Remdesivir Resistance Analyses From the PINETREE Study in Outpatients With COVID-19

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Introduction

- Remdesivir (RDV) is a broad-spectrum nucleotide analog prodrug approved for the treatment of coronavirus disease 2019 (COVID-19) in hospitalized and nonhospitalized patients[1]
- The clinical benefit of RDV for patients with COVID-19 has been demonstrated in multiple clinical trials, including the phase 3, randomized, double-blind, placebo-controlled PINETREE study conducted in outpatients[2]

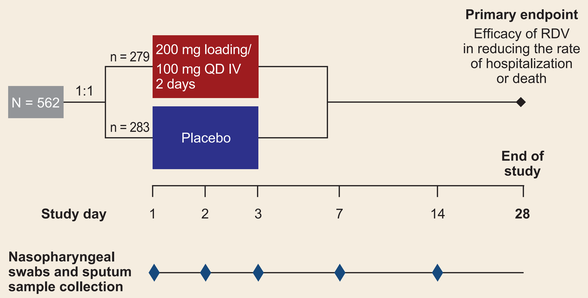
Objective

- To present severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) resistance analyses from the phase 3 PINETREE study

Methods

- PINETREE was a double-blind, placebo-controlled study wherein nonhospitalized participants (N = 562) aged ≥12 years who had COVID-19 and ≥1 risk factor for disease progression were randomly assigned (1:1) to receive RDV or placebo once daily for 3 days (**Figure 1**)

Figure 1. Study Design



Arm	COVID-19–related hospitalization or death from any cause by Day 28, n (%)	Hospitalization for any cause by Day 28, n (%)	Serious AE, n (%)
RDV	2 (0.7)	5 (1.8)	5 (1.8)
Placebo	15 (5.3)	18 (6.4)	19 (6.7)
	$P = 0.008$		

QD, once daily; IV, intravenous; RDV, remdesivir; COVID-19, coronavirus disease 2019; AE, adverse event.

- The SARS-CoV-2 genome was deep sequenced using Illumina NextSeq (DDL Diagnostic Laboratory)
- Sequencing of SARS-CoV-2 samples collected at baseline, Day 3 (or Day 2 if viral load did not meet the sequencing threshold), Day 7, and Day 14 was conducted for all participants in the RDV group and 50% of those in the placebo group with a viral load above the lower limit of quantification of the viral load assay (2,228 copies/mL)
- Amino acid substitutions that emerged in SARS-CoV-2 Nsp8, Nsp10, Nsp12, Nsp13, and Nsp14 were tested for effect on RDV efficacy by generating site-directed mutants in a SARS-CoV-2 subgenomic replicon system[3,4]
  - Replication capacity was calculated by dividing the luciferase signal of the mutant replicon by the wild-type (WT) replicon
- A biochemical assay used an RNA template and primer sequence with incorporated [α-^{32}P] cytidine triphosphate to detect RNA products synthesized by the WT, A376V, and A376V/P323L mutant RNA-dependent RNA polymerase (RdRp)

Results

Participants

- Of 562 participants treated in total, 281 participants met the criteria for sequencing
  - Of these, baseline and postbaseline sequencing data were available for 115/130 (88.5%) participants in the RDV group and 129/151 (85.4%) participants in the placebo group (**Table 1**)

Table 1. PINETREE Sequencing Data

	Number of participants	
	RDV	Placebo
Treated population	279	283
Met resistance analysis criteria and sequencing attempted, n/N (%)	130/279 (46.6)	151/283 (53.4)
Sequencing data available, n/N (%)		
Baseline	119/130 (91.5)	138/151 (91.4)
Postbaseline	122/130 (93.8)	137/151 (90.7)
Baseline + postbaseline, n/N (%)	115/130 (88.5)	129/151 (85.4)
Participants with emergent Nsp12 substitutions	8/115 (7.0)	7/129 (5.4)
Participants with Nsp12 substitutions affecting RDV in vitro activity	1/115 (0.9)	NA

RDV, remdesivir; NA, not applicable.

