Viral Resistance Analyses From the Obeldesivir OAKTREE Study in Low-Risk Nonhospitalised Patients With COVID-19

P0418

OAKTREE

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Conclusions

- In an analysis of nasal swab samples collected during the Phase 3 OAKTREE trial, emergent amino acid substitutions in the SARS-CoV-2 RNA-dependent RNA polymerase Nsp12 were observed in similar frequencies in participants who received obeldesivir twice daily for 5 days and in those who received the matched placebo
- Only 1 participant who received obeldesivir had an emergent Nsp12 substitution (C799F) associated with reduced (3.4-fold) in vitro susceptibility to obeldesivir
- The low-to-no change in obeldesivir susceptibility among the treatment-emergent Nsp12 substitutions indicated a high barrier to the development of obeldesivir resistance in low-risk, nonhospitalised patients with COVID-19

Plain Language Summary

- Obeldesivir is an oral antiviral drug that stops the replication of SARS-CoV-2, the virus that causes COVID-19
- Viruses can sometimes produce new copies of themselves that are less vulnerable to the effects of antiviral drugs, making it harder to treat the infection
- This study aimed to determine if SARS-CoV-2 can become resistant to obeldesivir treatment in people who have COVID-19 and a low risk of becoming severely ill from the disease
- Here, laboratory analyses showed that SARS-CoV-2 is not likely to develop resistance to obeldesivir in people who have COVID-19

Introduction

- Obeldesivir (ODV) is an oral nucleoside analogue prodrug inhibitor of the SARS-CoV-2 RNA-dependent RNA polymerase Nsp12 that has demonstrated activity against a broad spectrum of RNA viruses[1]
- The antiviral activity of ODV in patients with COVID-19 has been demonstrated in clinical trials, including the Phase 3 OAKTREE study conducted in nonhospitalised participants without risk factors for progression to severe COVID-19[2]

