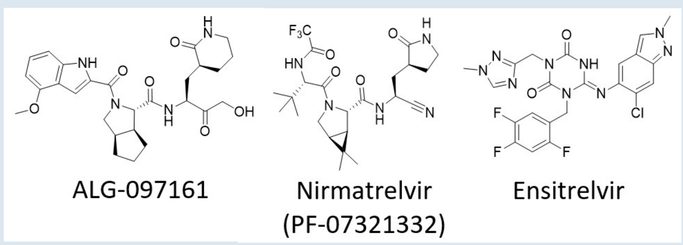
SARS-CoV-2 Viruses with Cross-Resistance to 3CLpro Inhibitors can be Selected *In Vitro*, and can Replicate and Transmit in a Hamster Model

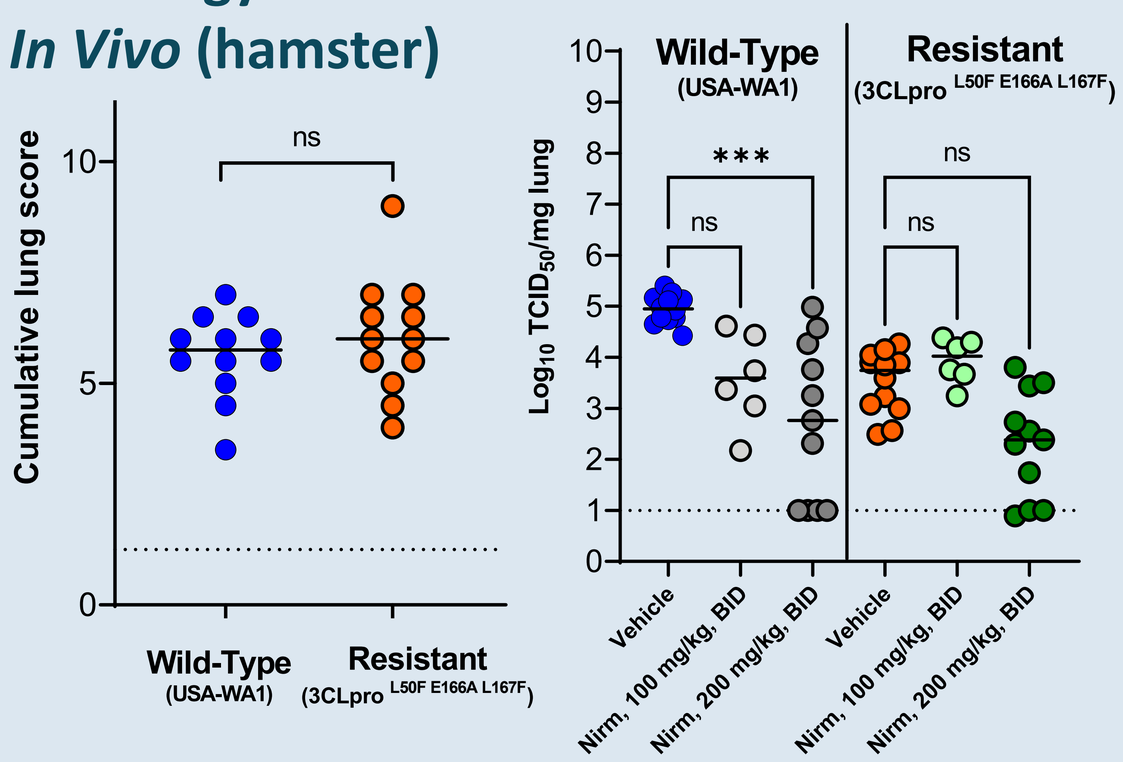
R Abdelnabi[1], K Donckers[1], C Liu[2], A Stoycheva[2], S Boland[4], SK Stevens[2], C De Vita[2], B Trüeb[3], N Ebert[3], V Thiel[3], S De Jonghe[1], M Laporte[1], D Bardiot[4], A Jekle[2], LM Blatt[2], L Beigelman[2], JA Symons[2], P Chaltin[1], A Marchand[4], J Deval[2], K Vandyck[2], J Neyts[1], D Jochmans[1] (dirk.jochmans@kuleuven.be)
[1]KU Leuven, Rega Institute, Belgium; [2]Aligos Therapeutics, USA and Belgium; [3]University of Bern, Switzerland; [4]CISTIM, Belgium

