Remdesivir Retains Potent Antiviral Activity Against SARS-CoV-2 Variants of Concern

562

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Introduction

- Omicron and its subvariants are the current predominant severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern (VOCs) circulating worldwide[1,2]
- Continued evolution of SARS-CoV-2 has led to the emergence of Omicron subvariants with progressive loss of therapeutic monoclonal antibody neutralizing activity due to amino acid changes in the Spike protein[3-5]
- Remdesivir (RDV) is a broad-spectrum nucleotide analog prodrug approved for the treatment of coronavirus disease 2019 (COVID-19) in nonhospitalized and hospitalized adult and pediatric patients,[6] with clinical benefits demonstrated in multiple clinical trials[7-10]
- The RNA-dependent RNA polymerase (RdRp) Nsp12, the target of RDV, is highly conserved across VOCs to date, and RDV antiviral activity against previous VOCs (Alpha to Omicron BA.1) has been maintained[11]

Objectives

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

Methods

- The prevalence of Nsp12 substitutions in Omicron subvariants was evaluated by an analysis of sequences from the Global Initiative on Sharing Avian Influenza Data (GISAID) EpiCoV™ database
- Structural analysis of identified substitutions was conducted on a prior cryo-electron microscopy–based model of the replication complex[12]
- Antiviral activity of RDV against SARS-CoV-2 Omicron subvariant clinical isolates was assessed by nucleoprotein enzyme-linked immunoassay (ELISA) in A549-hACE2-TMPRSS2 cells (**Table 1**)
- The impact of specific substitutions in the replication complex on RDV antiviral activity was further assessed by SDMs in a replicon system with Huh7-1CN cells, which overexpress the SARS-CoV-2 WA1 nucleoprotein

Table 1. SARS-CoV-2 Clinical Isolates

Variant	Isolate	Source
WA1 (lineage A)	hCoV-19/USA-WA1/2020	BEI Resources Cat# NR 52281
BA.2	hCoV-19/USA/WA-CDC-UW22012361822/2022	University of Washington
BA.2.12.1	hCoV-19/USA/NY-MSHSPSP-PV56475/2022	BEI Resources Cat# NR 56781
BA.4	hCoV-19/South Africa/CERI-KRISP-K040015/2022	University of Washington
BA.5	hCoV-19/South Africa/CERI-KRISP-K040013/2022	University of Washington
BA.2.75	Isolate name in progress	Emory University
BA.4.6	hCoV-19/USA/MD-HP35538/2022	BEI Resources Cat# NR-58715
BF.5	hCoV-19/USA/MD-HP34985/2022	BEI Resources Cat# NR-58716
XBB	hCoV-19/USA/CA-Stanford-109_S21/2022	BEI Resources Cat# NR-58925
BQ.1.1	hCoV-19/USA/MD-HP38861/2022	BEI Resources Cat# NR-58976

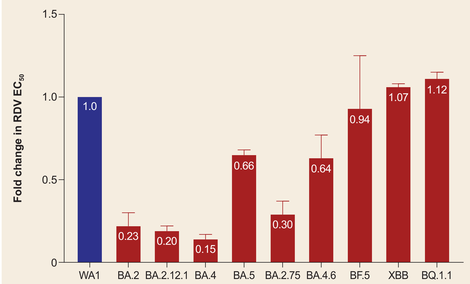
BEI Resources, Biodefense and Emerging Infections Research Resources Repository.

Results

RDV Retains Potent Antiviral Activity Against Clinical Isolates of Omicron Subvariants