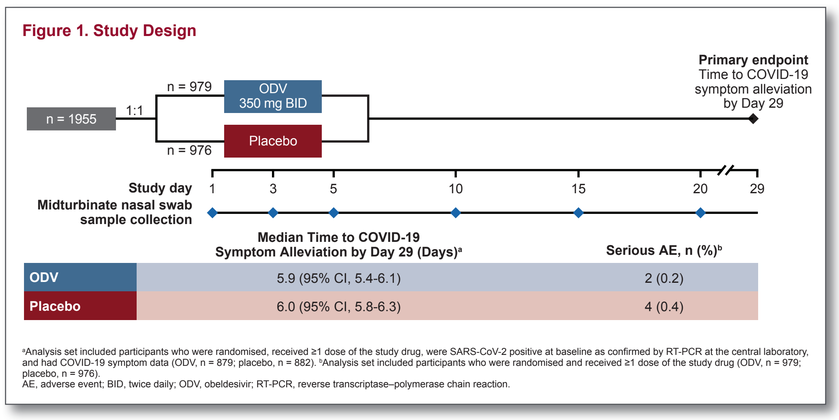
Objective

- To evaluate SARS-CoV-2 ODV resistance in the Phase 3 OAKTREE study

Methods

- OAKTREE was a multicentre, randomised, double-blind, placebo-controlled study wherein nonhospitalised participants aged ≥12 years who had COVID-19 and no risk factors for disease progression were randomly assigned to receive ODV or placebo twice daily for 5 days (**Figure 1**)
- The whole genome of SARS-CoV-2 was deep sequenced using Illumina MiSeq or NextSeq (DDL Diagnostic Laboratory) on all samples with viral load ≥2228 copies/mL that met the sequencing analysis criteria:
  - All participants in the ODV group and 50% of participants in the placebo group at baseline and on the last day of treatment
  - Participants with viral load increase of ≥1 $\log_{10}$ copies/mL compared to the previous time point
  - Participants with progression to hospitalisation or all-cause death by Day 29
  - Participants who did not achieve COVID-19 symptom alleviation or had symptom relapse by Day 29
  - Participants with positive rapid antigen test beyond Day 15 or viral antigen rebound
- The emergent Nsp12 substitutions observed in the ODV group were modelled on the cryo-electron microscopy structure of the SARS-CoV-2 replicase complex to determine the location of the substitutions in relation to the active site of Nsp12
- Amino acid substitutions in SARS-CoV-2 Nsp12 observed at baseline in ≥3 participants and Nsp12 substitutions that emerged post baseline in the ODV group (not observed in the placebo group) were phenotyped in a replicon system to determine if they altered susceptibility to ODV
  - Additionally, phenotyping was performed on amino acid substitutions that emerged post baseline in SARS-CoV-2 Nsp8, Nsp9, Nsp10, Nsp13, and Nsp14 if present in ≥2 participants in the ODV group but not in the placebo group

Figure 1. Study Design



	Median Time to COVID-19 Symptom Alleviation by Day 29 (Days)[a]	Serious AE, n (%)[b]
ODV	5.9 (95% CI, 5.4-6.1)	2 (0.2)
Placebo	6.0 (95% CI, 5.8-6.3)	4 (0.4)

[a]Analysis set included participants who were randomised, received ≥1 dose of the study drug, were SARS-CoV-2 positive at baseline as confirmed by RT-PCR at the central laboratory, and had COVID-19 symptom data (ODV, n = 879; placebo, n = 882). [b]Analysis set included participants who were randomised and received ≥1 dose of the study drug (ODV, n = 979; placebo, n = 976).
AE, adverse event; BID, twice daily; ODV, obeldesivir; RT-PCR, reverse transcriptase–polymerase chain reaction.

Results

Participants

- Of the 1955 participants randomised and treated, 287 participants (ODV, 174; placebo, 113) met the sequencing analysis criteria and had sequencing data at both baseline and postbaseline (**Table 1**)

Table 1. OAKTREE Sequencing Data

	ODV	Placebo
Treated population, n[a]	979	976
Participants with sequencing data available at baseline, n	714	700
Participants with sequencing data available at postbaseline, n	192	118
Participants with sequencing data available at both baseline and postbaseline, n	174	113
Participants with emergent Nsp12 substitutions, n/N (%)	30/174 (17.2)	23/113 (20.4)
Participants with Nsp12 substitutions with reduced susceptibility to ODV in vitro, n/N (%)	1/174 (0.6)	NA

[a]Analysis set included randomised participants who received ≥1 dose of study drug.
NA, not applicable; ODV, obeldesivir.

Baseline Virology Analysis

- Of the 1414 participants with baseline sequencing data, 1401 participants (ODV, 711; placebo, 690) had SARS-CoV-2 lineage determined
  - The following SARS-CoV-2 variants of interest and variants under monitoring as defined by the World Health Organization as of 5 March 2024 were observed: any XBB sublineage (observed in 96.1% of participants), XBB.1.5 (11.2%), XBB.1.6 (4.0%), XBB.2.3 (2.2%), XBB.1.9.1 (2.2%), and EG.5.1 (1.6%)
  - In the most prevalent Omicron subvariants (found in ≥20 participants), the Nsp12 substitutions observed were A250T, P323L, and G671S
- In total, 34 Nsp12 substitutions were observed in ≥3 participants at baseline (**Table 2**)
- Phenotyping of Nsp12 substitutions detected at baseline resulted in half-maximal effective concentration (EC_{50}) fold changes ≤2.1, suggesting that ODV maintains similar antiviral activity against replicons containing these substitutions compared with a replicon containing the wildtype reference sequence (**Figure 2**)

Postbaseline Amino Acid Substitutions in Nsp12

- Among participants with baseline and postbaseline sequencing data, rates of emergent amino acid substitutions in Nsp12 were similar between the ODV and placebo groups (**Table 1**)
- There were 20 Nsp12 substitutions observed in 229 postbaseline sequences in the ODV group (8.7%) and 12 Nsp12 substitutions observed in 126 postbaseline sequences in the placebo group (9.5%; **Table 3**)

