Supplementary Material: Methods

PCR primers sequences for second PCR

GT1a-F	CCTTTGAAAAACACGATAATACCATGGCGCCCATCACGGCGTACGCCCAGC
GT1a-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGGGATCTCATGGTTGTCCCTAGGTTC
GT1b-F	CCTTTGAAAAACACGATAATACCATGGCGCCTATTACGGCCTACTCCCAAC
GT1b-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGGGACCGCATAGTGGT
GT2a-F	GTTTTCCTTTGAAAAACACGATACGCGTATGGCTCCCATCACTGCTTATGCCCAGC
GT2a-R	GTGGCGTGCTGTTGTCACTGAAAGTGGGAGACCTTGTAACAACGTCGAGTGTCTC
GT2b-F	GGACGTGGTTTTCCTTTGAAAAACACGATACGCGTATGGCTCCCATTACTGCTTACA
GT2b-R	GCGGCGTACTGTTGTCAGAGAAACTGGGCGTCCGTGTGGTGACATCGAGTGATTCGAC
GT3a-F	CCTTTGAAAAACACGATAATACCATGGCCCCGATCACAGCATACGCCCAGCAAAC
GT3a-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGAGACCTAGCCTGTGTGCTAAGGGTTTCCAC
GT4a-F	CCTTTGAAAAACACGATAATACCATGGCCCCCATCACAGCATACGCGCAGCAGACC
GT4a-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGTGATCTCATGGTAGTCTCAAGAGATTCAACC
GT4d-F	CCTTTGAAAAACACGATAATACCATGGCCCCCATCACTGCGTATGCGCAACAGACC
GT4d-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGAGACCTCATGGTGGTTTCAAGAGATTCAACC
GT4n-F	CCTTTGAAAAACACGATAATACCATGGCTCCCATCACCGCGTACGCGCAGCAGACC
GT4n-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGAGACCTCATGGATGTTTCCAGGGACTCGACT
GT5a-F	CCTTTGAAAAACACGATAATACCATGGCCCCTATCACCGCGTATGCGCAGCAGAC
GT5a-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGCGAGCGCATCGTGGTTTCCAGGTTTTCAAC
GT6a-F	GACGTGGTTTTCCTTTGAAAAACACGATACGCGTATGGCTCCCATAACTGCGTACGCAC
GT6a-R	GCGTAGAGTTGTCTGTGAATGAGGGAGAGCGCATGGTCGTCTCCATATTCTC
GT6e-F	CCTTTGAAAAACACGATAATACCATGGCCCCTATCACCGCATACCATCAGCAAAC
GT6e-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGGGACCGCATTGTGGTT
GT6l-F	CCTTTGAAAAACACGATAATACCATGGCACCGATCACGGCATATGCTCAGCAGAC
GT6l-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGTGAGCGTGCGGTCGTCTGCATGCTCTCTAC
GT6p-F	CCTTTGAAAAACACGATAATACCATGGCCCCCATTACAGCCTATCATCAACAG
GT6p-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGAGACCGCATGGTAGTCTCCATGTTCTCGAC
GT6v-F	CCTTTGAAAAACACGATAATACCATGGCGCCTATAACAGCTTATGCGCAGCAG
GT6v-R	GCGGTACGGCCGGAGGCGCGCCGTTGTCCGTGAAGACCGGAGATCTCATGCTAGTTTCCATGTTTTCCAC

Construction of Genotype 1b, 2a, 2b, and 6a HCV Subgenomic Replicons

Replicon Con1/SG-PI-hRluc was derived from the plasmid I389luc-ubineo/NS3-3'/ET, which encodes a GT1b (Con-1 strain) subgenomic replicon and was obtained from ReBLikon (1, 2).

Replicon JFH-1/SG-Rluc neo was derived from the plasmid pLucNeo2a containing the nonstructural genes of GT2a JFH-1 strain and a renilla luciferase reporter {Cheng et al 2010}.

