Phenotyping and genotyping analysis using baseline swab samples in ensitrelvir clinical studies

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Background

COVID-19 is still public health concern and new variants have been emerging one after another. Ensitrelvir is an oral SARS-CoV-2 3CL protease ($3CL^{pro}$) inhibitor developed as a treatment for COVID-19. As surveillance to monitor susceptibility of recent variants to ensitrelvir, genotyping and phenotyping analysis was conducted using baseline (pre-treatment) swab samples in ensitrelvir SCORPIO-SR and SCORPIO-HR clinical studies, which were conducted in 2022 and 2022-2023, respectively.

Methods

For genotyping analysis, whole genome sequencing (WGS) was conducted using baseline swab samples and sequence of $3CL^{pro}$ region was analyzed. For phenotyping analysis, viruses were isolated and propagated in VeroE6/TMPRSS2 cells from baseline swab samples. Susceptibility of isolated viruses and a reference strain (Ancestral) to ensitrelvir was measured in VeroE6/TMPRSS2 cells with ViroSpot Immunostaining method, which was also used to measure viral titers in clinical studies. The fold change in EC_{50} was calculated by the following formula: EC_{50} against viruses from baseline swab samples / EC_{50} against reference virus.

Results

2375 baseline samples were analyzed for $3CL^{pro}$ region, and none had an amino acid mutation associated with reduced susceptibility to ensitrelvir. Total of 562 isolated viruses were used for phenotyping assay and these included various virus clades containing major omicron variants of BA.1, BA.2, BA.5, BA.2.86, EG.5.1, XBB.1.16, HK.3 etc. Ensitrelvir showed potent antiviral activities with <10-fold change in EC_{50} compared to the reference strain against 556 isolates. Regarding 6 isolates which showed >10-fold change in EC_{50} (placebo group: 2 isolates, ensitrelvir group: 4 isolates), analysis for anti-viral effects in clinical studies or genotyping analysis of baseline samples were conducted. From genotyping analysis, no amino acid mutation associated with reduced susceptibility to ensitrelvir was detected. For 4 participants in ensitrelvir groups, whose isolates showed >10-fold change in EC_{50}, viral RNA and titer decreased rapidly in clinical studies.

Table 1. Amino acid polymorphism at baseline

Amino acid polymorphism in $3CL^{pro}$	SCORPIO-SR	SCORPIO-HR
N*	920	1455
L75F	46	3
K90R	7	13
T93A	4	0
A94G	0	6
P96S	0	4
V104I	0	4
I106V	0	4
P108S	4	4
S123Y	45	0
P132H**	920	1452
T169S	124	0
F223L	0	35
P241L	1	2
A260V	1	2
V297I	0	4

*N: number of participants with WGS data available for baseline swab samples, or number of participants with amino acid variants
Polymorphic amino acid (AA) variants in $3CL^{pro}$ detected from ≥ 3 participants total in SCORPIO-SR Ph3 and SCORPIO-HR Ph3 study were described (AA variants from reference [SARS-CoV-2 isolate Wuhan-Hu-1, GenBank ID: MN908947] at baseline) This analysis was targeted for the polymorphic AA substitutions with mutation frequency ≥15% by WGS. All polymorphic AA variants described in this table are not located within 5Å from ensitrelvir binding site in $3CL^{pro}$.
**enzyme inhibitory assay showed that the inhibitory effects of ensitrelvir against P132H mutant were comparable to those against the WT (fold change: 1.12) [1].

Conclusion

Almost all Omicron variants detected in SCORPIO-SR and SCORPIO-HR were confirmed to be susceptible to ensitrelvir, and ensitrelvir showed potent antiviral activity against Omicron strains. In addition to surveillance using database, susceptibility testing of ensitrelvir to newly emerged major SARS-CoV-2 variants will be continually conducted.

Acknowledgement

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Reference

[1] Kawashima et al. Biochem Biophys Res Commun 2023 Feb 19:645:132-136.