The SARS-CoV-2 main protease (3CLpro) is a therapeutic target for the treatment of COVID-19 and the potential of 3CLpro-inhibitors to select for drug-resistant variants needs to be established. Therefore, SARS-CoV-2 was passaged *in vitro* in the presence of increasing concentrations of ALG-097161, a probe compound designed in the context of a 3CLpro drug discovery program. We identified a combination of amino acid substitutions in 3CLpro (L50F E166A L167F) that is associated with > 10x increase in EC_{50} values for ALG-097161, nirmatrelvir (PF-07321332) and ensitrelvir. *In vivo* (hamster) experiments show that this virus retains sufficient replication capacity, is less susceptible to nirmatrelvir treatment and is efficiently transmitted. These observations will be important for the interpretation of resistance development to 3CLpro inhibitors in the clinical setting.

Details of this work are available at:
bioRxiv 2022.06.07.495116 and bioRxiv 2022.09.28.509903

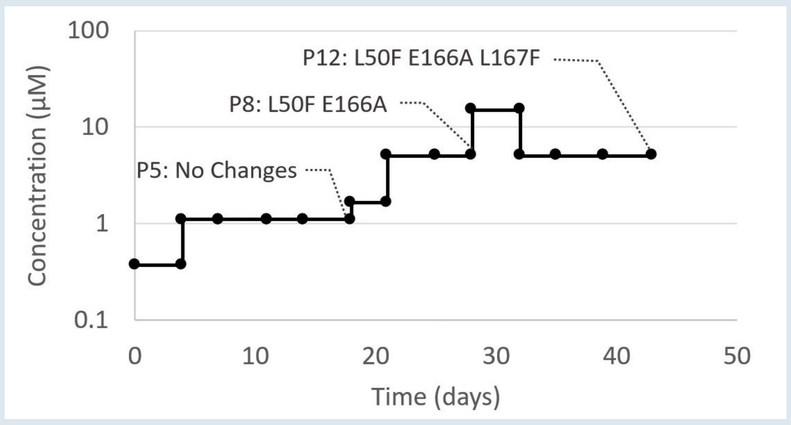


3CLpro$^{L50F\ E166A\ L167F}$ SARS-CoV-2 shows Pathology and Nirmatrelvir Resistance *In Vivo* (hamster)



In vitro Selection of 3CLpro Inhibitor Resistance

Passaging SARS-CoV-2-GHB (Wuhan) in VeroE6 cells in the presence of increasing concentrations of ALG-097161 was initiated at 0.4 µM. At passage 5, 8 and 12, vRNA in the cell culture medium was sequenced, and all substitutions in the 3CLpro are shown.

