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Non-Structural 5AResistance-Associated Substitutions and Interleukin28B in HCV Genotype 3b Decompensated Cirrhosis Patients with Sofosbuvir Plus Velpatasvir

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1. Abstract

Background: HepatitisCVirus(HCV)Genotype(GT)infection is still regarded as one of the more “difficult-to-treat”GTs in the era of IFN-free treatments. The viral factors involved in this unfavorable outcome have yet to be clarified.

GT3HCVstrainsconsistofvarioussubgenotypes,andonlyafew studies on genome sequences of GT3 HCV have been published, sinceuniversalprimerssuitableforanysubgenotypestraincannot be designed.

Case Summary: We describe two cases of HCV GT 3b decompensated liver cirrhosis treated with sofosbuvir plus velpat- asvir (SOF/VEL) for 12 weeks resulting in breakthrough and re- lapse.

Case1 (breakthrough): A 71-year-old man with Child-Pugh-Turcotte (CPT) score 7 demonstrated negative HCV RNAafter 4 weeksoftreatmentandat8weeksthereafter;however,HCVRNA turned positive 12 weeks post-treatment.

Case2(relapse):A60-year-oldmanwithCPTscore8demon-

stratednegativeHCVRNAafter12weeksoftreatment;16weeks post-treatment, HCV RNAturned positive. Despite breakthrough andrelapse,hepaticreservefunctionsincludingalbumin,bilirubin and prothrombin time improved in both cases without change in the CPT score.

Analysisofbothcases,revealednoresistance-associatedsubstitutions (RAS) within NS3 or NS5B, whereas non-structural protein 5A (NS5A) paired RASs (A30K, L31M), except M28 and Y93, were in evidence indicating strong resistance to any NS5A; inter- leukin28B (IL28B) single nucleotide polymorphisms (SNP) was heterogenous (rs8099917 TG).

Conclusion: To the best of our knowledge, this is the first analysisofRASandIL28BSNPinGT3bdecompensatedcirrhosis patients demonstrating breakthrough and relapse after SOF/VEL treatment.

2. Introduction

In Phase 3 studies, Sofosbuvir (SOF) (400 mg/day)/Velpatasvir (VEL)(100mg/day)treatedfor12weekshasbeenevaluatedin

57patientswithhepatitisCvirus(HCV)genotypes(GTs)andde-compensated liver cirrhosis [Child-Pugh-Turcotte (CPT)] class B or C [1].

Ofthe51patientsenrolledwithoutribavirinadministration,77%, 16%and1%showGT1,2and3HCVinfection,respectively,and 77% and 20% show CPT class B and C cirrhosis, respectively. SustainedViralResponse(SVR)12ratesare98%,89%and0%in GT1,2and3respectively[1].ThenumberofGT3patientsbeing verysmall(2patients)andtheSVRratebeing0%,exactdatasuch as CPT class, subtype, Resistant Associated Substitutions (RAS) and Interleukin28B (IL28B) Single Nucleotide Polymorphisms (SNP) regarding GT3 is unclear.

In 2019, SOF/VEL is approved in Japan, and Japan Society of Hepatology guidelines recommend SOF/VEL treatment for patients with decompensated cirrhosis.

WeencounteredtwocasesofHCVGT3bdecompensatedliv- er cirrhosis treated with SOF/VEL for 12 weeks resulting in breakthrough and relapse, and analyzed RAS and IL28B SNP (rs8099917).

3. Case Reports

Case1 (breakthrough)

A71-year-oldtreatment-naivemanwasreferredtoourhospital forthetreatmentofHCV-relatedlivercirrhosis.

SOF(400mg/day)plusVEL(100mg/day)wasfullyprescribedfor 12 weeks without interruption. HCVRNAwas negative at weeks 4oftreatmentandpersistedfor8weeksthereafter,however,HCV RNAturnedpositive12weeksafterthetreatment.Laboratorydata at baseline and at 12 weeks are shown in Table 1.

Case2 (relapse)

A60-year-oldtreatment-naivemanwasreferredtoourhospitalfor the treatment of HCV-related liver cirrhosis.