Table 2. Number and virus clade of samples used for phenotyping assay

SCORPIO-SR Ph2b study

Nextstrain clade	Pango lineage	N
Overall		251
21J (Delta)	**P.1**	1
21K	**BA.1**	247
21L	**BA.2**	1
Unknown	**-**	2

SCORPIO-SR Ph3 study

Nextstrain clade	Pango lineage	N
Overall		118
21K	**BA.1**	38
21L	**BA.2**	52
21M	**B.1.1.529**	1
22A	**BA.4**	2
22B	**BA.5**	6
22C	**BA.2.12.1**	2
recombinant		17

SCORPIO-HR Ph3 study

Nextstrain clade	Pango lineage*	N
Overall		193
22B	**BA.5**	6
22D	**BA.2.75**	2
22E	**BQ.1**	16
22F	**XBB**	5
23A	**XBB.1.5**	21
23B	**XBB.1.16**	51
23C	**CH.1.1**	3
23D	**XBB.1.9**	25
23E	**XBB.2.3**	1
23F	**EG.5.1**	25
23G	**XBB.1.5.70**	7
23H	**HK.3**	10
23I	**BA.2.86**	1
recombinant	**XBL.2, XCP, etc.**	3
Not specified		18

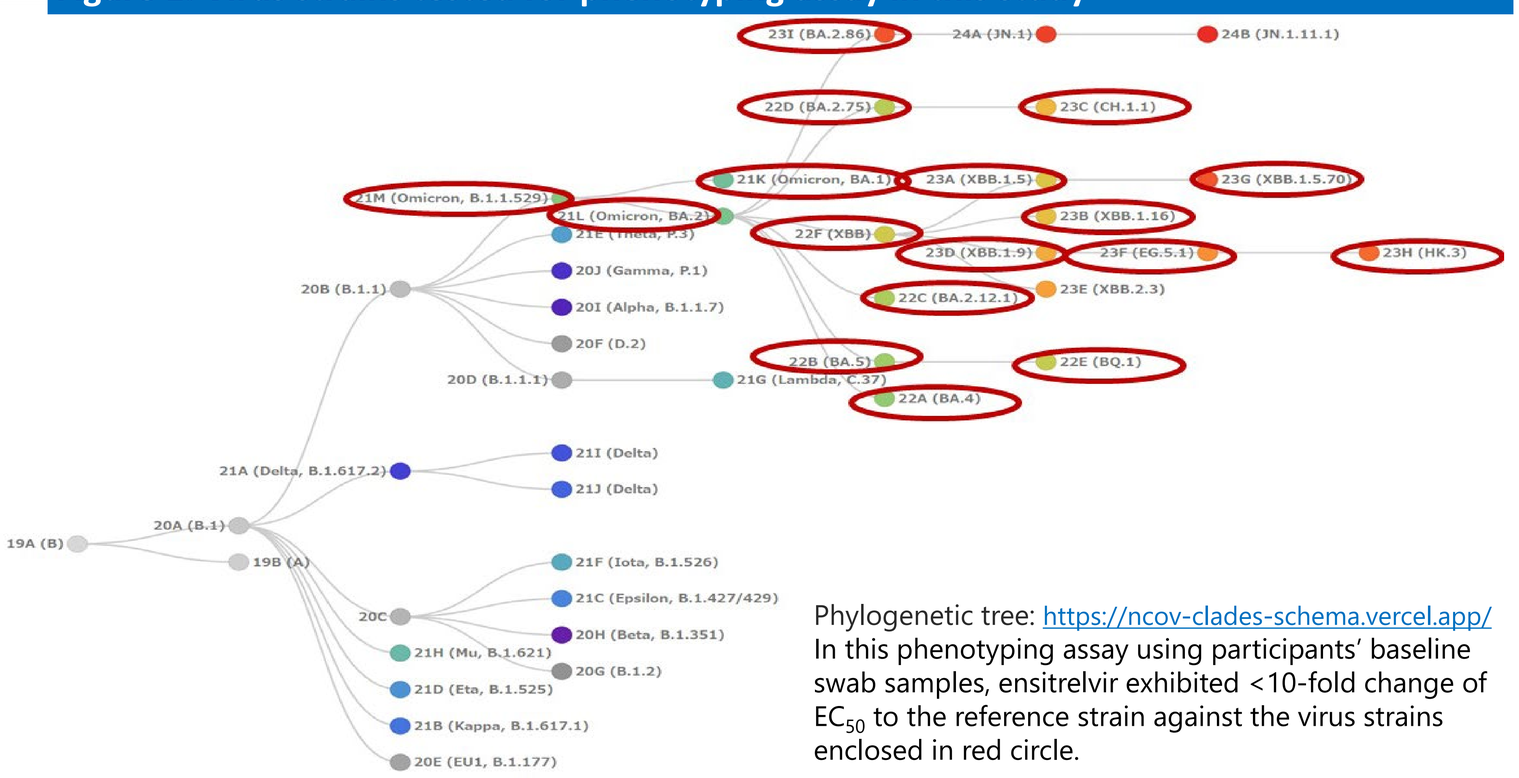
N: number of participants. *Representative Pango lineages are described.
Samples for phenotyping analysis were selected from baseline samples of both ensitrelvir and placebo groups.