Substitutions in Nsp12

- Among participants with both baseline and postbaseline data, rates of emerging Nsp12 substitutions were similar in those treated with RDV compared with those who received placebo (**Table 1**)
- A total of 7 emergent amino acid substitutions in Nsp12 were observed in the RDV group but not in the placebo group (**Table 2**)

Table 2. Amino Acid Substitutions Detected in Nsp12 Postbaseline

Nsp12 substitutions from baseline, n (%)	Number of participants, n (% out of participants with both baseline and postbaseline sequencing data available)		
	RDV n = 115	Placebo n = 129	Total n = 244
S6L	1 (0.9)	0	1 (0.4)
G44V	0	1 (0.8)	1 (0.4)
K103M	0	1 (0.8)	1 (0.4)
E144G	0	1 (0.8)	1 (0.4)
D155H	0	1 (0.8)	1 (0.4)
T206I	1 (0.9)	0	1 (0.4)
P232L	1 (0.9)	0	1 (0.4)
A376V	1 (0.9)[a]	0	1 (0.4)
T394M	1 (0.9)[a]	0	1 (0.4)
P505S	0	1 (0.8)	1 (0.4)
A526S	1 (0.9)	0	1 (0.4)
R631G[b]	0	1 (0.8)	1 (0.4)
A634S	1 (0.9)	0	1 (0.4)
S649*	0	1 (0.8)	1 (0.4)
F694Y	1 (0.9)	2 (1.6)	3 (1.2)
S835L	0	1 (0.8)	1 (0.4)

RDV, remdesivir.
*One participant had a viral load >5 $\log_{10}$ copies/mL at Day 7.
[a]Participant had Nsp12 substitutions E144G, D155H, R631G, and S835L emerge postbaseline.
*Denotes translational termination (stop) codon.

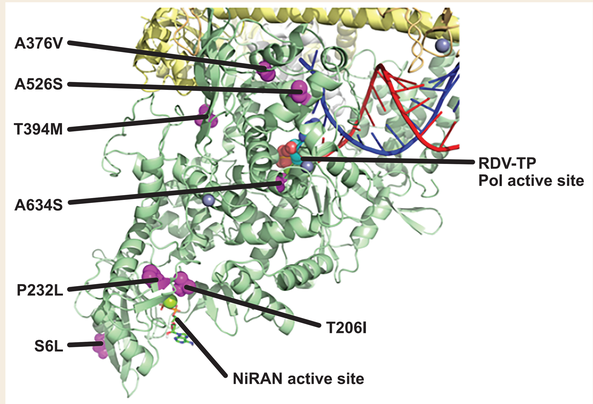
Structural Analysis of the Emergent Nsp12 Amino Acid Substitutions in Participants Treated With RDV

- None of the Nsp12 substitutions observed in the RDV group (S6L, T206I, P232L, A376V, T394M, A526S, and A634S) were found to be in direct contact with the incoming nucleoside triphosphate substrate or the RNA (**Figure 2**)
  - A376V was located close to the RNA template 5' overhang

Phenotype of Emergent Substitutions in the RDV-treated Group

- Emergent substitutions in Nsp8, Nsp10, Nsp13, or Nsp14 were detected in 10/115 (8.7%) participants in the RDV group and 10/129 (7.8%) participants in the placebo group
- The majority of the successfully phenotyped substitutions in Nsp8, Nsp10, Nsp12, Nsp13, or Nsp14 were equally susceptible to RDV as to the WT reference replicon (**Table 3**)
- An emergent Nsp12 substitution associated with reduced susceptibility to RDV (A376V) was observed in only 1 participant treated with RDV
  - P323L was observed at baseline in this participant and remained susceptible to RDV[5]
  - The SARS-CoV-2 viral load was reduced by 4 $\log_{10}$ copies/mL from baseline to Day 14 in this participant, and self-reported symptoms were alleviated by Day 7 (**Figure 3**)

Figure 2. Map of Observed Amino Acid Substitutions of Nsp12 From the RDV Group on the Cryo-EM Structure of the SARS-CoV-2 Polymerase Complex



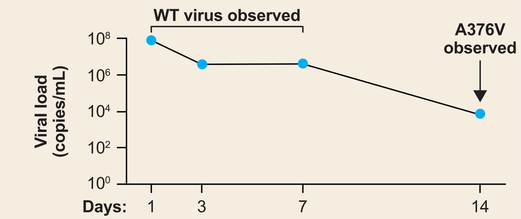
RDV, remdesivir; Cryo-EM, cryo-electron microscopy; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; RDV-TP, triphosphate form of remdesivir; Pol, polymerase; NiRAN, nidovirus RNA-dependent RNA polymerase-associated nucleotidyltransferase.