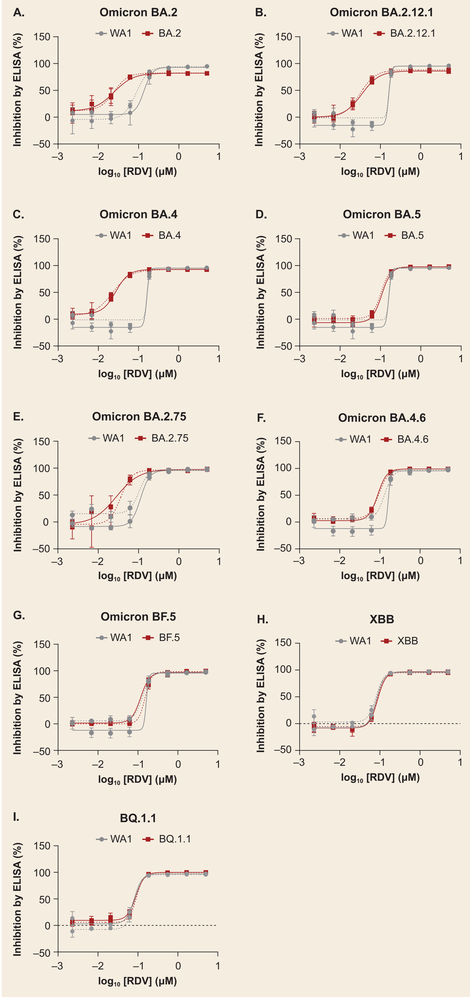
- Mean RDV half-maximal effective concentration (EC_{50}) values for all tested Omicron subvariants ranged from 24.5 nM (BA.2) to 134.1 nM (BF.5), representing a 0.2- to 1.1-fold change compared with the reference ancestral strain, WA1 (mean EC_{50} = 121.6 nM; **Figures 1** and **2**)

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



WT, wild-type; SD, standard deviation.
Values are the mean ± SD of the results of at least 2 independent experiments.

Figure 2. RDV Retains Potency Against Omicron Subvariants (A) BA.2, (B) BA.2.12.1, (C) BA.4, (D) BA.5, (E) BA.2.75, (F) BA.4.6, (G) BF.5, (H) XBB, and (I) BQ.1.1[a]



[a]Two replicates of each subvariant or reference strain were included in each assay (displayed in the same color curves).

Antiviral Potencies Were Consistent Between Infectious Isolates and SDMs Evaluated in the Replicon System

- Mean RDV EC_{50} values were consistently lower in the replicon system; however, the fold changes remained similar between the variants tested in both assays, suggesting that the replicon system could be a valuable surrogate assay (**Table 2**)

Table 2. RDV EC_{50} and Fold Change From WT Reference for SARS-CoV-2 VOCs Tested Using Clinical Isolates and SDMs in the Replicon System

Variant lineage	Lineage-defining substitutions in the replication complex[a]	RDV EC_{50}, nM		Fold change from WT reference	
		Clinical isolate	Replicon	Clinical isolate	Replicon
WT	–	110	7.7	1.0	1.0
Alpha B.1.1.7	Nsp12 P323L	192[11]	7.2	1.6[11]	0.9
Delta B.1.617.2	Nsp12 P323L G671S Nsp13 P77L Nsp14 A394V	70[11]	10.1	0.6[11]	1.2
Omicron BA.1	Nsp12 P323L Nsp14 I42V	42[11]	4.8	0.5[11]	0.6
Omicron BA.2	Nsp12 P323L Nsp13 R392C Nsp14 I42V	25	4.7	0.2	0.4
Omicron BA.2.75	Nsp8 N118S Nsp12 P323L G671S Nsp13 R392C Nsp14 I42V	32	9.6	0.3	1.1

The WA1 strain (lineage A) was used as the WT reference for the clinical isolates and the SH01 strain (lineage B) was used as the WT reference in the replicon system. The EC_{50} values are the mean of at least 2 independent experiments. The fold change values were calculated by dividing the variant mean EC_{50} by the reference strain mean EC_{50}.
[a]The characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in >75% of sequences within that lineage.

RDV Retains Potent Antiviral Activity Against the Omicron BQ.1 Substitutions in the Replicon System

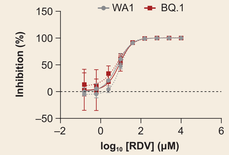
- The Omicron BQ.1 variant was tested in the replicon system by introducing all defining amino acid substitutions in the replication complex (Nsp12 to Nsp14) by SDMs
- The Omicron BQ.1 variant was inhibited by RDV with an EC_{50} of 7.9 nM, representing a 0.95-fold change compared with the reference ancestral strain (EC_{50} = 8.3 nM; **Table 3** and **Figure 3**)

Table 3. RDV EC_{50} and Fold Change From WT Reference for SARS-CoV-2 VOC Tested in the Replicon System

Variant lineage	Lineage-defining substitutions in the replication complex[a]	RDV EC_{50}, nM	Fold change from WT reference
WT	–	8.3	1.0
Omicron BQ.1	Nsp12 P323L Y273H Nsp13 M233I R392C Nsp14 I42V	7.9	0.95

[a]The characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in >75% of sequences within that lineage.