Phenotype of the Emergent Nsp12 Amino Acid Substitutions in Participants Treated With ODV

- Only 1 Nsp12 substitution from 1 participant from the ODV group (C799F; first detected at Day 15) was associated with reduced in vitro susceptibility to ODV, with an EC_{50} fold change of 3.4 compared with the wildtype reference (**Figure 3**)
  - The participant reported that all symptoms resolved by Day 10 and that they did not experience symptom relapse
  - None of the other substitutions impacted ODV susceptibility (EC_{50} fold change ≤1.3)
  - This result aligns with what has previously been observed in Phase 3 clinical trials investigating remdesivir: C799F was identified in in vitro resistance selection experiments in the presence of the parent nucleoside, GS-441524, in 1 participant from the ACTT-1 study[3] and in 1 participant from the REDPINE study[4]

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

- Most of the Nsp12 substitutions that emerged in the ODV group were located on the protein surface, with no expectation of an impact on ODV activity (**Figure 4**)
  - The substitutions closest to the active site were T556S, A558S, H572R, and C799F

Postbaseline Substitutions in Nsp8, Nsp9, Nsp10, Nsp13, and Nsp14

- Of 287 participants with both baseline and postbaseline data, 54 participants (18.8%) had emergent substitutions in the replication complex genes Nsp8, Nsp9, Nsp10, Nsp13, or Nsp14
- Thirty-three participants of 174 in the ODV group (19.0%) and 21 participants of 113 in the placebo group (18.6%) had substitutions in any of these genes
- Of the 28 substitutions observed across the replication complex components, only 1 substitution, Nsp14 I42V, met the criteria for phenotyping
  - Phenotyping of Nsp14 I42V resulted in an ODV EC_{50} fold change of 0.6 compared with the wildtype reference, indicating no impact on antiviral activity (**Figure 5**)

Table 2. Baseline Amino Acid Substitutions[a] Detected in Nsp12

Baseline Nsp12 Substitution, n (%)	ODV (n = 714[b])	Placebo (n = 700[b])	Total (n = 1414[b])
D63N	15 (2.1)	10 (1.4)	25 (1.8)
H82R	1 (0.1)	2 (0.3)	3 (0.2)
K91R	0	3 (0.4)	3 (0.2)
A97A/V	4 (0.6)	1 (0.1)	5 (0.4)
A130T	1 (0.1)	3 (0.4)	4 (0.3)
R132R/K	1 (0.1)	4 (0.6)	5 (0.4)
N158N/S	2 (0.3)	1 (0.1)	3 (0.2)
Y163C	3 (0.4)	2 (0.3)	5 (0.4)
A185V	1 (0.1)	2 (0.3)	3 (0.2)
A250T	13 (1.8)	15 (2.1)	28 (2.0)
V257F	4 (0.6)	3 (0.4)	7 (0.5)
D258D/Y	3 (0.4)	0	3 (0.2)
Y273H	14 (2.0)	17 (2.4)	31 (2.2)
T276M	2 (0.3)	1 (0.1)	3 (0.2)
E277V	5 (0.7)	1 (0.1)	6 (0.4)
Y289H	2 (0.3)	3 (0.4)	5 (0.4)
T293T/I	3 (0.4)	0	3 (0.2)
P323L	714 (100)	698 (99.7)	1412 (99.9)
V354L	2 (0.3)	3 (0.4)	5 (0.4)
A382T	3 (0.4)	0	3 (0.2)
A382V	3 (0.4)	1 (0.1)	4 (0.3)
P461S	2 (0.3)	1 (0.1)	3 (0.2)
V473F	2 (0.3)	2 (0.3)	4 (0.3)
K478N	2 (0.3)	3 (0.4)	5 (0.4)
A529V	2 (0.3)	5 (0.7)	7 (0.5)
H613Y	1 (0.1)	4 (0.6)	5 (0.4)
M666I	1 (0.1)	3 (0.4)	4 (0.3)
G671S	686 (96.1)	677 (96.7)	1363 (96.4)
F694F/Y	7 (1.0)	0	7 (0.5)
T739I	6 (0.8)	4 (0.6)	10 (0.7)
M818I	2 (0.3)	2 (0.3)	4 (0.3)
Q822H	2 (0.3)	6 (0.8)	8 (0.6)
G823GinsD	2 (0.3)	3 (0.4)	5 (0.4)
R914S	2 (0.3)	1 (0.1)	3 (0.2)
Other[c]	67 (9.4)	50 (7.1)	117 (8.3)

