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CARAVAN

Resistance Analyses From the Remdesivir Phase 2/3 CARAVAN Study in Pediatric and Neonatal Participants Hospitalized With COVID-19

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Conclusions

- Among 28 pediatric participants treated with remdesivir with baseline and postbaseline SARS-CoV-2 sequencing data, 5 substitutions in Nsp12 emerged in 3 participants as mixtures with wildtype
- Among these, Nsp12 V166V/L and V792V/I were observed post baseline in the same participant
  - Relative to the wildtype reference strain, V792I alone and in combination with V166L showed 3.20- and 6.12-fold reduced susceptibility to remdesivir, respectively
  - The participant recovered clinically and was discharged
- All other observed baseline and postbaseline substitutions showed no change in remdesivir susceptibility
- These analyses from the CARAVAN study support a high barrier to clinically meaningful SARS-CoV-2 resistance to remdesivir in pediatric patients with COVID-19

Plain Language Summary

- Remdesivir is an antiviral drug that stops the replication of SARS-CoV-2, the virus that causes COVID-19
- Antiviral drug resistance occurs when a virus mutates and no longer responds to a drug, causing the drug to lose its effectiveness
- This study aimed to evaluate the potential for SARS-CoV-2 to become resistant to remdesivir in infants and children with COVID-19
- This study found that, although several mutations in the virus did occur after remdesivir treatment, these mutations had very little effect on remdesivir's ability to stop the virus from replicating
- This shows that remdesivir is unlikely to lose its effectiveness against SARS-CoV-2 in infants and children

Introduction

- Remdesivir (RDV) is a broad-spectrum nucleotide analog prodrug approved for the treatment of COVID-19 in adults and pediatric patients (birth to <18 years of age, weighing >1.5 kg) who:
  - Are hospitalized, or
  - Are not hospitalized, have mild-to-moderate COVID-19, and are at high risk for progression to severe COVID-19, including hospitalization or death[1]
- RDV targets the highly conserved RNA-dependent RNA polymerase of SARS-CoV-2, Nsp12[1]
- Phase 3 studies of adolescent and adult participants with COVID-19 who received RDV showed that treatment-emergent Nsp12 amino acid substitutions resulted in low-to-minimal changes in RDV susceptibility[2-4]
- The Phase 2/3, single-arm, open-label CARAVAN study demonstrated the safety of RDV in children hospitalized with COVID-19 and no identified resistance in pediatric participants aged ≥28 days (Cohorts 1-4 and 8)[5]

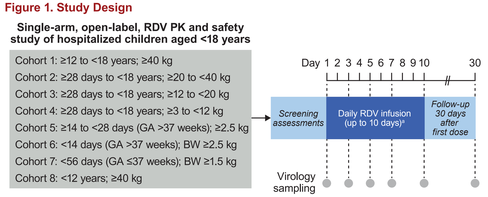
Objective

- To present integrated SARS-CoV-2 resistance analyses from the Phase 2/3 CARAVAN study, which included pediatric participants aged ≥28 days (Cohorts 1-4 and 8) and term and preterm neonates (Cohorts 5-7)

Methods

- CARAVAN was a Phase 2/3, single-arm, open-label study wherein pediatric participants (birth to <18 years of age) hospitalized with COVID-19 were enrolled in 8 age- and weight-based cohorts and received RDV for ≤10 days (**Figure 1**)

Figure 1. Study Design



Gray circles indicate days when assessments were performed.
[a]Cohorts 1 and 8: RDV 200 mg on Day 1 followed by RDV 100 mg QD up to Day 10; Cohorts 2 to 5: RDV 5 mg/kg on Day 1 followed by RDV 2.5 mg/kg QD up to Day 10; Cohorts 6 and 7: RDV 2.5 mg/kg on Day 1 followed by RDV 1.25 mg/kg QD up to Day 10.
BW, birth weight; GA, gestational age; PK, pharmacokinetics; QD, once daily; RDV, remdesivir.

