Supplementary Material.

Nirmatrelvir-Ritonavir in Kidney Transplant Recipients Infected with SARS-CoV-2: Safety, Efficacy and Relapse in a case-series from Belgium.

1. Supplementary Materials and Methods

SARS-CoV-2 variants sequencing

Positive samples presenting Ct values < 25 were then sequenced starting from the same Nasopharyngeal swabs, conserved in 1.5 ml of Universal Transport Media (COPAN Diagnostic) at −80°C until sequencing. Total nucleic acid was extracted starting from 200 µl of the transport media using the MagMAX™ Viral/Pathogen II Nucleic Acid Isolation Kit according to the manufacturer instructions (Cat. No. A48383, ThermoFisher Scientific). Samples were then sequenced using the amplicon-based Illumina COVIDSeq protocol (Illumina Inc, USA) in combination with the ARTIC v4 primers pools (https://artic.network/) following the manufacturer's instructions. Briefly, after the first strand cDNA synthesis, the obtained cDNA was amplified using a multiplex polymerase chain reaction (PCR) protocol, producing amplicons across the whole SARS-CoV-2 genome. The PCR amplified product was later processed for tagmentation and adapter ligation using IDT for Illumina Nextera UD Indexes (Illumina Inc, USA). After additional PCR amplification of the tagmented amplicons and a bead-based cleanup, libraries were pooled together in a single tube. The final pool was quantified using Qubit 2.0 fluorometer (Invitrogen Inc.) and fragment size was evaluated using an Agilent 2100 Bioanalyzer (Agilent Inc). The pooled library was diluted to a final concentration of 100pM for a single read (1 x 150bp) sequencing on a NextSeq 1000 instrument. Generated fastq files were uploaded on the cloud-based ASP-IDNS®−5 analysis software (SmartGene). Analysis was made using the "SARS-CoV-2 full genome" pipeline version 2.5.0_COV_v0.2.

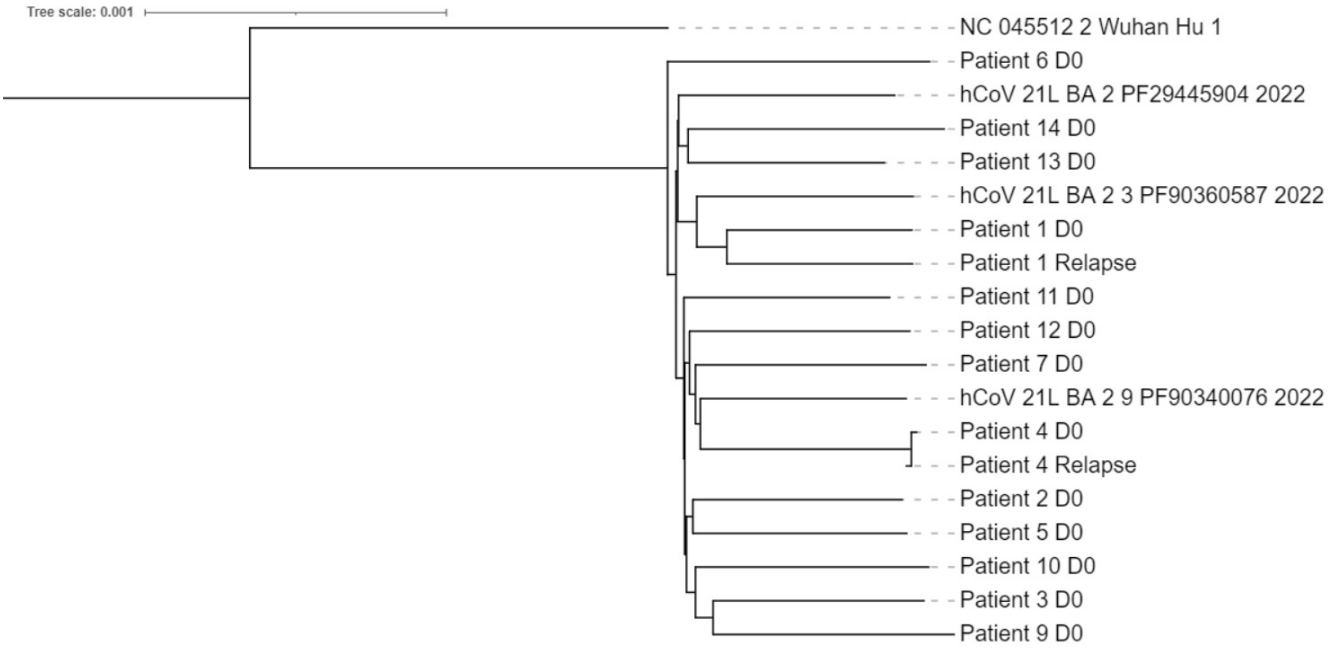
Briefly, reads were automatically filtered for low quality sections. The resulting reads were mapped against the SARS-CoV-2 (Wuhan-hu-1/2019 (MN908947)) reference genome and mutations were detected in a quantitative manner (% reads aligned). A consensus genome was generated using a 40% cut-off for base determination and a minimal number of 30 reads per position. Online Nextclade version 2.2.0 software was used as a first sequence aligner, allowing comparison to the Wuhan-hu-1/2019 (MN908947) SARS-CoV-2 reference genome and permitting a clade assignment (https://clades.nextstrain.org). FASTA sequences were also submitted to the Pangolin COVID-19 Lineage Assigner. Whole-genome sequences analyzed here were finally submitted to the GISAID platform and are accessible through the following identifiers: EPI_ISL_12587374, EPI_ISL_12587390, EPI_ISL_12660432, EPI_ISL_13017210, EPI_ISL_13017212, EPI_ISL_13017215, EPI_ISL_13017221, EPI_ISL_13017301, EPI_ISL_13017302, EPI_ISL_13204422, EPI_ISL_13204423, EPI_ISL_13204424, EPI_ISL_13424225, EPI_ISL_13424237, EPI_ISL_13424238.

Phylognentic Tree was generated by submitting the Fasta files to the NGPhylogeny web interface.[S1] The workflow included: sequence alignment using the MAFFT software [S2], curation of the sequences with the block mapping and gathering with entropy (BMGE) software [S3], tree generation using the fast distance-based phylogeny inference program FastME 2.0 [S4], and tree output formatted with the Newick display [S5].

2. Supplementary Figure.

Figure S1: Phylogeny of the 14 patients at day 0 and of the 2 patients (patient 1 and 4) at relapse.

In patient 1, both strains (the one of the initial infection compared to the one during relapse) were very close making the hypothesis of an infection by a new strain unlikely.



3. Supplementary references

S1. Lemoine F, Correia D, Lefort V, et al. NGPhylogeny.fr: new generation phylogenetic services for non-specialists. *Nucleic Acids Res.* 2019;47:W260-W265.

S2. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol.* 2013;30:772-780.

S3. Criscuolo A, Gribaldo S. BMGE (Block Mapping and Gathering with Entropy): a new software for selection of phylogenetic informative regions from multiple sequence alignments. *BMC Evol Biol.* 2010;10:210.

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