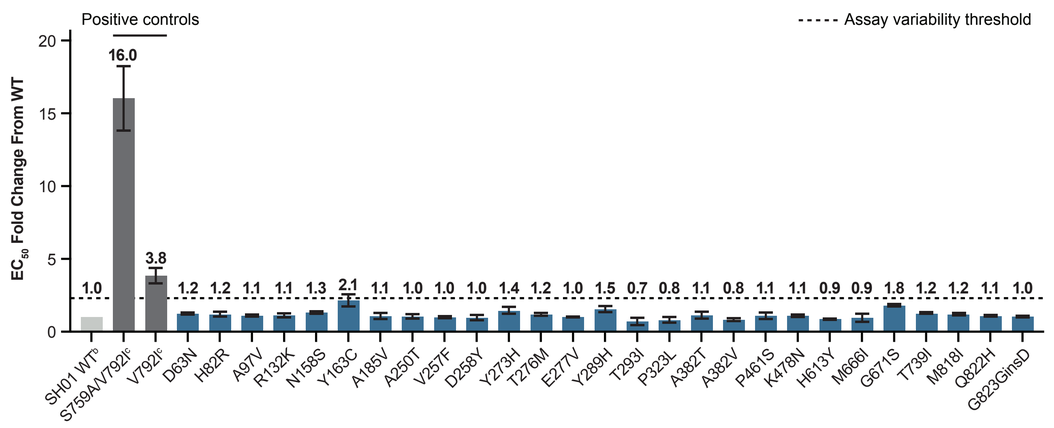
[a]Baseline sequences were compared with the WT reference sequence Wuhan-Hu-1, NC_045512.2. [b]Number of participants with baseline sequencing data available. [c]123 Nsp12 substitutions were observed in <3 participants. None of these substitutions have been associated with resistance to ODV.
ODV, obeldesivir; WT, wildtype.

Table 3. Emergent Postbaseline Amino Acid Substitutions in Nsp12

Emergent Nsp12 Substitution, n (%)	ODV (n = 174[a])	Placebo (n = 113[a])	Total (n = 287[a])
A34A/V	0	1 (0.9)	1 (0.3)
Y38Y/H	1 (0.6)	0	1 (0.3)
F70F/S	1 (0.6)	0	1 (0.3)
T76T/I	0	1 (0.9)	1 (0.3)
D92D/G	1 (0.6)	0	1 (0.3)
D140Y	1 (0.6)	0	1 (0.3)
A185A/V	1 (0.6)	0	1 (0.3)
G200G/D	1 (0.6)	0	1 (0.3)
G203S	1 (0.6)	0	1 (0.3)
Y273H	2 (1.1)	3 (2.7)	5 (1.7)
K288K/*	0	1 (0.9)	1 (0.3)
I307I/S	1 (0.6)	0	1 (0.3)
A311A/S	0	1 (0.9)	1 (0.3)
V338A	1 (0.6)	0	1 (0.3)
S364S/F	1 (0.6)	0	1 (0.3)
A382A/S	0	1 (0.9)	1 (0.3)
F422Y	1 (0.6)	0	1 (0.3)
K478N	1 (0.6)	0	1 (0.3)
A526A/V	0	1 (0.9)	1 (0.3)
T556T/S	1 (0.6)	0	1 (0.3)
A558A/S	1 (0.6)	0	1 (0.3)
H572H/R	1 (0.6)	0	1 (0.3)
V637V/A	0	1 (0.9)	1 (0.3)
M666M/I	0	1 (0.9)	1 (0.3)
G671S	5 (2.9)	2 (1.8)	7 (2.4)
F694F/Y	3 (1.7)	1 (0.9)	4 (1.4)
L707L/F	1 (0.6)	0	1 (0.3)
D711D/V	0	1 (0.9)	1 (0.3)
T739I	1 (0.6)	0	1 (0.3)
T739T/S	0	1 (0.9)	1 (0.3)
R750R/H	0	1 (0.9)	1 (0.3)
E796E/K	0	3 (2.7)	3 (1.0)
C799C/F	1 (0.6)	0	1 (0.3)
T806A	1 (0.6)	0	1 (0.3)
Q822H	1 (0.6)	0	1 (0.3)
G823GinsD	1 (0.6)	2 (1.8)	3 (1.0)

