Viral Resistance Analyses From the Obeldesivir BIRCH Study in High-Risk Nonhospitalised Patients With COVID-19

P0425

BIRCH

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Conclusions

- In an analysis of nasal swab samples collected during the Phase 3 BIRCH trial, emergent amino acid substitutions in the SARS-CoV-2 RNA-dependent RNA polymerase Nsp12 were observed in both the obeldesivir group and the placebo group
- Only 1 participant who received obeldesivir had an emergent Nsp12 substitution (V792I) associated with reduced susceptibility to obeldesivir (4.0-fold)
  - The substitution was first detected at Day 15
  - This participant was never hospitalised for COVID-19
- The other emergent substitutions did not confer reduced susceptibility to obeldesivir in vitro (half-maximal effective concentration fold change ≤2.0)
- 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 high-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
- 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 determine if SARS-CoV-2 is likely to become resistant to obeldesivir treatment in people who have COVID-19 and who have a high risk of becoming severely ill from the disease
- Here, viral 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 antiviral 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 BIRCH study that was conducted in nonhospitalised participants with risk factors for progression to severe COVID-19[2]
  - BIRCH showed greater decreases in SARS-CoV-2 viral load and infectious viral titre with ODV compared with placebo

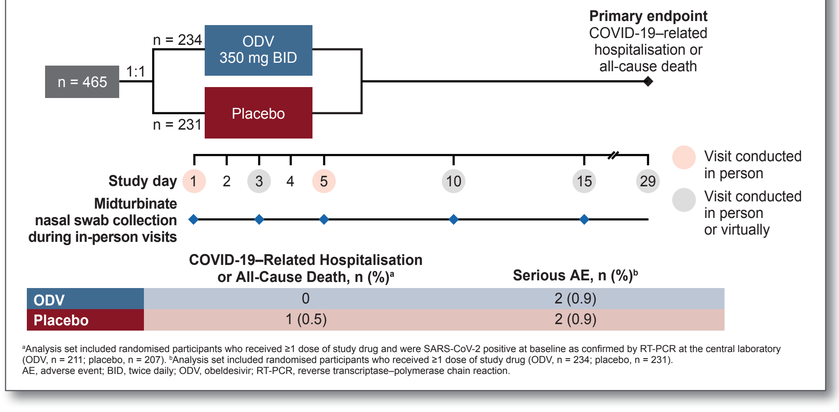
Objective

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

Methods

- BIRCH was a multicentre, double-blind, placebo-controlled study wherein nonhospitalised participants aged ≥18 years who had COVID-19 and risk factors for progression to severe disease were randomised 1:1 to receive ODV or placebo twice daily for 5 days (**Figure 1**)
  - Enrolment was halted early due to a lower-than-expected event rate for the primary endpoint in the context of a dynamic COVID-19 landscape
- The SARS-CoV-2 genome 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 with the previous time point
  - Participants with progression to COVID-19–related hospitalisation or all-cause death by Day 29
- The emergent Nsp12 substitutions observed in the ODV group were modelled on the cryo-electron microscopy (EM) 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



	COVID-19–Related Hospitalisation or All-Cause Death, n (%)[a]	Serious AE, n (%)[b]
ODV	0	2 (0.9)
Placebo	1 (0.5)	2 (0.9)

[a]Analysis set included randomised participants who received ≥1 dose of study drug and were SARS-CoV-2 positive at baseline as confirmed by RT-PCR at the central laboratory (ODV, n = 211; placebo, n = 207). [b]Analysis set included randomised participants who received ≥1 dose of study drug (ODV, n = 234; placebo, n = 231).
AE, adverse event; BID, twice daily; ODV, obeldesivir; RT-PCR, reverse transcriptase–polymerase chain reaction.

Results

Participants

- Of the 465 participants randomised and treated, 127 participants (ODV, 73; placebo, 54) met the sequencing analysis criteria and had sequencing data at both baseline and postbaseline (**Table 1**)

Table 1. BIRCH Sequencing Data

	ODV	Placebo
Treated population, n[a]	233	232
Participants with sequencing data available at baseline, n	190	62
Participants with sequencing data available at postbaseline, n	79	56
Participants with sequencing data available at both baseline and postbaseline, n	73	54
Participants with emergent Nsp12 substitutions, n/N (%)	12/73 (16.4)	5/54 (9.3)
Participants with Nsp12 substitutions with reduced susceptibility to ODV in vitro, n/N (%)	1/73 (1.4)	NA

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

Baseline Virology Analysis

- The SARS-CoV-2 lineages observed in this study included Omicron subvariants, with sublineages of the XBB variant being the most prevalent
- Among the 252 participants with sequencing data at baseline, 5 Nsp12 substitutions were observed in ≥3 participants (**Table 2**)

