Resistance Analyses From the Remdesivir Phase 3 REDPINE Study in Participants With Severely Reduced Kidney Function Who Were Hospitalized for COVID-19

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Key Findings

- In participants with severe kidney impairment who progressed to all-cause death or invasive mechanical ventilation (IMV), or who had a viral load greater than the lower limit of quantification (LLOQ) at Day 14, emerging Nsp12 substitutions postbaseline were observed in both the remdesivir (RDV) and placebo groups
- Nsp12 substitutions with low reductions in susceptibility to RDV (≤3.4-fold change) were observed in 4 participants in the RDV group 9 days after cessation of treatment
  - Three of these 4 participants were solid organ transplant recipients and received concomitant immunosuppressive therapies during the study
  - The clinical significance of these substitutions is unknown

Conclusion

Resistance analyses from the REDPINE study in participants with severely reduced kidney function who were hospitalized for COVID-19 support a high barrier to high-level resistance to RDV

References: **1.** Veklury® (remdesivir) injection, for intravenous use [package insert]. Gilead Sciences, Inc; 2023. **2.** Beigel JH, et al. *N Engl J Med*. 2020;383(19):1813-1826. **3.** Spinner CD, et al. *JAMA*. 2020;324(11):1048-1057. **4.** Goldman JD, et al. *N Engl J Med*. 2020;383(19):1827-1837. **5.** Gottlieb RL, et al. *N Engl J Med*. 2022;386(4):305-315. **6.** Santos JR, et al. Presented at: 33rd European Congress of Clinical Microbiology and Infectious Diseases (ECCMID); April 15-18, 2023; Copenhagen, Denmark. Poster P2635. **7.** Xie X, et al. *Nat Commun*. 2020;11(1):5214. **8.** Zhang Y, et al. *Antiviral Res*. 2021;185:104974. **9.** Malone BF, et al. *Nature*. 2023;614(7949):781-787. **10.** Pitts J, et al. *Antimicrob Agents Chemother*. 2022;66(6):e00222-22. **11.** Global Initiative on Sharing All Influenza Data (GISAID). Accessed August 31, 2023. https://gisaid.org.

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Introduction

- RDV is a nucleotide analog prodrug approved for the treatment of COVID-19 in nonhospitalized and hospitalized adult and pediatric patients[1]
- The clinical benefit of RDV in patients with COVID-19 has been demonstrated in multiple Phase 3 trials[2-5]
- The Phase 3 REDPINE study, although underpowered for assessment of efficacy, demonstrated the safety of RDV for the treatment of COVID-19 in participants with kidney impairment, including those on dialysis, without the requirement for dose adjustment[6]

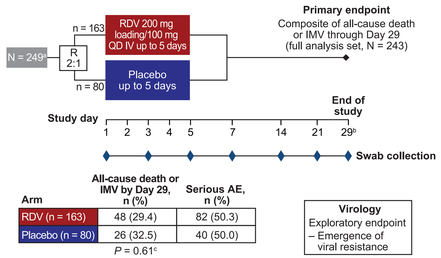
Objective

- To present SARS-CoV-2 resistance analyses from the Phase 3 REDPINE study

Methods

- REDPINE (ClinicalTrials.gov Identifier: NCT04745351) was a randomized, double-blind, placebo-controlled, parallel-group, multicenter study in participants aged ≥12 years with severely reduced kidney function (estimated glomerular filtration rate <30 mL/min/1.73 m^2) who were hospitalized for COVID-19[6] (**Figure 1**)
- Participants were randomly assigned (2:1) to receive RDV or placebo for 5 days

Figure 1. REDPINE Study Design[6]



Arm	All-cause death or IMV by Day 29, n (%)	Serious AE, n (%)
RDV (n = 163)	48 (29.4)	82 (50.3)
Placebo (n = 80)	26 (32.5)	40 (50.0)
	P = 0.61[c]	

Virology
Exploratory endpoint – Emergence of viral resistance

R, randomization; RDV, remdesivir; QD, once daily; IV, intravenous; IMV, invasive mechanical ventilation; AE, adverse event.
[a]249 participants were randomized, but 6 were not treated.
[b]If a participant was discharged prior to Day 29, a phone follow-up was completed on Days 29 and 60.
[c]The study was underpowered for efficacy due to insufficient enrollment.