Table 3. RDV EC_{50} Against SARS-CoV-2 Reference and Mutant Replicons

Gene	Substitution	Average RDV EC_{50}, nM ± SD	EC_{50} fold-change from WT
	SH01 WT	10.0 ± 2.17	1.0
Nsp12	S759A/V792I (positive control)	99.1 ± 10.1	9.9
Nsp8	S41F	7.8 ± 0.1	0.8
Nsp10	A1D	23.2 ± 0.6	2.3
Nsp10	T102I	18.3 ± 3.0	1.8
Nsp12	S6L	NA	NA
Nsp12	T206I	8.4 ± 6.7	0.8
Nsp12	P232L	7.3 ± 1.1	0.7
Nsp12	A376V	126.4 ± 34.8	12.6
Nsp12	T394M	12.0 ± 2.7	1.2
Nsp12	A526S	10.9 ± 0.8	1.1
Nsp12	A634S	14.0 ± 0.8	1.4
Nsp13	T137M	10.7 ± 4.3	1.1
Nsp13	G196V	NA	NA
Nsp13	E341D	8.5 ± 3.2	0.9
Nsp13	S577F	NA	NA
Nsp13	A598V	10.7 ± 0.4	1.1
Nsp14	A214S	14.5 ± 3.3	1.4
Nsp14	K362R	11.5 ± 0.8	1.4

RDV, remdesivir; EC_{50}, half-maximal effective concentration; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; SD, standard deviation; WT, wild-type; NA, not applicable.
SH01 WT is a WT reference SARS-CoV-2 replicon generated from clinical isolate from Shanghai (lineage B).

Figure 3. Viral Load Decrease and Symptom Alleviation in Participant With Nsp12 Substitution A376V

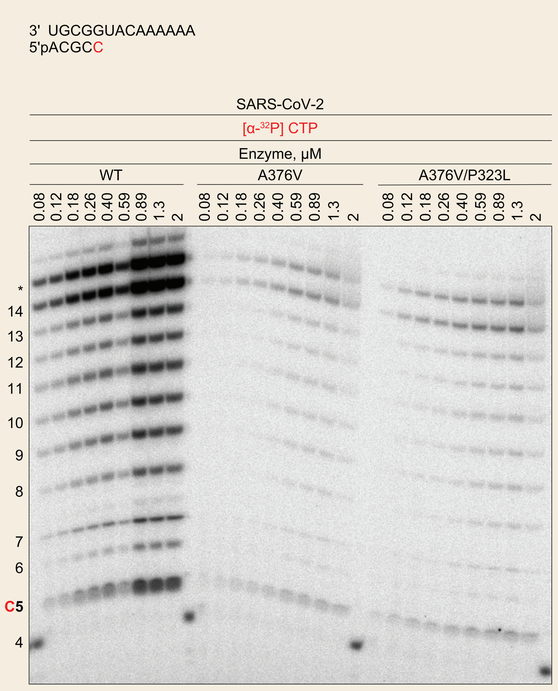


WT, wild-type; RDV, remdesivir.
[a]Self-reported symptoms scale: (4) very much, (3) quite a bit, (2) somewhat, (1) a little bit, (0) not at all.

A376V Is Associated With Diminished Fitness

- The A376V substitution is rare in sequences in the Global Initiative on Sharing All Influenza Data database as of January 6, 2023 (3 of 14 million sequences)
- A SARS-CoV-2 replicon containing the A376V substitution in Nsp12 showed a replication capacity of 2.98% compared to the WT control replicon
- Both the A376V mutant RdRp and the A376V/P323L double mutant RdRp were compromised in their ability to promote RNA extension (**Figure 4**)

Figure 4. A376V Mutant Enzyme Showed Compromised Activity in a Biochemical Assay



SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CTP, cytidine triphosphate; WT, wild-type; RdRp, RNA-dependent RNA polymerase.
Panel A shows the primer sequence with incorporated [α-^{32}P] CTP in red.
Panel B shows the RNA products synthesized by the WT, A376V, and A376V/P323L mutant RdRps with the lengths of extended RNA products shown on the left.
*Indicates likely SARS-CoV-2 RdRp slippage.

Conclusions

- Emergent substitutions in the SARS-CoV-2 replication complex, including Nsp12, were observed with similar frequency in the RDV and placebo groups
- One RDV-treated participant had emergence of an Nsp12 substitution (A376V) associated with 12.6-fold reduced susceptibility to RDV in vitro, possibly due to its proximity to the RNA template 5' overhang; however, this substitution demonstrated diminished fitness
  - This participant had an alleviation of baseline symptoms by Day 7 and a 4-log reduction in viral load by Day 14
- The resistance analysis results from the PINETREE study support a high barrier to the development of RDV resistance in patients with COVID-19

Disclosures: LR, JL, RM, DH, SX, JKP, RHH, DPP, MA, and CH are stockholders and employees of Gilead Sciences, Inc. HWL has nothing to disclose. GC is a shareholder and former employee of Gilead Sciences, Inc., and an employee of Vir Biotechnology, Inc. MG received funding from Gilead Sciences, Inc., for studies on the mechanism of action of RDV.

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