SOF (400 mg/day) / VEL (100 mg/day) was fully prescribed for 12 weeks without interruption. HCV RNA was negative after 12 weeksoftreatment,however,HCVRNAturnedpositive16weeks post-treatment. Laboratory data at baseline and at 16 weeks are shown in Table 1.

In both cases, hepatic reserve functions improved, however, the CPT B class did not change (score: 7 for case 1 and 8 for case 2) (Table 1).

Thepatientshadnopasthistoryofbloodtransfusionordrugabuse, anddidnottakedrugs,suchasproton-pumpinhibitors,thatimpact theefficacyofSOF/VEL.Theyhadneverbeenabroad,makingthe transfection route unclear.

IL28BSNPwasheterogenous(TG)inbothpatients(Table1).

Table1:Laboratorydata

	Case 1		Case 2	
Parameters	At baseline	12weekspost-treatment	At baseline	16weekspost-treatment
Totalprotein	6.8 g/dl	6.8 g/dl	6.8 g/dl	7.0 g/dl
Albumin	3.2 g/dl	3.3 g/dl	2.5 g/dl	2.9 g/dl
TotalBilirubin	1.98 mg/dl	2.45 mg/dl	3.09 mg/dl	2.5 mg/dl
Prothrombin time	82.30%	83.80%	64.40%	63.40%
Child-Pugh-Turcotteclass	B (score 7)	B (score 7)	B (score 8)	B (score 8)
Genotype	3b		3b	
IL28B	heterogenous(CT)		heterogenous(CT)	
HCVRNA	5.8 Log IU/ml	1.4 Log IU/ml	5.7 Log IU/ml	5.3 Log IU/ml

IL28B: interleukin28B.

RAS analysis

MethodsandResults

Briefly,themethodforRASanalysiswasasfollows:

ViralRNAwasextractedandreversetranscribedasdescribed.

Primers GT3.5AF2 and GT3.5AR2 were used for non-structural protein 5A (NS5A) regions as described.

The NS5B regions were amplified into two overlapping regions withtwopairsofprimers,theformer(fromS76ofNS5BtoA338); forward: 5'- AAGGAGGTGAAGGAGCGAGCA-3', reverse:5'-GTACCTGGTCATAGCCTCCGTG-3', and the latter (from T227 of NS5B to S549); forward: 5'-TGATACCCGCTGYTTT-GACTC-3', reverse: 5'- GTGATAAATGTCGTTCCCGCC-3'.

AmplifiedproductswerepurifiedwithmagneticbeadsAMPure XP(BeckmanCoulter,Indiana,UnitedStates),andterminated withtheBigDyeTerminatorv3.1CycleSequencingKit(Thermo FisherScientific)accordingtothemanufacturer'sprotocol.

The terminal products were sequenced with 3500 Genetic Analyzer (Thermo Fisher Scientific) after purification with magnetic beads(AgencourtCleanSEQBeckmanCoulter).Theresultingnu-cleotidesequencedatawereassembledwiththeuseofATGCver.6 (GENETYX, Tokyo, Japan).

Paired RASs (A30K + L31M) in NS5A region were observed in bothpatient'spost-treatment(case1:12weeksafterthetreatment, case 2: 16 weeks after the treatment) and at baseline.

NootherRASinNS5Aregion,suchasM28Y93,wasobservedin either patient post-treatment, or at baseline.

No RASs, including LI59, S282, or V323 in NS5B region was observed post-treatment or at baseline (Figure 1).