- Nasopharyngeal swab samples were collected on Days 1 (baseline), 3, 5, 7, 14, 21, and/or 29
- The criteria for sequencing analysis included (1) all participants with all-cause death or IMV; or (2) all participants with a SARS-CoV-2 viral load >LLOQ (2228 copies/mL) on Day 14
- Full-genome deep sequencing of SARS-CoV-2 from nasopharyngeal swab samples was performed using Illumina MiSeq or NextSeq (DDL Diagnostic Laboratory)
- Sequencing was performed on samples collected on Day 1, on the last available day on treatment, and on Day 14 or the last available visit with a SARS-CoV-2 viral load >LLOQ
- Participants who did not have a viral load >LLOQ at Day 1 and ≥1 qualifying postbaseline sample were excluded from resistance analyses
- Emergent amino acid substitutions in SARS-CoV-2 Nsp12 were evaluated for RDV susceptibility by phenotypic testing of site-directed mutants in a replicon system[7,8]
  - Emergent substitutions in Nsp8, Nsp10, Nsp13, and Nsp14 were also phenotypically evaluated if detected in the same sample as emergent Nsp12 substitutions

Results

Participants

- Of the 243 participants treated in total, 82 met the criteria for sequencing
  - 15 participants with missing viral load data or a baseline viral load <LLOQ were excluded (9 participants in the RDV group and 6 participants in the placebo group)
  - Sequencing data at both the baseline and postbaseline time points were available for 41/46 (89.1%) participants in the RDV group and 19/21 (90.5%) participants in the placebo group (**Table 1**)

Table 1. REDPINE Sequencing Data

	RDV (n = 163)	Placebo (n = 80)
Met resistance analysis criteria and sequencing attempted, n/N (%)	46/163 (28.2)	21/80 (26.3)
All-cause death or IMV by Day 29	39/163 (23.9)	20/80 (25.0)
Viral load >LLOQ at Day 14 without death or IMV	7/163 (4.3)	1/80 (1.3)
Sequencing data available, n/N (%)		
Baseline	45/46 (97.8)	20/21 (95.2)
Postbaseline	42/46 (91.3)	20/21 (95.2)
Baseline and postbaseline, n/N (%)	41/46 (89.1)	19/21 (90.5)
Participants with emergent Nsp12 substitutions	8/41 (19.5)[a]	1/19 (5.3)[a]

RDV, remdesivir; IMV, invasive mechanical ventilation; LLOQ, lower limit of quantification.
[a]*P* = 0.25.

Substitutions in Nsp12

- A total of 8 participants who received RDV had emergent postbaseline Nsp12 substitutions (**Table 1**)
  - Among participants with both baseline and postbaseline data, there was a trend for a higher rate of emerging Nsp12 substitutions in those treated with RDV compared with those who received placebo; however, this was not statistically significant
- In the RDV group, a lower proportion of participants with Nsp12 substitutions met the resistance analysis criterion of all-cause death or IMV by Day 29 compared to those without the substitutions; however, this was not statistically significant (**Table 2**)

Table 2. Resistance Analysis Criteria in Participants Treated With RDV With or Without Nsp12 Substitutions

Resistance analysis criteria, n (%)	With Nsp12 substitutions (n = 8)	Without Nsp12 substitutions (n = 33)
All-cause death or IMV by Day 29	5 (62.5)[a]	30 (90.9)[a]
SARS-CoV-2 viral load >LLOQ on Day 14	3 (37.5)[a]	3 (9.1)[a]

RDV, remdesivir; IMV, invasive mechanical ventilation; LLOQ, lower limit of quantification.
[a]*P*=0.08.

- A total of 6 emergent amino acid substitutions in Nsp12 were observed in the RDV group but not in the placebo group (**Table 3**)

Table 3. Amino Acid Substitutions Detected in Nsp12 Postbaseline

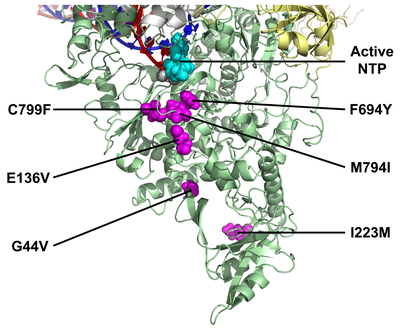
Emergent Nsp12 substitutions, n (%)[a]	RDV (n = 41)	Placebo (n = 19)	Total (n = 60)
F694Y	3 (7.3)	0	3 (5.0)
M794I	2 (4.9)	0	2 (3.3)
C799F	1 (2.4)	0	1 (1.7)
G44V	1 (2.4)	0	1 (1.7)
E136V + I223M	1 (2.4)	0	1 (1.7)
P384S	0	1 (5.3)	1 (1.7)

RDV, remdesivir.
[a]N = participants with both baseline and postbaseline sequencing data available.

Structural Analysis of Emergent Nsp12 Substitutions in Participants in the RDV Group

- None of the Nsp12 substitutions observed in the RDV group (F694Y, M794I, C799F, G44V, E136V, and I223M) were found to be in direct contact with the incoming active nucleoside triphosphate substrate or the RNA (**Figure 2**)
  - F694Y was located close to the nucleoside triphosphate substrate
  - M794I and C799F were part of an active site motif that governs the closing of the active site

Figure 2. Map of RDV Emergent Nsp12 Substitutions on Cryo-EM Structure of SARS-CoV-2 Polymerase Complex[9]



RDV, remdesivir; Cryo-EM, cryo-electron microscopy; NTP, nucleoside triphosphate.

