SARS-CoV-2 ensitrelvir treatment-emergent resistance-associated mutations in Phase III randomized clinical trial

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Background

- The potential for treatment-emergent drug resistance following SARS-CoV-2 antiviral therapy is not yet well understood.
- Ensitrelvir, an oral main protease inhibitor for SARS-CoV-2, showed strong antiviral activity in the Phase 3 SCORPIO-HR/ACTIV-2d/A5407 trial.
- This exploratory analysis examines the emergence of treatment resistance-associated mutations following ensitrelvir treatment.

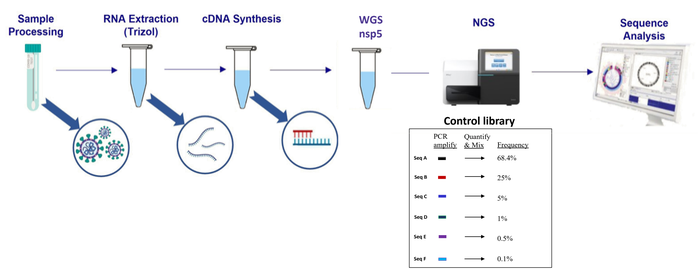
SCORPIO-HR/ACTIV-2d/A5407 STUDY DESIGN

Eligibility Criteria:

- Age: ≥18 years old
- Confirmed SARS-CoV-2 Infection:
  - ➢ Reverse transcriptase polymerase chain reaction (RT-PCR) or rapid antigen test
- Disease Severity: Mild-to-moderate COVID-19
- Symptom Onset (mITT): ≤3 days before treatment initiation
- Symptoms: At least one of 15 COVID-19 symptoms

Sample Collection & Laboratory Assays

- Study participants: mITT population
  1888 participants enrolled between Aug 2022-Dec 2023
  - ➢Placebo (n=943)
  - ➢Ensitrelvir (n=945)
- SARS-CoV-2 viral load testing
  Nasopharyngeal swabs collected on days 1, 4, 8, and 16
- SARS-CoV-2 whole genome & nsp5 sequencing



- Ensitrelvir treatment emergent resistance-associated mutations were defined as changes that reduced susceptibility by ≥3-fold.
- For the calculation of the frequency of treatment-emergent mutations, the denominator included all mITT participants regardless of sequence availability. Comparisons were conducted using the Fisher's exact test and Wilcoxon rank-sum test.

Table 1: Participant baseline characteristics

	Treatment (n=945)	Placebo (n=943)
Median age (Q1, Q3), y	40 (30-51)	39 (30-51)
Female sex, %	57	54
Ethnicity, %		
Hispanic or Latino	46	45
Not Hispanic or Latino	54	54
Not reported/unknown	1	1
Race		
Asian	41	42
American Indian or Alaska Native	1	1
Black or African American	8	10
White	43	41
Multiple races reported	1	<1
Other/unknown	4	5
Not reported	2	2
Median days from symptom onset to enrollment (Q1, Q3)	2.0 (2.0, 3.0)	2.0 (2.0, 3.0)
Symptom score at enrollment (study day 1) (Q1, Q3)	11 (6,17)	11 (6,16)
Median NP SARS-CoV-2 viral load at enrollment (Q1, Q3), log_{10} copies/mL	5.54 (3.50, 7.11)	5.61 (3.51, 6.99)

NP = nasopharyngeal; Q = quartile

RESULTS

- The distribution of SARS-CoV-2 variants was consistent across study groups (**Figure 1**).
- Treatment-emergent resistance-associated mutations (T25A/N, M49I/L) were observed more frequently in the ensitrelvir-treated group than in the placebo group (**Figure 2a,b**). This pattern was consistent with the rise in T25A/N and M49I/L cases in Japan following ensitrelvir's approval (**Figure 2c**).
- One participant developed E166A treatment-emergent resistance-associated mutation (at <10% of the total viral population), which leads to reduced ensitrelvir susceptibility (**Figure 2d**).
- Ensitrelvir-treated participants with treatment-emergent resistance-associated mutations (n=18; T25A/N: n=4, M49I/L: n=14) had higher SARS-CoV-2 RNA levels at baseline and on study days 4 and 8 compared to those without mutations (n=917) and the placebo group (n=934) **(Figure 3)**.

