Successful retreatment of a patient with chronic hepatitis C genotype 2k/1b virus with ombitasvir/paritaprevir/r plus dasabuvir

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32Running title: Re-treatment in case of an HCV intergenotypic recombinant

Dear Editor-in-Chief,

Hepatitis C virus (HCV) is an enveloped, positive-strand RNA virus and a major causative agent of acute and chronic hepatitis worldwide. Due to error-prone replication, HCV is highly variable and based on phylogenetic analysis, viral isolates can be grouped into seven major genotypes (GTs). Genetic recombination is a further mechanism of increasing RNA virus diversity and thereby constitutes an escape strategy from the host immune response as well as antiviral treatments. For HCV, naturally occurring recombinants are rare *in vivo,* but probably also underestimated due to genotyping of single genomic regions in HCV diagnostics. However, several epidemiologically important hybrid strains with different breakpoints have been described[1] including the homologous recombinant of GT 2k/1b initially discovered in St. Petersburg.[2] The 2k/1b chimeric virus is found at higher prevalence in countries belonging to the former Soviet Union.[3] This variant can be missed by most routine genotyping and can only be detected by specific targets or extended sequencing of the HCV genome.[4] It has been shown in humanized mice infected with the 2k/1b variant that viral RNA levels drop in response to pegylated IFN-α (peg-IFN-α) treatment.[5] However, new regimens with direct-acting antivirals (DAAs) targeting the HCV non-structural proteins are not all pan-genotypic and data in patients infected with recombinant viruses are limited.

Here, we report a case of a male patient with a history of intravenous drug abuse. Before first antiviral treatment he had a fibroscan of 26.1 kPa suggesting liver cirrhosis although there had not been any events of cirrhotic decompensation. According to Versant HCV Genotype 2.0 (LiPA) he had genotype 2a/2c. His first antiviral treatment with peg-IFN-α2b and ribavirin was terminated prematurely after 3 months due to insufficient virological response (Fig. 1A). After approval of DAA-based therapy, the patient received standard treatment for genotype 2 with sofosbuvir and ribavirin for 3 months according to current guidelines.[6] While HCV-RNA became negative at week 4 of treatment the patient experienced viral relapse at follow-up week 4. Viral suppression was associated with a marked decrease in liver enzymes

59whereas viral relapse was followed by an increase of liver enzymes (Fig. 1A). Standard 60sequencing of the NS5B polymerase region for potential sofosbuvir resistance mutation 61revealed a GT1b-specific coding sequence indicative for an HCV chimeric strain. By 62applying total RNA next-generation sequencing, we identified the presence of an HCV 2k/1b 63variant at the time point before initial therapy and after sofosbuvir plus ribavirin treatment 64confirming the initial misgenotyping (Fig. 1A, B) As depicted in Fig. 1B, the 5'UTR to NS2 65regions of both analysed viral genomes (day 101 and day 630) showed highest nucleotide 66sequence similarity to HCV subtype 2k, whereas NS2 to 3'UTR was most similar to subtype 671b. The breakpoint between GT 2k and 1b was pinpointed to a region in the NS2 gene 68between nucleotide 3175 and 3176, as previously observed in the reference 2k/1b genome 69sequence (GenBank accession number AY587845). Phylogenetic analysis revealed that both 70recombinant viral sequences cluster together with other 2k/1b chimera archived in the NCBI 71data base in a distinct clade between genotype 1 and genotype 2 (Fig. 1C). As the DAA target 72regions NS3/4A, NS5A and NS5B all lie within the genotype 1b section of 2k/1b chimera, a 73treatment regimen suitable for genotype 1b rather than genotype 2 was supposed to be a 74sufficient antiviral treatment. In consequence, the patient was retreated with the genotype 1 75specific so-called "3D" combination containing the NS3/4A protease inhibitor paritaprevir 76(ABT-450), boosted by ritonavir, the NS5A inhibitor ombitasvir and the non-nucleotide 77NS5B polymerase inhibitor dasabuvir. The patient became HCV-RNA negative at week 4 of 78treatment and achieved a sustained virological response (SVR) at follow-up week 12 (Fig. 791A).

80Due to limited information on the prevalence and response of the HCV 2k/1b recombinant to 81novel DAA treatment, no clear recommendations on the therapy regimen and duration exists. 82Recently, lower SVR rates of sofosbuvir plus ribavirin treatment among patients infected 83with 2k/1b chimera were reported,[7] which is in line with the relapse observed in the patient 84here. The standard treatment for genotype 2 sofosbuvir plus ribavirin given for 3 months

showed only 10% SVR in genotype 1 patients who failed previous peg-IFN-α based therapy.[8] The detection of these cases demonstrates the importance of determining the HCV genotype in case of unsuccessful antiviral therapy in genotype 2. This can be achieved by NS5B sequencing, next-generation sequencing of the full genome or via RT-PCR methods, detecting parts of the 5'-UTR and NS5B domain, like employed in commercially available genotyping kits. It also highlights that genotype 1 therapies without genotype 2 approval are sufficient for the 2k/1b chimeric strains. However, it would be useful to have pan-genotypic therapies, which will not depend on the HCV genotype, subtype or intergenotypic recombinants. The recently approved combination sofosbuvir plus velpatasvir closes this gap.[9] However, costs and access have to be considered if a one pill fits all regimen or individualized approaches based on genotype and other factors are used.[10] To our knowledge this is the first report of a successful antiviral therapy with the "3D" combination regimen in 2k/1b chimera after failure of treatment with sofosbuvir and ribavirin.