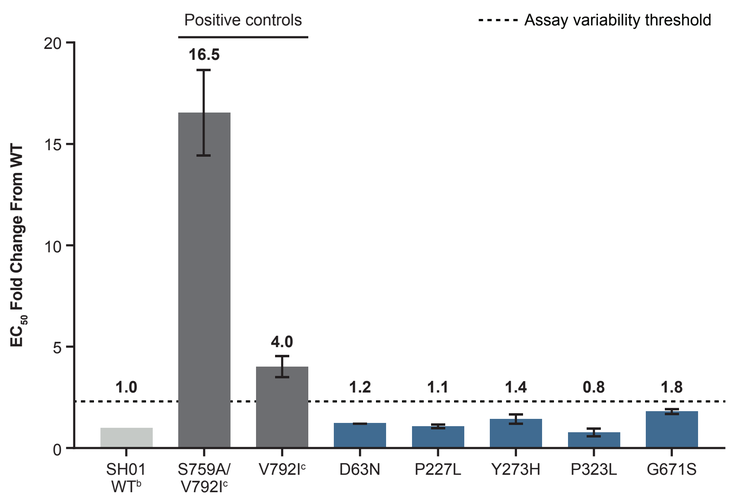
Table 2. Baseline Amino Acid Substitutions[a] Detected in Nsp12

Baseline Nsp12 Substitution, n (%)	ODV (n = 190[b])	Placebo (n = 62[b])	Total (n = 252[b])
D63N	5 (2.6)	2 (3.2)	7 (2.8)
P227L	11 (5.8)	1 (1.6)	12 (4.8)
Y273H	6 (3.2)	1 (1.6)	7 (2.8)
P323L	190 (100)	62 (100)	252 (100)
G671S	183 (96.3)	60 (96.8)	243 (96.4)

[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.
ODV, obeldesivir; WT, wildtype.

- Phenotyping of Nsp12 substitutions detected at baseline resulted in mean half-maximal effective concentration (EC_{50}) fold changes ≤1.8, suggesting that ODV maintains similar antiviral activity against replicons containing these substitutions compared to a replicon containing the wildtype (WT) reference sequence (**Figure 2**)

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



Data are represented as mean ± SD.
[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.

Postbaseline Amino Acid Substitutions in Nsp12

- Among participants with baseline and postbaseline sequencing data, there was a trend for a higher rate of emergent amino acid substitutions in those who received ODV compared with those who received placebo; however, this difference was not statistically significant (**Table 1**)
- There were 9 and 5 Nsp12 substitutions unique to the ODV and placebo groups, respectively (**Table 3**)

Table 3. Postbaseline Amino Acid Substitutions Detected in Nsp12

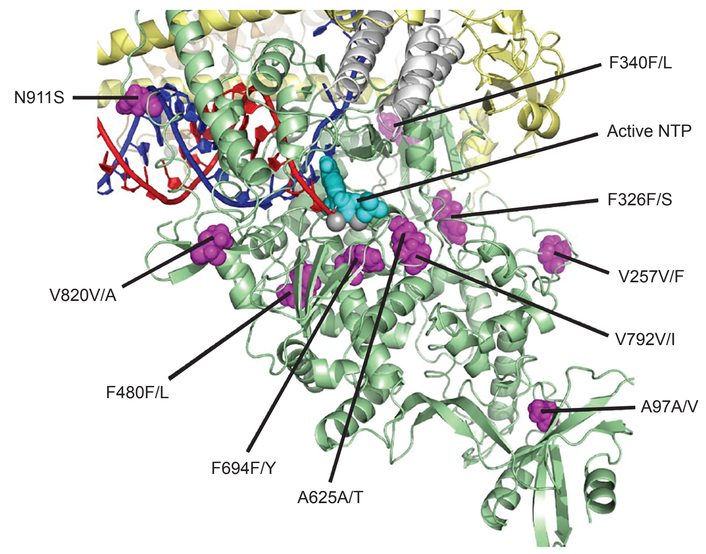
Emergent Nsp12 Substitution, n (%)	ODV (n = 73[a])	Placebo (n = 54[a])	Total (n = 127[a])
S15S/N	0	1 (1.9)	1 (0.8)
D62D/H	0	1 (1.9)	1 (0.8)
A97A/V	1 (1.4)	0	1 (0.8)
V257V/F	1 (1.4)	0	1 (0.8)
F326F/S	1 (1.4)	0	1 (0.8)
F340F/L	1 (1.4)	0	1 (0.8)
A400A/T	0	1 (1.9)	1 (0.8)
S433S/C	0	1 (1.9)	1 (0.8)
F480F/L	1 (1.4)	0	1 (0.8)
T565T/I	0	1 (1.9)	1 (0.8)
A625A/T	1 (1.4)	0	1 (0.8)
F694F/Y	4 (5.5)	1 (1.9)	5 (3.9)
V792V/I	1 (1.4)	0	1 (0.8)
V820V/A	1 (1.4)	0	1 (0.8)
N911S	2 (2.7)	0	2 (1.6)

[a]Number of participants with baseline and postbaseline sequencing data available.
ODV, obeldesivir.