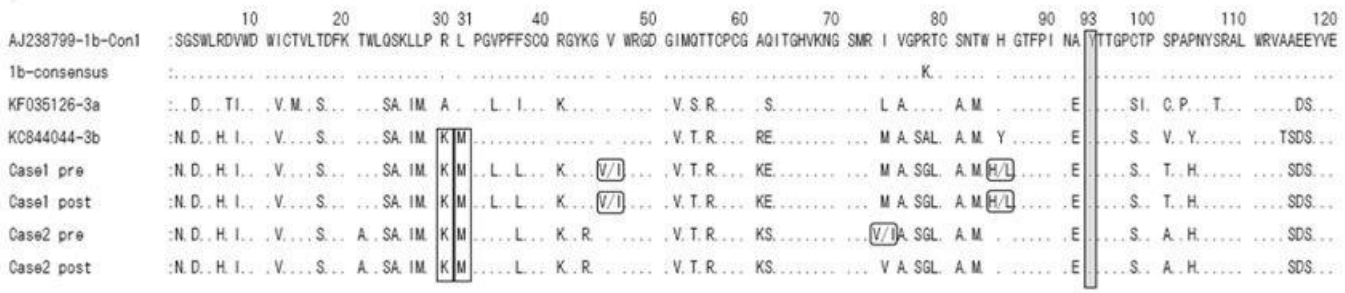

Figure1: NS5AclassRASat baselineandatpost-treatmentinthebreakthroughandrelapse patients.

4. Discussion

SOF/VEL is recommended in the US, the European Union, and otherareasforthetreatmentofGTs1-6chronicHCVinfectionin patients with or without compensated liver cirrhosis, and for use withribavirininpatientswithdecompensatedlivercirrhosis[2,5]. TheASTRAL-4studyassesses12and24weeksoftreatmentwith SOF/VELwithorwithoutribavirininHCV-infectedpatientswith CPTclass B decompensated liver cirrhosis in the US [5]: SVR12 in 83% of patients taking 12 weeks of SOF/VEL, in 94% of patientstaking12weeksofSOF/VEL plusribavirin,andin86% of patients taking 24 weeks of SOF/VEL, SVR rates are lower in HCVGT3patientsthaninthosewiththeotherGTs,particularlyin treatment-experienced liver cirrhotic patients [2, 3, 6].

HCV GT3 is the second-most prevalent genotype, accounting for 25%ofallinfectedpatients,andhasaparticularlyhighpercentage among European drug abusers and in SouthernAsia [7, 8]. Moreover, recent data indicate that HCV GT3 infection is related to higher rates of hepatic steatosis, more rapid fibrosis progression, andoccurrenceofhepatocellularcarcinomacomparedtoinfection with other HCV genotypes [9, 11].

From view point of history, HCVGT3 infection treated with IFN is thought an easy-to-treat GTwith SVR rates of about 70% after 24-48 weeks of IFN-based therapy in non-liver cirrhotic patients [12,13].Mostfirst-generationHCVproteaseandNS5Ainhibitors are,however,lesseffectiveinGT3infection,especiallywithother negativepredictivefactors,includinglivercirrhosis,previousIFN therapyfailureand/orResistance-AssociatedSubstitutions(RASs) [8, 14].

Thus, HCV GT3 infection is still estimated as one of the more "difficult-to-treat"GTsintheageofIFN-freetreatments.Theviral factors involved in this unfavorable result remain to be clarified.

GT3 HCV strains are composed of various subgenotypes, and a fewstudiesongenomesequencesofGT3HCVhavebeenreport- ed, since universal primers suitable for any subgenotype strain cannot be developed [15].

HCV-resistant patients in a network of the world of cohorts of GT3a infected patients, DAA-naïve patients (n=315, 51.8%), shownoNS5BRASs,whereasNS3RASsaredemonstratedin 8.9%(e.g.Q80K,Q168Q/R,A166T)andNS5ARASsin25.1% ofpatients(withA30KandY93H,at4.4%and6.0%respectively) [16]. Of DAA-treated patients (n=293, 48.2%) failing NS5A inhibitor-basedtherapy,78.8%harboredNS5ARASsatfailureofall NS5inhibitor-containingregimens,exceptledipasvir,followedby NS5AA30K/S(17%).AcombinationofNS5AA30Kandy93His seldomdetected.ThepercentageofNS5BS282C/Tislow(1.6%) in patients failing SOF-containing regimens. NoNS5B polymorphisms (including at newly reported positions 150 and 206) are related to SOF-containing regimen failures [16]. The authors did not, however, include GT3b in the study.

TheA30K+L31McombinationisshowninallsamplesofGTs3b and3g,demonstratinghighfrequenciesofRASsinnon-structural protein 5A inhibitors of GT3 HCV and in vitro analysis shows that these subtypes may be inherently resistant to all approved non-structural protein 5A inhibitors for GT3 HCV [17, 20].

