions observed in Omicron subvariants remained susceptible to RDV and ODV

Conclusions

These data support the continued use of RDV to treat people infected with currently circulating Omicron subvariants

These data also indicate that ODV is effective against currently circulating variants and support its continued clinical development for the treatment of COVID-19

References: **1.** Centers for Disease Control and Prevention. SARS-CoV-2 variant classifications and definitions. Accessed July 7, 2023. https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html. **2.** World Health Organization. Updated working definitions and primary actions for SARS-CoV-2 variants. Accessed July 7, 2023. https://www.who.int/publications/m/item/updated-working-definitions-and-primary-actions-for--sars-cov-2-variants. **3.** Centers for Disease Control and Prevention. COVID data tracker. Accessed July 7, 2023. https://covid.cdc.gov/covid-data-tracker. **4.** World Health Organization. Tracking SARS-CoV-2 variants. Accessed July 7, 2023. https://www.who.int/en/activities/tracking-SARS-CoV-2-variants. **5.** Veklury® (remdesivir) injection, for intravenous use [package insert]. Gilead Sciences, Inc.; 2023. **6.** Martinez DR, et al. *bioRxiv*. Preprint posted online June 28, 2023. doi:10.1101/2023.06.27.546784. **7.** Rodriguez L, et al. Presented at: Conference on Retroviruses and Opportunistic Infections (CROI); February 19-22, 2023; Seattle, WA, USA. Poster P562. **8.** Malone BF, et al. *Nature*. 2023;614(7949):781-787.

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Introduction

- New variants of SARS-CoV-2 continue to emerge, including VOCs, VOIs, or VUMs, with the potential for increased transmission, more severe disease, enhanced antibody evasion, and reduced effectiveness of vaccines and therapeutics[1,2]
- Omicron subvariants are the predominant VOIs and VUMs currently circulating in the United States and worldwide[3,4]
- RDV is a nucleotide analog prodrug approved to treat COVID-19 in both hospitalized and nonhospitalized adult and pediatric patients[5]
- ODV is an oral GS-441524 prodrug currently being evaluated in Phase 3 studies and has the potential to be a simple twice-daily treatment regimen with limited drug-drug interactions
- RDV (intravenous) and ODV (oral), through different metabolic pathways, generate the same active nucleoside triphosphate (active NTP), which acts as a SARS-CoV-2 RNA-dependent RNA polymerase (Nsp12) inhibitor[6]
- The antiviral activity of RDV and ODV against previous Omicron subvariants (BA.1 to BQ.1.1) has been maintained relative to the ancestral WA1 strain[7]

Objectives

- To characterize the in vitro antiviral activity of RDV and ODV against recent SARS-CoV-2 Omicron VOCs/VOIs/VUMs using clinical isolates and/or site-directed mutants in a replicon system
- To conduct a structural analysis of Nsp12 substitutions observed in recent Omicron VOCs/VOIs/VUMs with respect to RDV and ODV susceptibility

Methods

- The prevalence of Nsp12 substitutions in Omicron subvariants was evaluated by an analysis of sequences from the Global Initiative on Sharing All Influenza Data EpiCoV™ database
- A structural analysis of identified substitutions was conducted on a prior cryo-electron microscopy–based model of the replication-transcription complex[8]
- Antiviral activity of RDV and ODV against subvariant clinical isolates (**Table 1**) was assessed by nucleoprotein enzyme-linked immunoassay in A549-hACE2-TMPRSS2 cells and by site-directed mutants in the replicon system

Table 1. SARS-CoV-2 Clinical Isolates

Variant	Isolate	Source
WA1 (lineage A)	hCoV-19/USA-WA1/2020	BEI Resources Cat# NR-52281
BF.7	hCoV-19/USA/MD-HP38288/2022	BEI Resources Cat# NR-58974
BQ.1	hCoV-19/USA/MD-HP38960/2022	BEI Resources Cat# NR-58975
XBB.1.5	hCoV-19/USA/MD-HP40900/2022	BEI Resources Cat# NR-59104
CH.1.1	hCoV-19/USA/MD-HP41275/2022	BEI Resources Cat# NR-59204
XBF	hCoV-19/Japan/TY41-831/2022	NIID, Japan
XBB.1.16	hCoV-19/Japan/TY41-984/2023	NIID, Japan
XBB.1.9.1	hCoV-19/Japan/TY41-951/2023	NIID, Japan

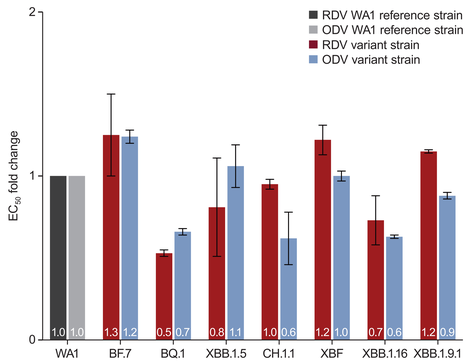
BEI Resources, Biodefense and Emerging Infections Research Resources Repository; NIID, National Institute of Infectious Diseases.