- Full-genome deep sequencing of SARS-CoV-2 was performed on respiratory samples collected on Days 1 (baseline), 3, 5, 7, and/or 10
- A subgenomic replicon system in Huh7-1CN cells was used to assess the antiviral activity of RDV against site-directed mutants bearing substitutions observed in the replication complex that met any of the following criteria: (1) baseline Nsp12 substitutions detected in ≥3 participants; (2) baseline replication complex substitutions detected in ≥2 participants with viral RNA increase post baseline; or (3) treatment-emergent postbaseline replication complex substitutions
- Structural analysis of identified substitutions was conducted on a prior cryo-electron microscopy–based model of the replication-transcription complex[6]

Results

Participants

- Demographic and baseline characteristics for each cohort are listed in **Table 1**

Table 1. Participant Demographic and Baseline Characteristics

Characteristic	Cohort 1 (n = 12)	Cohort 2 (n = 12)	Cohort 3 (n = 12)	Cohort 4 (n = 12)	Cohort 5 (n = 3)	Cohort 6 (n = 1)	Cohort 7 (n = 1)	Cohort 8 (n = 5)	Total (n = 58)
Age,[a] mean (range)	15 (12-17) years	10 (4-16) years	4 (2-7) years	0.4 (0.1-0.9) years	16 (15-16) days	12 (12-12) days	30 (30-30) days	10 (8-11) years	7 (0-17) years
Weight, kg, median (Q1, Q3)	83.5 (56.8, 106.9)	26.5 (25.0, 30.9)	14.6 (13.4, 18.2)	5.0 (4.4, 8.5)	3.5 (2.8, 3.5)	3.5 (3.5, 3.5)	2.2 (2.2, 2.2)	73.0 (55.1, 80.0)	20.7 (9.0, 52.0)
Nasal/OP SARS-CoV-2 viral RNA copy number									
n	5	5	4	3	1	0	0	1	19
Log_{10} copies/mL, median (Q1, Q3)	5.7 (4.6, 6.4)	3.1 (2.7, 7.7)	2.7 (2.7, 3.7)	5.1 (3.4, 7.7)	5.0 (5.0, 5.0)	–	–	6.2 (6.2, 6.2)	4.6 (2.7, 6.4)
NP/OP SARS-CoV-2 viral RNA copy number									
n	5	4	5	7	2	1	1	4	29
Log_{10} copies/mL, median (Q1, Q3)	5.1 (5.0, 6.4)	6.0 (4.1, 6.6)	6.6 (4.0, 6.8)	6.4 (3.9, 7.2)	6.0 (5.9, 6.1)	4.1 (4.1, 4.1)	8.5 (8.5, 8.5)	6.9 (5.2, 7.0)	6.4 (4.1, 6.8)
Endotracheal tube aspirate SARS-CoV-2 viral RNA copy number									
n	1	4	2	3	1	0	0	0	11
Log_{10} copies/mL, median (Q1, Q3)	5.4 (5.4, 5.4)	4.9 (2.7, 8.5)	6.1 (5.5, 6.7)	7.4 (4.9, 7.7)	4.6 (4.6, 4.6)	–	–	–	5.5 (4.6, 7.4)

[a]Age for term neonatal (Cohorts 5 and 6) and preterm neonate and infant (Cohort 7) cohorts is shown in days; age for pediatric participants (Cohorts 1-4 and 8) is shown in years.
NP, nasopharyngeal; OP, oropharyngeal.