Table 3. EC_{50} and fold change of the EC_{50} to the reference strain of the phenotyping assay

Study	N	Mean EC_{50} against baseline samples*	Mean EC_{50} against reference strain*	Fold change** in EC_{50}
SCORPIO-SR Ph2b	251	**0.066**	0.042	**1.565**
SCORPIO-SR Ph3	118	**0.085**	0.043	**1.978**
SCORPIO-HR Ph3	193	**0.134**	0.051	**2.614**

*EC_{50} of ensitrelvir is represented as μg/mL. **Compared with the EC_{50} against reference strain
N: number of participants. Reference strain: BetaCoV/Germany/BavPat1/2020 (Ancestral, Wuhan),

Figure 1. Virus strains tested for phenotyping assay in this study



Phylogenetic tree: https://ncov-clades-schema.vercel.app/
In this phenotyping assay using participants' baseline swab samples, ensitrelvir exhibited <10-fold change of EC_{50} to the reference strain against the virus strains enclosed in red circle.

Table 4. Sample information which showed >10 fold-change in EC_{50} compared to reference strain

Study	Sample group	Sample ID*	Detected variants in $3CL^{pro}$ by WGS**	Fold change*** in EC_{50}	Nextstrain clade	Mean fold change*** in EC_{50}	Number of samples in the same viral clade (/total)
SCORPIO-SR Ph2b	Ensitrelvir 125mg	1SA001	N.T.	**19**	**21K**	1.518	**2/247**
		1FI004	N.T.	**13**			
	Ensitrelvir 250mg	1FK017	N.T.	**11**	**21J**	11.333	**1/1**
SCORPIO-SR Ph3	Placebo	1FK107	N.T.	**18.5**	**21K**	1.229	**1/38**
SCORPIO-HR Ph3	Ensitrelvir 125mg	710006	P132H	**59.7**	**23D**	5.274	**1/25**
	Placebo	823010	P132H	**12.3**	**23A**	3.060	**1/21**

* Sample which showed >10 fold-change in EC_{50} compared to reference strain. N.T.: not tested
**Amino acid variants detected at baseline swab samples compared with reference strain (Ancestral, Wuhan).
***Compared with the EC_{50} against reference strain (Ancestral, Wuhan).
The phenotyping assay was conducted for multiple samples in the same Nextstrain clade as 5 samples from 6 samples which showed >10-fold increase in EC_{50} of ensitrelvir compared to the reference strain, and for those, ensitrelvir showed <10-fold increase in EC_{50} compared to the reference. For samples in the 21J clade, only one sample was evaluated, but the fold change in EC_{50} compared to the reference was 11 and did not show a significant decrease.

Table 5. Virus load reduction in clinical studies for participants whose sample showed >10-fold change in EC_{50}

	SCORPIO-SR Ph2b						SCORPIO-HR Ph3		
	Ensitrelvir					Placebo	Ensitrelvir		Placebo
Reduction	125mg			250mg		-	125mg		-
(day4 or day5)	**1SA001**	**1FI004**	Mean***	**1FK017**	Mean***	Mean***	**710006**	Mean***	Mean***
viral RNA*	**-3.59**	**-3.25**	-2.69	**-3.81**	-2.54	-1.4	**-6.87**	-2.74	-1.96
viral titer**	**-0.7**	**-1.4**	-1.7	**-1.7**	-1.4	-1.1	**-1.5**	-1.49	-1.21

Anti-viral effects in clinical studies were analyzed for 4 participants in ensitrelvir group from 6 participants which showed >10-fold increase in EC_{50} of ensitrelvir compared to the reference strain.
*Viral RNA reduction from baseline (log_{10} copies/mL). ** Viral titer reduction from baseline (log_{10} $TCID_{50}$/mL).
***Mean: mean viral load reduction for all of ITT1.
If viral RNA was negative or less than the lower limit of quantification, the viral RNA was imputed by 2.27 or 2.08 in SCORPIO-SR study, and 1.7 or 0 in SCORPIO-HR study, respectively. If viral titer was less than the lower detection limit, the viral titer was imputed by 1.1 and 0.7 in SCORPIO-SR Ph2b study and in SCORPIO-HR Ph3 study, respectively.