Results

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

- Mean RDV half-maximal effective concentration (EC_{50}) values for all tested Omicron subvariants ranged from 50.8 nM (BQ.1) to 155.1 nM (CH.1.1), representing a 0.5- to 1.3-fold change compared with the reference ancestral strain, WA1 (EC_{50} = 114.6 nM; **Figure 1**; **Table 2**)
- Mean ODV EC_{50} values for all tested Omicron subvariants ranged from 1.4 µM (BQ.1) to 2.6 µM (BF.7), representing a 0.6- to 1.2-fold change compared with WA1 (EC_{50} = 2.5 µM; **Figure 1**; **Table 2**)
- These data confirm similar antiviral activity of RDV and ODV against Omicron subvariants compared to the WA1 reference strain

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



RDV, remdesivir; ODV, obeldesivir; EC_{50}, half-maximal effective concentration; WT, wild-type.
A fold change was calculated for each experiment, and a mean (standard deviation) fold change was calculated with these values; values below 2.8-fold were within the variability of the assay.

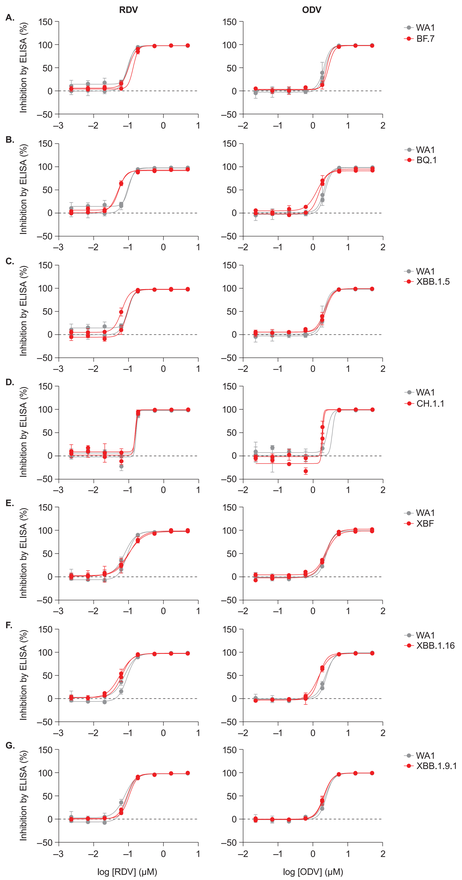
Table 2. RDV and ODV EC_{50} Values and Fold Changes From WT Reference for SARS-CoV-2 VOCs, VOIs, and VUMs Tested Using Clinical Isolates

Variant lineage	Lineage-defining substitutions in the replication complex[a]	RDV		ODV	
		RDV EC_{50} (nM)[b]	Fold change from WT[c] reference	ODV EC_{50} (µM)[b]	Fold change from WT[c] reference
WT reference	–	114.6	1.0	2.5	1.0
BF.7	Nsp12 P323L Nsp13 R392C Nsp14 I42V	119.6	1.3	2.6	1.2
BQ.1	Nsp12 P323L, Y273H Nsp13 M233I, R392C Nsp14 I42V	50.8	0.5	1.4	0.7
XBB.1.5	Nsp12 P323L, G671S Nsp13 S36P, R392C Nsp14 I42V	76.8	0.8	2.3	1.1
CH.1.1	Nsp8 N118S Nsp12 P323L, G671S Nsp13 R392C Nsp14 I42V, V182I	155.1	1.0	1.8	0.6
XBF	Nsp8 N118S Nsp12 P323L, G671S Nsp13 R392C Nsp14 I42V, V182I	102.3	1.2	2.3	1.0
XBB.1.16	Nsp12 P323L, G671S Nsp13 S36P, R392C Nsp14 I42V, D222Y	60.4	0.7	1.5	0.6
XBB.1.9.1	Nsp9 T35I Nsp12 P323L Nsp13 S36P, R392C Nsp14 I42V	96.9	1.2	2.1	0.9

RDV, remdesivir; ODV, obeldesivir; EC_{50}, half-maximal effective concentration; WT, wild-type; VOC, variant of concern; VOI, variant of interest; VUM, variant under monitoring.
[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.
[c]The WA1 strain (lineage A) was used as the WT reference for the clinical isolates.