Baseline Virology Analysis

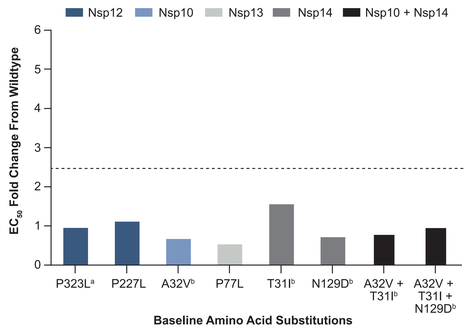
- Of the 58 participants enrolled and treated, baseline sequencing data were obtained from 37 participants (**Table 2**)
- Six baseline amino acid polymorphisms met the criteria for phenotyping

Table 2. Participants With Baseline Amino Acid Substitutions That Met the Criteria for Phenotyping

	Cohort 1 (n = 12)	Cohort 2 (n = 12)	Cohort 3 (n = 12)	Cohort 4 (n = 12)	Cohort 5 (n = 3)	Cohort 6 (n = 1)	Cohort 7 (n = 1)	Cohort 8 (n = 5)	Total (n = 58)
Participants with sequencing data at baseline, n	7	5	6	9	3	1	1	5	37
Nsp12 substitution detected in ≥3 participants, n (%)									
P227L	0	0	0	2 (22)	1 (33)	0	0	0	3 (8)
P323L	7 (100)	5 (100)	6 (100)	9 (100)	3 (100)	1 (100)	1 (100)	5 (100)	37 (100)
Replication complex substitution detected in ≥2 participants with viral RNA increase post baseline, n (%)									
Nsp10 A32V	0	2 (40)	0	0	0	0	0	0	2 (5)
Nsp13 P77L	1 (14)	0	0	0	1 (33)	0	0	0	2 (5)
Nsp14 T31I	0	2 (40)	0	0	0	0	0	0	2 (5)
Nsp14 N129D	0	2 (40)	2 (33)	1 (11)	0	0	0	0	5 (14)

- Phenotypic evaluation demonstrated that no baseline substitutions impacted RDV susceptibility (range of 0.53- to 1.55-fold change in half-maximal effective concentration [EC_{50}] compared to wildtype reference; **Figure 2**)

Figure 2. RDV EC_{50} Fold Change From Wildtype Against Baseline Amino Acid Substitutions



N ≥2 experiments were performed in technical triplicate to obtain EC_{50} values. Fold change values were calculated by dividing the mean EC_{50} of the variant by the mean EC_{50} of the SH01 reference strain. EC_{50} fold change values <2.5 (dashed line) are within the variability of the assay, indicating no change in susceptibility to RDV compared to the SH01 reference strain.
[a]Nsp12 P323L resistance was assessed previously using a recombinant SARS-CoV-2 WA1 virus containing a nanoluciferase transgene.[7]
[b]The Nsp10 A32V, Nsp14 T31I, and Nsp14 N129D substitutions were observed in the same 2 participants at baseline and were tested alone and in combination.
EC_{50}, half-maximal effective concentration; RDV, remdesivir; SH01, wildtype SARS-CoV-2 replicon generated from clinical isolate from Shanghai (lineage B).

Postbaseline Virology Analysis

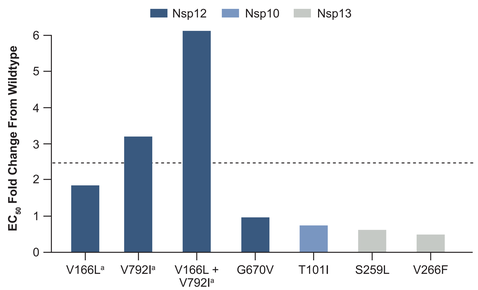
- Baseline and postbaseline sequencing data were obtained from 28 participants (**Table 3**)
  - Five postbaseline substitutions emerging in Nsp12 were identified in 3 participants as mixtures with wildtype Nsp12
  - Six postbaseline substitutions emerging in Nsp9, Nsp10, and Nsp13 were identified in 3 participants
- Of the postbaseline substitutions, only Nsp12 V792I alone and in combination with V166L showed low-level reduced susceptibility to RDV, with EC_{50} fold changes from wildtype of 3.20 and 6.12, respectively (**Figure 3**)