Substitutions in Nsp8, Nsp10, Nsp13, and Nsp14

- Emergent substitutions in Nsp13 were detected in 4/41 (9.8%) participants in the RDV group and in 1/19 (5.3%) participants in the placebo group
  - None of the emergent substitutions in Nsp13 coemerged with Nsp12 substitutions; thus, they were not assessed for phenotypic susceptibility to RDV
- No emergent substitutions in Nsp8, Nsp10, or Nsp14 were detected in participants from either the RDV or placebo group

Phenotype of Emergent Substitutions in the RDV Group

- F694Y, I223M, and G44V had fold changes similar to wild type (WT; ≤1.3 fold), indicating no impact on antiviral activity
- C799F, M794I, and E136V showed low-level reduced susceptibility to RDV (2.9- to 3.4-fold change compared to WT; **Table 4**)

Table 4. RDV EC_{50} Against SARS-CoV-2 Reference and Mutant Replicons

Nsp12 substitutions	Average RDV EC_{50} (nM ± SD)	EC_{50} fold change from WT	Testing system
SH01 WT	9.5 ± 0.5	1.0	Replicon
G44V	8.0 ± 0.9	0.8	
E136V + I223M	27.2 ± 1.8	2.9	
E136V	27.2 ± 1.2	2.9	
I223M	12.1 ± 0.3	1.3	
M794I	27.6 ± 1.3	2.9	
C799F	49.9 ± 2.5[a]	3.4	Replicon[a]
F694Y	112.3 ± 1.6[b]	1.1	Recombinant[b]

RDV, remdesivir; EC_{50}, half-maximal effective concentration; SD, standard deviation; WT, wild type.
[a]C799F was tested separately in the replicon assay, and the EC_{50} fold-change value was calculated using the WT from that experiment, with a mean ± SD EC_{50} value of 14.7 ± 3.0 nM.
[b]F694Y was tested using a recombinant SARS-CoV-2 WA1 virus containing a nanoluciferase transgene, as described previously.[10] EC_{50} fold change was calculated from the WA1 WT strain, with a mean ± SD EC_{50} value of 99 ± 2 nM. F694Y has been tested in the replicon assay and did not replicate.

Details of Participants With Emergent Nsp12 Substitutions Conferring Reduced RDV Susceptibility

- Four participants had emergence of substitutions with low-level reduced susceptibility to RDV
  - All experienced a viral load decline while receiving RDV, and no substitutions were detected at Day 5 (the last day of RDV treatment)
  - These participants had varying levels of kidney disease and other risk factors for progression to severe COVID-19 (**Figure 3**)
  - The emergent substitutions were present as mixtures with the WT amino acid and were detected in samples collected 9 days after cessation of RDV treatment (**Figure 4**)
  - Three of these 4 (75.0%) participants had received solid organ transplants and were on concomitant immunosuppressive therapies versus 35/163 (21.5%) participants in the overall study population
- As of July 8, 2023, the prevalence of the Nsp12 substitutions with reduced susceptibility in the GISAID database is 0.00106% for C799F, 0.003% for M794I, and 0.00013% for E136V[11]

Figure 3. Medical History of Participants With Nsp12 Substitutions Associated With Reduced Susceptibility to RDV

Participant 1
Medical history
- CKD
- Hypertension
- Diabetes
- Cardio/cerebrovascular disease
- Transplant (lung)

Resistance analysis criteria
- All-cause death or IMV by Day 29

Nsp12 substitution
- M794M/I

Participant 2
Medical history
- ESKD
- Hypertension
- Chronic lung disease
- Transplant (kidney)

Resistance analysis criteria
- Viral load >LLOQ on Day 14

Nsp12 substitution
- M794M/I

Participant 3
Medical history
- ESKD
- Hypertension
- Diabetes
- Cardio/cerebrovascular disease
- BMI ≥30 kg/m^2
- Current cancer
- Transplant (kidney)

Resistance analysis criteria
- Viral load >LLOQ on Day 14

Nsp12 substitution
- C799C/F

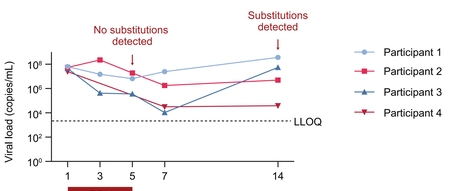
Participant 4
Medical history
- AKI
- Chronic liver disease
- BMI ≥30 kg/m^2

Resistance analysis criteria
- All-cause death or IMV by Day 29

Nsp12 substitution
- E136E/V

RDV, remdesivir; CKD, chronic kidney disease; IMV, invasive mechanical ventilation; ESKD, end-stage kidney disease; LLOQ, lower limit of quantification; BMI, body mass index; AKI, acute kidney injury.

Figure 4. Viral Load Dynamics in Participants With Nsp12 Substitutions Associated With Reduced Susceptibility to RDV



RDV, remdesivir; LLOQ, lower limit of quantification.

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