Subgenomic replicon GT2b J8-PI-hRluc, encoding a bicistronic replicon with the humanized renilla luciferase (hRluc) reporter gene downstream of the poliovirus IRES (PI), and the GT2b J8 (GenBank accession number JQ745652) nonstructural genes (NS3-NS5B, including adaptive mutations A178T and F438L in NS3, A15S in NS4A, I253V in NS4B, E287V in NS5A, and H480R and D559G in NS5B) downstream of the EMCV IRES was constructed. The 5'UTR and 3'UTR included the GT2b J8 sequence.

Subgenomic replicon GT6a-PI-hRluc, encoding a bicistronic replicon with the humanized renilla luciferase (hRluc) reporter gene downstream of the poliovirus IRES (PI) and the genotype 6a (SUBID 6636, GSI6a-1) nonstructural genes (NS3-NS5B, including an NS3 adaptive mutation K272R and 2 NS5A

adaptive mutations S232G and P237L) downstream of the EMCV IRES was constructed. The 5'UTR and 3'UTR included the genotype 2a JFH-1 sequence.

Cell Lines

1C cells were derived from a lunet-based genotype 1a replicon clone. The clone was cured by IFN + BILN2061+VX222 to generate the 1C cell line. 1C cells have been intensively validated for a complete cure and no surviving colonies have been observed after G418 treatment (3). 1C cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM; GIBCO, Carlsbad, CA) supplemented with fetal bovine serum (FBS; Hyclone, Logan, UT). Cells were maintained at 37°C in humidified incubators (85% humidity) and 5% CO2.

Deep sequencing

Reverse transcription polymerase chain reaction (RT-PCR) products targeting the entire NS3/4A, from patient plasma with HCV RNA >1000 IU/mL were amplified and sequenced on the Illumina MiSeq (Illumina, Inc., San Diego, CA) deep sequencing platform or by population sequencing at DDL Diagnostic Laboratory (Rijswijk, The Netherlands), WuXi AppTec (Shanghai, China) or Monogram Biosciences (San Francisco, CA). Internally-developed software pipeline (Gilead Sciences) was used to process and align sequencing data to identify the substitutions present at level above 1% (percent total of reads). The presence of variants in GT1 to 6 samples was established by comparison with the wild-type reference sequence: HCV1a_H77_NC_004102; HCV1b_con1_AJ238799, HCV2a_JFH_ AB047639, HCV3a_S52_ GU814263, HCV4a_ED43_GU814265, HCV5a_SA13_ AF064490, and HCV6a_EUHK2_ Y12083.

Site-directed Mutagenesis

Replicon NS3 variants were created by Gilead Sciences (Foster City, CA) or by Wuxi AppTec (Shanghai, China) by site-directed mutagenesis (SDM) using a QuikChange II Site-Directed Mutagenesis Kit (Stratagene, Cat# 200514). Following verification of mutation presence by sequencing, the NS3 region was sub-cloned back into wild-type Pi-Rluc plasmids. Sequencing analysis was performed again to confirm the correct construct was produced

Compounds

Voxilaprevir (VOX, GS-9857, lot w5) was synthesized by Gilead Sciences (Foster City, CA).

References

1. Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R. 1999. Replication of subgenomic hepatitis C virus RNAs in a hepatoma cell line. Science 285:110-113.
2. Vrolijk JM, Kaul A, Hansen BE, Lohmann V, Haagmans BL, Schalm SW, Bartenschlager R. 2003. A replicon-based bioassay for the measurement of interferons in patients with chronic hepatitis C. J Virol Methods 110:201-9.
3. Peng B, Yu M, Xu S, Lee YJ, Tian Y, Yang H, Chan K, Mo H, McHutchison J, Delaney W, Cheng G. 2013. Development of robust hepatitis C virus genotype 4 subgenomic replicons. Gastroenterology 144:59-61 e6.