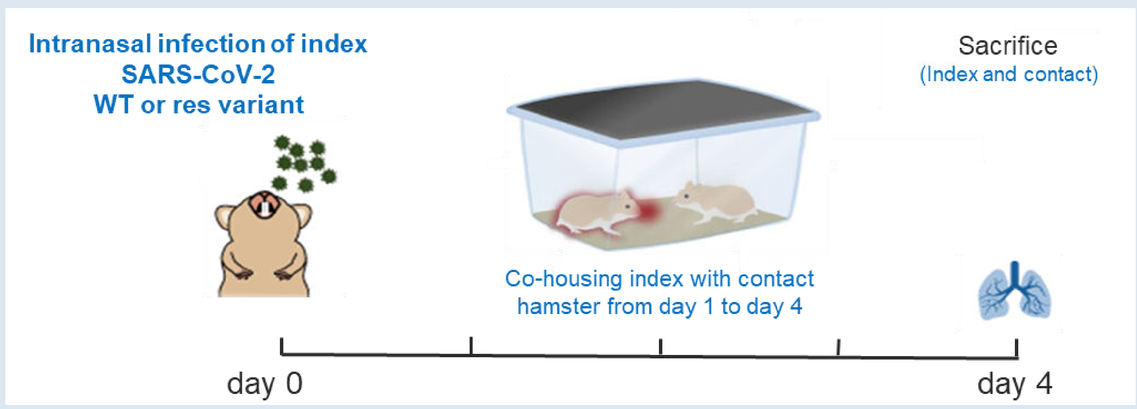


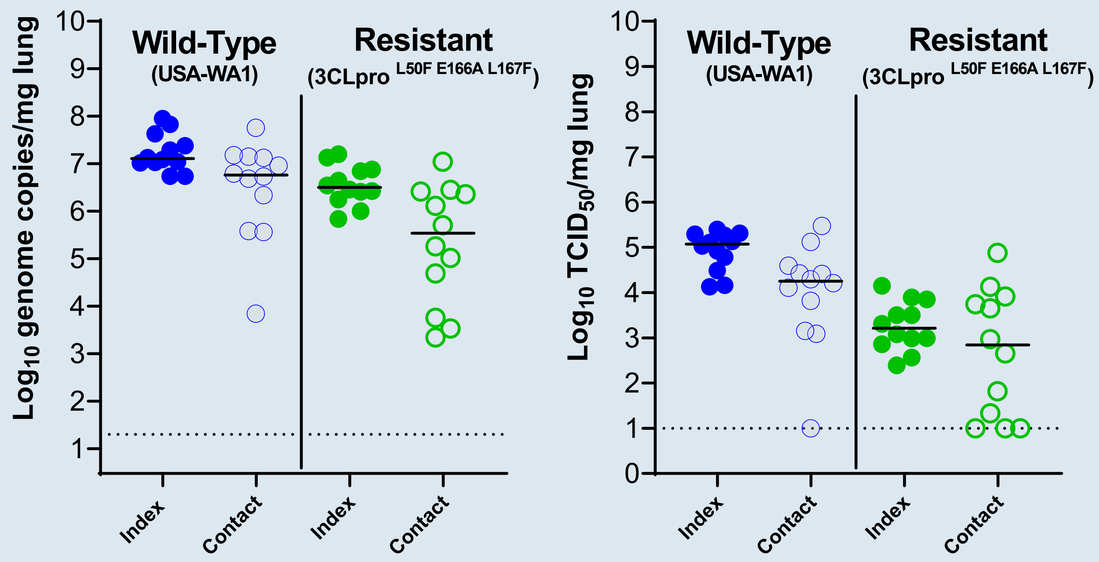
In vitro Resistance Confirmation Using Reverse Engineered SARS-CoV-2 viruses

	SARS-CoV-2 WT-USA-WA1 EC_{50} (µM)	EC_{50} (µM) [FC]***		
		L50F	E166A L167V	L50F E166A L167V
ALG-97161	0.60* (0.51-0.71)**	0.98 **[1.6]** (0.83-1.1)	2.9 **[4.6]** (1.5-4.5)	6.2 **[10.3]** (4.8-7.3)
Nirmatrelvir	0.11 (0.09-0.12)	0.16 **[1.5]** (0.12-0.2)	1.1 **[10.0]** (0.67-1.8)	3.2 **[29]** (2.3-3.5)
Ensitrelvir	0.21 (0.18-0.27)	0.24 **[1.1]** (0.18-0.31)	8.0 **[38]** (2.8-12.7)	9.3 **[44]** (3.5-15)
Remdesivir (GS-441524)**	0.86 (0.53-1.1)	1.1 **[1.3]** (0.8-1.1)	1.5 **[1.7]** (1.1-1.9)	1.2 **[1.4]** (0.91-1.7)

* Median value (n > 3); ** 25th – 75th percentile, EC_{50} = 50% effective concentration, ***FC = Fold Change of EC_{50}, **** GS-441524 is the parent nucleoside of remdesivir, it is intracellularly converted to the same active metabolite
EC50 (50% effective concentration)
note: all assays on VeroE6 cells are performed in the presence of 0.5 µM CP-100356

3CLpro$^{L50F\ E166A\ L167F}$ SARS-CoV-2 is Efficiently Transmitted *In Vivo* (hamster)





Deep sequencing analysis, of vRNA from lung tissue, revealed that the L50F E166A L167F substitutions are preserved in each contact hamster, that was co-housed with a hamster infected with the resistant virus. This demonstrates the genomic stability of these mutations.