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None to declare

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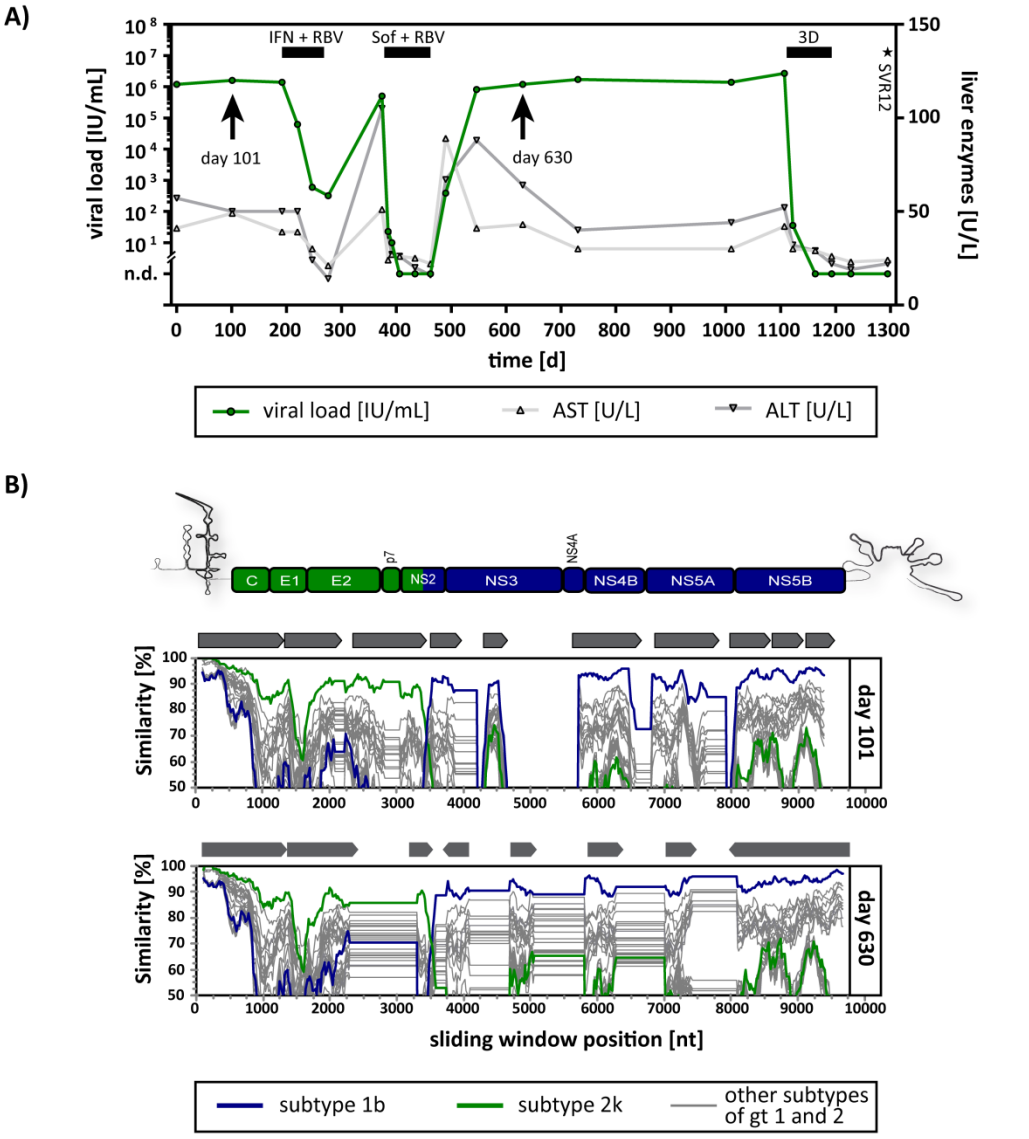
A)
IFN + RBV
Sof + RBV
3D
day 101
day 630
★ SVR12
viral load [IU/mL]
liver enzymes [U/L]
n.d.
time [d]
viral load [IU/mL]
AST [U/L]
ALT [U/L]
B)
C
E1
E2
p7
NS2
NS3
NS4A
NS4B
NS5A
NS5B
Similarity [%]
day 101
day 630
sliding window position [nt]
subtype 1b
subtype 2k
other subtypes of gt 1 and 2
C)
genotype 7
genotype 2
0.05
genotype 6
2k/1b chimera
genotype 1
genotype 5
genotype 3
genotype 4

Figure legend

A) Changes in HCV viral load (IU/L), Alanine transaminase and aspartate transaminase (U/mL) during three antiviral therapies (indicated by black horizontal bars). Serum samples for next generation sequencing of viral genomes were drawn at day 101 and 630, as highlighted by the black arrows. **B)** Nucleotide similarity comparison of available recombinant viral sequences at day 101 (upper line plot) and day 630 (lower line plot) and all subtypes of HCV genotypes 1 and 2 archived in the HCV database (http://hcv.lanl.gov/components/sequence/HCV/search/searchi.html). Similarities to subtype 1b (blue line) and subtype 2k (green line) are pronounced, the crossing point was identified at 3175/3176 bp (reference isolate H77). Gray arrows above the plots represent genome segments for which contigs were assembled during de-novo sequencing. Analyses and plotting was performed using the online available tool SimPlot version 3.5.1 (http://sray.med.som.jhmi.edu/SCRoftware/simplot/) with a sliding window size of 200 bp at a 20 bp step size using the 2-parameter Kimura approach. **C)** For phylogenetic classification a reduced dataset of 208 sequences from the HCV database (*vide supra*) containing all HCV genotypes was analyzed using MEGA6 software implementing the Neighbour-Joining method and Jukes-Cantor method for computing the evolutionary distances (scale bar reflects units of number of base substitutions per site).