Table 3. Participants With Postbaseline Amino Acid Substitutions

	Cohort 1 (n = 12)	Cohort 2 (n = 12)	Cohort 3 (n = 12)	Cohort 4 (n = 12)	Cohort 5 (n = 3)	Cohort 6 (n = 1)	Cohort 7 (n = 1)	Cohort 8 (n = 5)	Total (n = 58)
Participants with sequencing data at baseline and post baseline, n	5	4	4	6	2	1	1	5	28
Participants with no emergent substitutions in Nsp12, n (%)	4 (80)	4 (100)	3 (75)	6 (100)	2 (100)	0	1 (100)	5 (100)	25 (89)
Participants with emergent substitutions, n (%)									
Nsp12	1 (20)	0	1 (25)	0	0	1 (100)	0	0	3 (11)
V166V/L[a]	0	0	0	0	0	1 (100)	0	0	1 (4)
V495V/F (<15%)	0	0	1 (25)	0	0	0	0	0	1 (4)
A656A/P[b]	1 (20)	0	0	0	0	0	0	0	1 (4)
G670G/V[b]	1 (20)	0	0	0	0	0	0	0	1 (4)
V792V/I[a] (<15%)	0	0	0	0	0	1 (100)	0	0	1 (4)
Nsp8	0	0	0	0	0	0	0	0	0
Nsp9	1 (20)	0	0	0	0	0	0	0	1 (4)
N95N/D[c] (<15%)	1 (20)	0	0	0	0	0	0	0	1 (4)
Nsp10	0	0	1 (25)	0	0	0	0	0	1 (4)
D64D/Y[d]	0	0	1 (25)	0	0	0	0	0	1 (4)
T101T/I[d]	0	0	1 (25)	0	0	0	0	0	1 (4)
Nsp13	1 (20)	0	0	1 (17)	0	0	0	0	2 (7)
R248R/I[c]	1 (20)	0	0	0	0	0	0	0	1 (4)[e]
S259S/L	0	0	0	1 (17)	0	0	0	0	1 (4)[e]
V266V/F[c]	1 (20)	0	0	0	0	0	0	0	1 (4)[e]
Nsp14	0	0	0	0	0	0	0	0	0

[a]The Nsp12 V166V/L substitution was observed in the sample collected on Days 7 and 10 in 1 participant in Cohort 6. The Nsp12 V792V/I substitution was observed in the sample collected on Day 10 from the same participant at <15% of the virus population, which was below the cutoff for variant calling. Due to read length constraints, sequencing was unable to determine whether V166L and V792I were present on the same viral genomes.
[b]The Nsp12 A656A/P and G670G/V substitutions were observed in the sample collected on Day 3 from the same participant.
[c]The Nsp9 N95N/D and Nsp13 R248R/I and V266V/F substitutions were observed in the sample collected on Day 10 from the same participant.
[d]The Nsp10 D64D/Y and T101T/I substitutions were observed in the sample collected on Day 3 from the same participant.
[e]No Nsp13 sequence coverage was obtained for 1 participant at positions 248, 259, and 266.

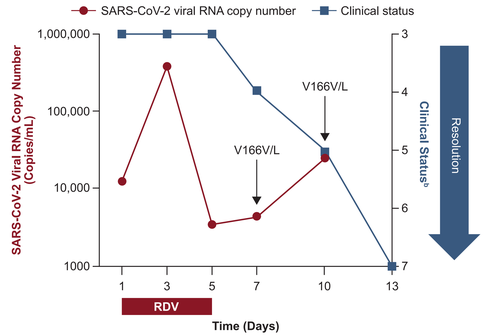
Figure 3. RDV EC_{50} Fold Change From Wildtype Against Postbaseline Amino Acid Substitutions



N ≥2 experiments were performed in technical triplicate to obtain EC_{50} values. Fold change values were calculated by dividing the mean EC_{50} of the variant by the mean EC_{50} of the SH01 reference strain. No replication was observed in the following replicons: Nsp9 N95D, Nsp10 D64Y, Nsp12 V495F, Nsp12 A656P, Nsp12 A656P + G670V, and Nsp13 R248I. EC_{50} fold change values <2.5 (dashed line) are within the variability of the assay, indicating no change in susceptibility to RDV compared to the SH01 reference strain.
[a]The Nsp12 V166L and V792I substitutions were observed in the same participant and were tested alone and in combination.
EC_{50}, half-maximal effective concentration; RDV, remdesivir; SH01, wildtype SARS-CoV-2 replicon generated from clinical isolate from Shanghai (lineage B).