Figure 1: Distribution of SARS-CoV-2 variants was consistent across study groups

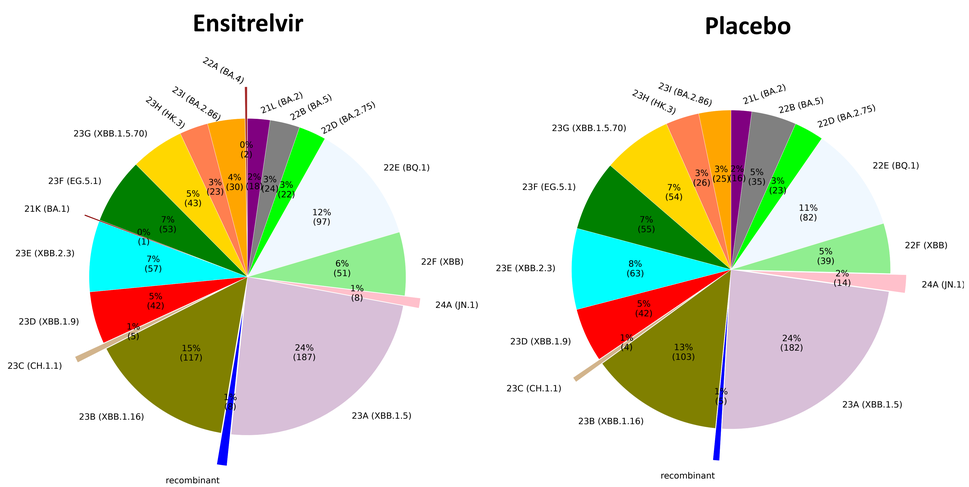


Figure 2: Treatment-emergent resistance-associated mutations were more frequent in the ensitrelvir-treated group

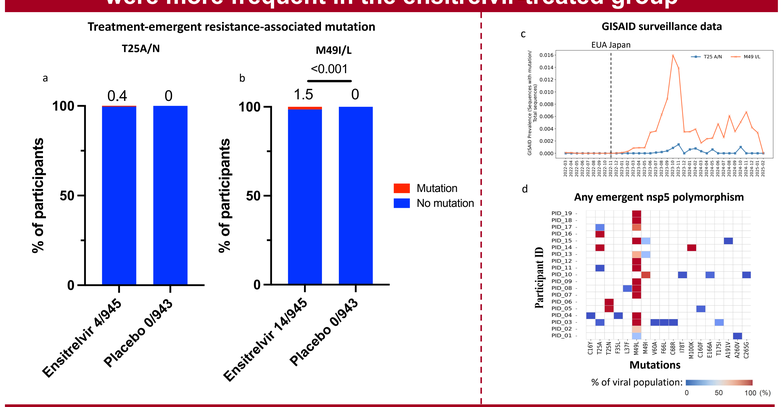
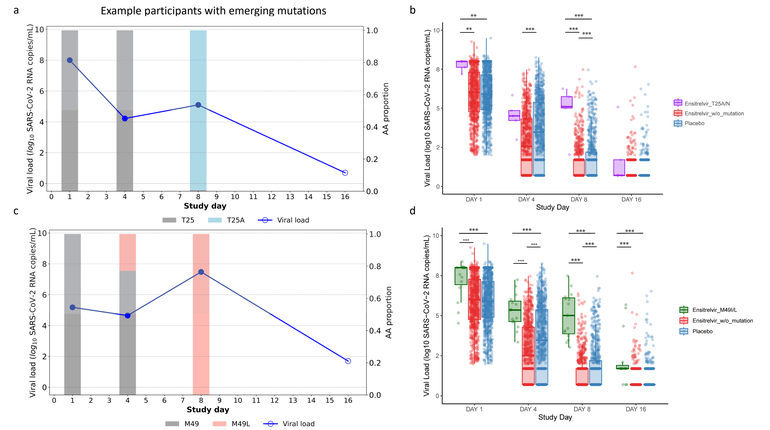


Figure 3: Higher RNA levels in ensitrelvir-treated participants with treatment-emergent resistance-associated mutations



CONCLUSIONS

- Treatment-emergent resistance-associated mutations were rare with ensitrelvir treatment and correlated with higher pre-treatment RNA levels, though their clinical significance remains uncertain.
- This exploratory analysis includes a small number of participants with resistance mutations, and findings should be interpreted with caution.
- Protease inhibitors represent an important treatment modality for COVID-19, but can be vulnerable to treatment-emergent mutations and additional monitoring is warranted

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