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

- In the cryo-EM SARS-CoV-2 replicase complex model, A97A/V and V257V/F were located on the surface of the Nsp12 protein, distant from the polymerase active site or viral RNA (**Figure 3**)
  - The substitutions closest to the active site were A625A/T and V792V/I
  - None of the substitutions had direct contact with the incoming nucleoside triphosphate substrate or the RNA

Figure 3. 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.[3] 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.

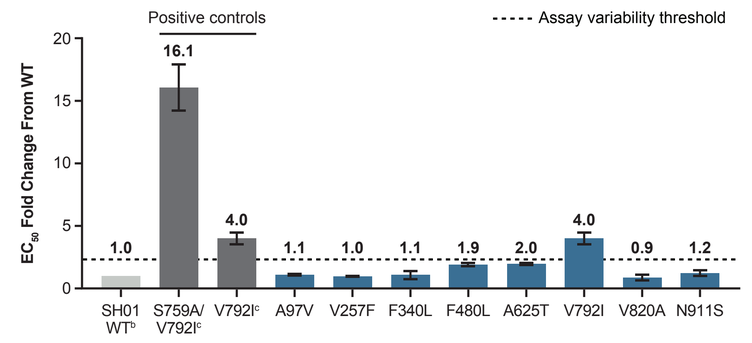
Postbaseline Amino Acid Substitutions in Nsp8, Nsp9, Nsp10, Nsp13, and Nsp14

- Of 127 participants with both baseline and postbaseline data, 23 participants (18.1%) had emergent substitutions in the replication complex genes Nsp8, Nsp9, Nsp10, Nsp13, or Nsp14
- Fourteen participants of 73 in the ODV group (19.2%) and 9 participants of 54 in the placebo group (16.7%) had substitutions in any of these genes

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

- Only 1 Nsp12 substitution from 1 participant in the ODV group (V792I; first detected at Day 15) was associated with reduced in vitro susceptibility to ODV, with an EC_{50} fold change of 4.0 compared with a WT reference (**Figure 4**)
  - None of the other substitutions impacted ODV susceptibility (EC_{50} fold change ≤2.0)
- None of the substitutions in Nsp8, Nsp9, Nsp10, Nsp13, or Nsp14 met the criteria for phenotyping, and none of the substitutions have been associated with resistance to ODV

Figure 4. ODV EC_{50} Fold Changes[a] From WT of Emergent Nsp12 Amino Acid Substitutions in the ODV Group

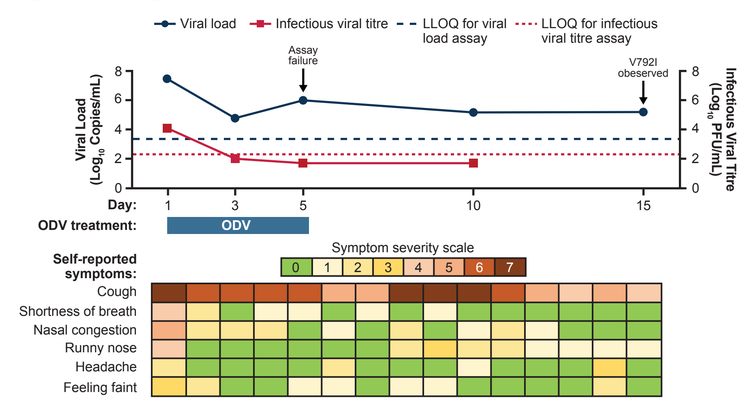


Data are represented as mean ± SD. For F326S, transfection of the mutant replicon was attempted twice, but a phenotypic result was unable to be generated due to lack of replication.
[a]A fold change was calculated for each experiment, and a mean fold change was calculated with these values. The variability 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.

Details of the Participant With Emergent Nsp12 Substitution V792I

- One emergent substitution, Nsp12 V792I, resulted in a low reduction in ODV susceptibility
- The participant with this substitution had a SARS-CoV-2 viral load of 7.46 $\log_{10}$ copies/mL at baseline, which was above the median viral load of the study; viral load decreased by 1.47 $\log_{10}$ copies/mL on Day 5 compared to baseline (**Figure 5**)
- Infectious viral titre reached negativity on Day 5 and remained negative on Day 10
- The symptoms that remained at Day 15 included cough and runny nose; the participant was never hospitalised for COVID-19

Figure 5. Viral Dynamics and Symptom Alleviation in Participant With Emergent Nsp12 Substitution V792I



Infectious viral titres reported as "<LLOQ-positive" and "<LLOQ-negative" were imputed as 100 PFU/mL and 50 PFU/mL, respectively. Symptom severity was evaluated using the Symptoms of Infection With Coronavirus-19 questionnaire in which respondents rated the severity of their symptoms using a 0-to-10 numeric rating scale.
LLOQ, lower limit of quantitation; ODV, obeldesivir; PFU, plaque-forming unit.

References: **1.** Martinez DR, et al. *Sci Transl Med*. 2024;16:eadj4504. **2.** Streinu-Cercel A, et al. *Open Forum Infect Dis*. 2025;12:ofae631.2182. **3.** Malone BF, et al. *Nature*. 2023;614:781-7.

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