- The participant in whom Nsp12 V166V/L and V792V/I substitutions were observed achieved clinical recovery and was released from the hospital on Day 13 (**Figure 4**)

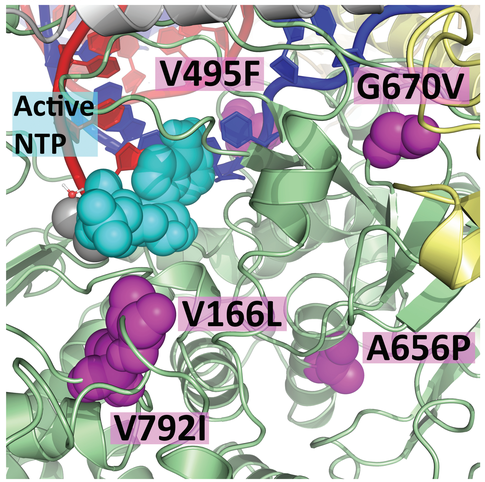
Figure 4. SARS-CoV-2 Viral RNA Copy Number and Clinical Status of Participant in Whom Nsp12 V166V/L and V792V/I[a] Substitutions Were Observed



NP/OP swab samples were collected on Days 1 (baseline), 3, 5, 7, and 10. Sequencing data from the Day 5 samples were not available due to assay failure.
[a]The Nsp12 V792V/I substitution was observed in a sample collected on Day 10 at <15% of the virus population.
[b]Clinical status was evaluated using a predefined 7-point ordinal scale. Category 1: death; Category 2: hospitalized, on invasive mechanical ventilation or extracorporeal membrane oxygenation; Category 3: hospitalized, on noninvasive ventilation or high-flow oxygen devices; Category 4: hospitalized, requiring low-flow supplemental oxygen; Category 5: hospitalized, not requiring supplemental oxygen, requiring ongoing medical care (COVID-19–related or otherwise); Category 6: hospitalized, not requiring supplemental oxygen, no longer requiring ongoing medical care (other than per-protocol RDV administration); Category 7: not hospitalized.
NP, nasopharyngeal; OP, oropharyngeal; RDV, remdesivir.

- Structural modeling suggests that V166L and V792I are located on or adjacent to motif D of Nsp12 and may alter the dynamics of nucleoside triphosphate incorporation (**Figure 5**)

Figure 5. Structural Analysis of Observed Postbaseline Nsp12 Amino Acid Substitutions



The structure is a model of the preincorporated active NTP of RDV in the SARS-CoV-2 polymerase active site based on the 6XEZ cryo-electron microscopy structure.[6]
NTP, nucleoside triphosphate; RDV, remdesivir.

References: **1.** VEKLURY® (remdesivir) for injection, for intravenous use [package insert]. Gilead Sciences, Inc.; 2024. **2.** Hedskog C, et al. *J Infect Dis*. 2023;228:1263-73. **3.** Hedskog C, et al. *Viruses*. 2024;16:546. **4.** Rodriguez L, et al. Presented at: Conference on Retroviruses and Opportunistic Infections (CROI); February 19-22, 2023; Seattle, WA, USA. Poster 561. **5.** Ahmed A, et al. *Pediatrics*. 2024;153:e2023063775. **6.** Malone BF, et al. *Nature*. 2023;614(7949):781-7. **7.** Pitts J, et al. *Antimicrob Agents Chemother*. 2022;66:e00222-22.

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Disclosure: **JM**, **JL**, **LR**, **DH**, **SX**, **PYH**, **NP**, **CM**, **SC**, **KK**, **JP**, **DP**, and **CH** are employees of and may own stock or stock options in Gilead Sciences, Inc.