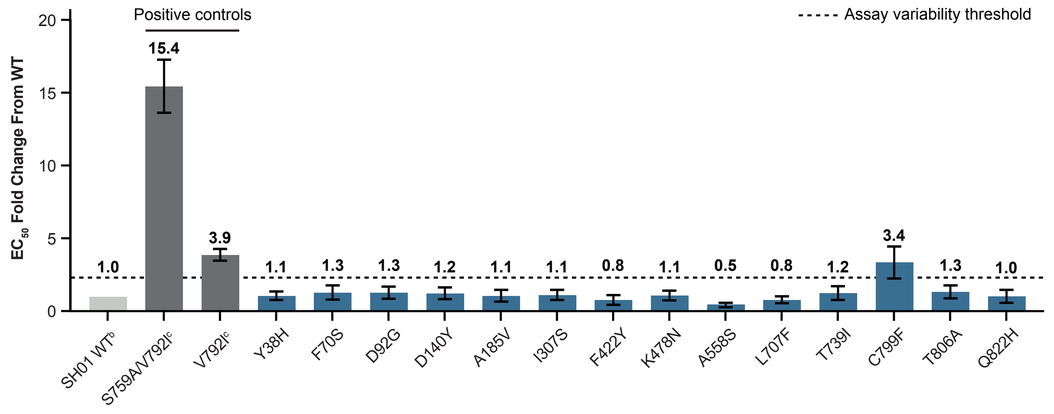
* denotes translational termination (stop) codon.
[a]Number of participants with baseline and postbaseline sequencing data available.
ODV, obeldesivir.

Figure 2. ODV EC_{50} Fold Changes[a] From WT of Baseline Nsp12 Amino Acid Substitutions



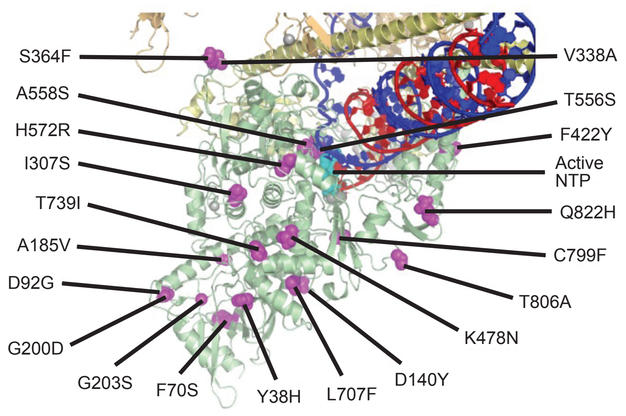
Data are represented as mean ± SD. For substitutions K91R, A130T, V354L, V473F, A529V, F694Y, and R914S, transfection of the mutant replicon was attempted twice, but a phenotypic result was unable to be generated due to lack of replication.
[a]A fold change was calculated for each experiment, and a mean fold change was calculated with these values. The variability of the replicon assay has been estimated to 2.3-fold (dashed line); thus, any fold change values below this threshold are within the variability of the assay. [b]WT SARS-CoV-2 replicon reference SH01 was generated from a clinical isolate from Shanghai (lineage B). [c]S759A/V792I and V792I were utilised as positive controls.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; WT, wildtype.