- The antiviral activity of RDV and ODV at different concentrations against the BF.7, BQ.1, XBB.1.5, CH.1.1, XBF, XBB.1.16, and XBB.1.9.1 variants is shown in **Figure 2**

Figure 2. RDV and ODV Retain Potency Against Omicron Subvariants (A) BF.7, (B) BQ.1, (C) XBB.1.5, (D) CH.1.1, (E) XBF, (F) XBB.1.16, and (G) XBB.1.9.1



RDV, remdesivir; ODV, obeldesivir; ELISA, enzyme-linked immunoassay.

Characterization of Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants

- SARS-CoV-2 genomic analysis of >2 million Omicron subvariant sequences revealed unique substitutions in Nsp12 versus WA1
- No new defining substitutions in Nsp12 were found in the analyzed sequences when compared with earlier Omicron variants
  - The Nsp12-defining amino acid substitutions (≥75% of sequences) included P323L, observed in all Omicron subvariants; Y273H, observed in BQ.1; and G671S, observed in XBB.1.5, CH.1.1, and XBF (**Table 3**)
- Less prevalent substitutions were observed with frequencies ranging from 0.5% to 3.3% (L247F, T248I, V257F, N507I, A529V, and F694Y; **Table 3**)
- When the Nsp12 substitutions were introduced in the replicon system, mean RDV EC_{50} values ranged from 5.3 to 15.5 nM, representing a 0.5- to 1.6-fold change compared with the reference strain (**Table 3**)
  - Mean ODV EC_{50} values ranged from 0.2 to 1.3 µM, representing a 0.5- to 1.8-fold change compared with the reference strain

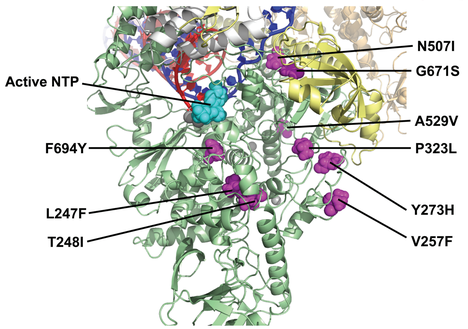
Table 3. Genotypic and Phenotypic Characterization of Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants at ≥1% Frequency in the Replicon System

Nsp12 substitution	Omicron lineage	Frequency,[a] % (n)	RDV		ODV	
			RDV EC_{50} (nM)[b]	Fold change from WT[c] reference[d]	ODV EC_{50} (µM)[b]	Fold change from WT[c] reference[d]
WT reference	B	–	8.2	1.0	0.7	1.0
L247F	BF.7	3.3 (1670)	6.0	0.5	0.7	0.8
T248I	BF.7	2.0 (1292)[e]	5.3	0.9	0.4	0.8
V257F	XBB.1.5	3.1 (4819)[e]	6.8	1.2	0.2	0.5
Y273H	BQ.1	98.0 (53,775)	10.4	1.2	0.2	0.5
P323L	Across Omicron subvariants	98.8 (15,244,818)	7.2	0.9	Ongoing	NA
N507I	XBF	1.8 (173)	6.7	0.9	0.4	0.7
A529V	BF.7	1.8 (676)	Did not replicate	NA	Did not replicate	NA
G671S	XBB.1.5	96.0 (175,776)	15.5	1.6	1.3	1.8
	CH.1.1	92.5 (18,798)				
	XBF	97.0 (9356)				
F694Y	CH.1.1	0.5 (104)	Did not replicate	NA	Ongoing	NA
	XBF	2.4 (236)				

RDV, remdesivir; ODV, obeldesivir; EC_{50}, half-maximal effective concentration; WT, wild-type; NA, not applicable; GISAID, Global Initiative on Sharing All Influenza Data.
[a]Frequency in the GISAID database as of July 8, 2023.
[b]The EC_{50} values are the mean of ≥2 independent experiments.
[c]The SH01 strain (lineage B) was used as the WT reference in the replicon system.
[d]Values below 2.5-fold were within the variability of the replicon assay.
[e]Frequency in the GISAID database as of April 6, 2023.

- None of the substitutions had direct interaction with the incoming active NTP or the viral RNA, except N507I, which interacts with the 5′ template overhang (**Figure 3**)

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



NTP, nucleoside triphosphate.

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