Figure 3. RDV Remains Effective Against Omicron BQ.1 in the Replicon System[a]



[a]Two replicates of each subvariant or reference strain were included in each assay (displayed in the same color curves).

Characterization of Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants

- SARS-CoV-2 genomic analysis of >1.4 million Omicron sequences revealed that in addition to Nsp12 P323L, which is present in all Omicron subvariants thus far, the only other Nsp12-defining amino acid substitutions (≥75% frequency) were Y273H, observed in BQ.1 and BQ.1.1, and G671S, observed in XBB.1.5, BJ.1, XBB, and BA.2.75 (**Table 4**)
- When the Nsp12 substitutions were introduced in the replicon system, mean RDV EC_{50} values ranged from 6.0 to 10.4 nM, representing a 0.5- to 1.2-fold change compared with the reference ancestral strain (**Table 4**)
- Phenotyping of F694Y was attempted twice but could not be assessed due to lack of replication; prior data using the recombinant virus showed that G671S and F694Y substitutions maintained full in vitro susceptibility to RDV[11]

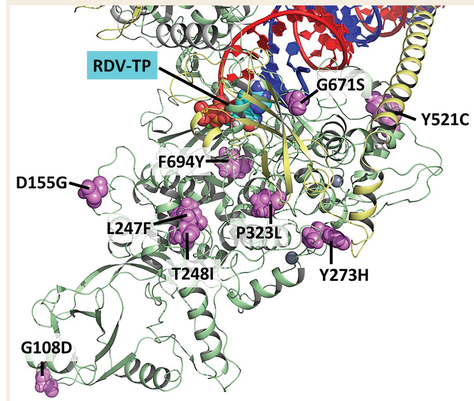
Table 4. Genotypic and Phenotypic Characterization of Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants at ≥1% Frequency in the Replicon System

Nsp12 substitution	Omicron lineage	Frequency,[a] % (n)	EC_{50}, nM	Fold change from WT[b] reference
WT	B	–	9.3	1.0
G108D	BJ.1	1.7 (3)	6.9	0.8
D155G	BA.2.3.20	2.9 (25)	Ongoing	NA
L247F	BF.7	2.4 (904)	6.0	0.5
T248I	BF.7	1.3 (503)	8.3	1.0
Y273H	BQ.1	99.6 (8,547)	10.4	1.2
	BQ.1.1	99.0 (14,261)		
P323L	Across Omicron subvariants	99.1-99.9 (172-1,100,040)	7.2	0.9
Y521C	BA.5	1.8 (20,079)	9.8	1.2
G671S	BJ.1	98.3 (170)	Ongoing	NA
	XBB	98.0 (2,000)		
	BA.2.75	96 (26,104)		
F694Y	BA.4	1.8 (3,217)	Did not replicate	NA
	BA.5	1.4 (15,260)		
	BA.2.75	1.1 (307)		

NA, not applicable.
[a]Frequency in the GISAID database as of 28 November 2022.
[b]WT SH01 (lineage B).

- Most of the Nsp12 substitutions identified were distant from the RdRp active site or viral RNA
- F694Y was measured to be closest to the critical 1'-cyano group of the pre-incorporated triphosphate form of RDV (9.7Å); however, the modest change from Phe to Tyr had minimal impact on the inhibitor's predicted affinity for the polymerase active site (**Figure 4**)

Figure 4. Structural Analysis of Identified Nsp12 Amino Acid Substitutions Observed in Omicron Subvariants at ≥1% Frequency



RDV-TP, active triphosphate metabolite of remdesivir.

Conclusions

- RDV retained potent in vitro antiviral activity against all recent Omicron subvariant clinical isolates evaluated, with potencies comparable to WT reference isolates
- Nsp12 substitutions observed in recent Omicron subvariants remain susceptible to RDV
- These data support the continued use of RDV to treat patients infected with currently circulating Omicron subvariants

Disclosures: LR, JL, DH, JM, XL, RM, NCR, JP, JKP, JPB, DPP, and CH are employees of Gilead Sciences, Inc. MG is a consultant for Merck, Biogen, and HDT Bio. T-YH declares no personal interests.

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