Figure 3. ODV EC_{50} Fold Changes[a] From WT of Emergent Nsp12 Amino Acid Substitutions in the ODV Group



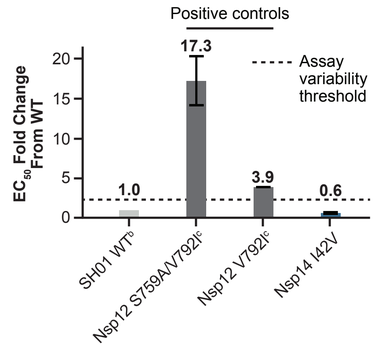
For substitutions G200D, G203S, S364F, V338A, T556S, and H572R, transfection of the mutant replicon was attempted twice, but a phenotypic result was unable to be generated due to lack of replication.
[a]A fold change was calculated for each experiment, and a mean fold change was calculated with these values. The variability of the replicon assay has been estimated to 2.3-fold (dashed line); thus, any fold change values below this threshold are within the variability of the assay. [b]WT SARS-CoV-2 replicon reference SH01 was generated from a clinical isolate from Shanghai (lineage B). [c]S759A/V792I and V792I were utilised as positive controls.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; WT, wildtype.

Figure 4. Map of Nsp12 Amino Acid Substitutions Observed in the ODV Group on the Cryo-EM Structure of the SARS-CoV-2 Polymerase Complex



The structure is a model of active NTP incorporation in the SARS-CoV-2 polymerase active site based on the cryo-EM structure.[5] The full Nsp12 protein is shown in green, with the locations of the observed postbaseline amino acid substitutions shown in magenta. White is Nsp7 and yellow is Nsp8 (2 subunits). The template RNA strand is shown in blue and nascent RNA strand in red.
EM, electron microscopy; NTP, nucleoside triphosphate form of obeldesivir; ODV, obeldesivir.

Figure 5. ODV EC_{50} Fold Changes[a] From WT of Emergent Replication Complex Amino Acid Substitutions in the ODV Group in ≥2 Participants



[a]A fold change was calculated for each experiment, and a mean fold change was calculated with these values. The variability of the replicon assay has been estimated to 2.3-fold (dashed line); thus, any fold change values below this threshold are within the variability of the assay. [b]WT SARS-CoV-2 replicon reference SH01 was generated from a clinical isolate from Shanghai (lineage B). [c]Nsp12 S759A/V792I and Nsp12 V792I were utilised as positive controls.
EC_{50}, half-maximal effective concentration; ODV, obeldesivir; WT, wildtype.

References: **1.** Martinez DR, et al. *Sci Transl Med*. 2024;16:eadj4504. **2.** Ogbuagu O, et al. *Open Forum Infect Dis*. 2025;12:ofae631.2192. **3.** Hedskog C, et al. *J Infect Dis*. 2023;228:1263-73. **4.** Andreatta K, et al. *Open Forum Infect Dis*. 2023;10:ofad500.575. **5.** Malone BF, et al. *Nature*. 2023;614:781-7.

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Disclosures: **LR**, **YH**, **J Li**, **DH**, **NP**, **CM**, **PYH**, **JKP**, **RM**, **AL**, and **CH** are stockholders and employees of Gilead Sciences, Inc. **OO** served on advisory boards for Gilead Sciences, Inc., Moderna, and ViiV Healthcare/GSK. **J Llewellyn** is a stockholder and former employee of Gilead Sciences, Inc.

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