Inthepresentstudy,thepairedA30KandL31Msubstitutionswere observedatbaseline,asinthepreviousreports.Thesignificanceof such substitutions has, however, not yet been clarified in clinical practice because of the difficulty in genome sequencing of GT3 HCV [15].

Nowadays,clinicaltrialswithSOF/VELhavebeenperformedfor difficult-to-treatGT3patientswithfirstgenerationHCVinhibitors and non-structural protein 5A inhibitors.

Of 478 patients (GT3a 472, GT3b 3, GT3g 2, GT3k 1) with GT3 infections,12%haveNS5AclassRASsatbaseline.TheSVRrate is 93% (53/57) and 98% (411/420) in patients with and without baselineNS5AclassRASs.OfthetestedSVRratesinthepresence ofY93H in patients with GT3 infections and liver cirrhosis, only 1.2% (6/478) with GT3 are liver cirrhotic, however, and harbor Y93Hatbaseline.FourofthesesixpatientsobtainSVRafterSOF/ VELtherapy. In the context of GT3b, the SVR rate is 100% (3/3) [6].

The authors demonstrate also 100% of SVR12 in 5 GT3 patients treatedwithSOF/VELfor12weeks,regardlessofbaselineNS5A RASs, such asA30K and L31M. Data with respect to decompensated liver cirrhosis and GT3b is not clear [6].

Analysisof293GT3patientsshows25.3%aslivercirrhoticand

21.8% as treatment-given, including 4.1% with DAAexperience. Baseline NS5ARASs (Y93H,A30K, L31M) are found in 11.2%. Ribavirin (RBV) added in 5% of non-liver cirrhotic and 58.9%of liver cirrhotic patients shows SVR12 rates for SOF/VEL/RBV at 95.9% (mITT) and 99.5% (PP). Only 1 virological relapse ex- periences in a liver cirrhotic patient previously treated with SOF/ RBV [21].

However,theydidnotincludeGT3bpatientsinthestudy.

A landmark discovery of SNPs in or near the IFNL3 and IFNL4 loci[22,23],firstreportedasIL28BSNP,isstronglyrelatedtothe responsetoIFN-basedtherapyforchronichepatitisC[22,25],and with spontaneous clearance of HCV [26, 28]. In the age of IFN- free treatment, a few papers reported IL28B SNP CC (major) as related to favorable early viral kinetics and effectiveness of DAA therapy of HCV GT1 patients [29, 30].

5. Conclusion

Inourstudy,3factorswererelatedtothebreakthroughandrelapse cases.

First, paired NS5A(A30K, L31M) at baseline was in evidence in both cases, indicating strong resistance to any NS5A inhibitors.

Second, decompensated cirrhosis itself (CPT B score 7 and score 8) may be related.

Third,IL28BSNPheterogenousmaybeafactor,althoughIL28B SNP analysis of GT3b cases has not been reported.

Inaddition,regardingthefactorsrelatedtothebreakthroughcase, NS5B mutation, including 150, 206 and 282 may have occurredin the course of treatment, although NS5B mutation was not ob- served at baseline or post treatment in our breakthrough and re- lapse patients.

TothebestofourknowledgeJthisisthefirstanalysisofRASand IR28B SNP in decompensated cirrhosis patients, demonstrating breakthrough and relapse with SOF/VEL treatment.

Furtherstudyisneededtoclarifythereasonfortreatmentfailurein GT3b decompensated liver cirrhosis patients.

6. AuthorContributions:

Kim SK conceived the study and wrote the manuscript; Kim SR, OgawaSandTanakaYobservedtheclinicalcourseofthepatients andmadethefigures;FujiiT,FujiiY,KobayashiH,HayakumoT, OkudaT,NakaiA,YuasaK,TakamiM,KimKI,OhtaniA,Nakao KandSaijoYobservedtheclinicalcourseofthepatient;OgawaS and Tanaka Y performed RAS and IL28B SNP analyses.

7. Conflictsofinterest:

TanakaY.Researchsupport:JanssenandGilead,Speaker:Gilead.
Theotherauthorshavenoconflictsofinteresttodeclare.

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