Natural Variation in Susceptibility of HCV Genotype 1 Patient Isolates to Direct Acting Antiviral Agents

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Direct-acting antiviral (DAA) agents that target HCV replication offer significant promise for the curative treatment of HCV infected individuals.

In an effort to support and standardize the pre-clinical and clinical evaluation of DAA agents, we generated genotypic and phenotypic assays to assess resistance to NS3 protease, NS5A and NS5B polymerase inhibitors.

In this study we surveyed a large panel of DAA treatment naïve genotype (GT) 1a and 1b patient viruses to assess natural variation in DAA susceptibility.
Methods

• Genotype 1a and 1b NS3/4A, NS5A and NS5B sequences were amplified from patient plasma and subjected to conventional sequence analysis.
• A Con1 luciferase-reporter replicon was engineered to generate replicons that enable the incorporation of patient-derived NS3 protease, NS5A or NS5B sequences.
• One or more DAA resistance associated mutations were introduced into reference replicons by site-directed mutagenesis (SDM).
• Replicons containing SDM or patient virus sequences were used to evaluate DAA susceptibility (fold change (FC) in IC$_{50}$ or IC$_{95}$) and replication capacity (RC) relative to wild type reference replicons.
HCV reporter replicons

5’ UTR

C Luciferase

poliovirus IRES

EMCV IRES

NS3 protease

PDS

3’ UTR

NS5A

NS5B

HDV ribozyme

PDS = patient derived sequences

5’ UTR

C Luciferase

poliovirus IRES

EMCV IRES

NS3 protease

PDS

3’ UTR

NS5A

NS5B

HDV ribozyme

5’ UTR

C Luciferase

poliovirus IRES

EMCV IRES

NS3 protease

PDS

3’ UTR

NS5A

NS5B

HDV ribozyme
NS3 protease inhibitors

(n=107, 1a=52, 1b=55)
Replication capacity of replicons containing patient-derived NS3 sequences

Median (range) RC (% of Con1)
1a+1b = 9% (0.2-85%)
1a = 6% (0.2 to 27)
1b = 19% (0.34 to 85%)
Susceptibility of replicons containing patient virus derived NS3 sequences to protease inhibitors

Median FC (range) relative to Con1
BOC FC = 0.48 (0.16-2.15)
TVR FC = 0.52 (0.17-1.58)
Drug 1 FC = 0.65 (0.14-7.14)

Samples with DRMs in NS3 (red):
1 (1a). V36L
2 (1a). T54S, V55I
3 (1a). T54S, V55I
4 (1a). T54T/S
NS5A inhibitors

(n=109, 1a=71, 1b=38)
Replication capacity of replicons containing patient-derived NS5A sequences

Median (range) RC (% of Con1)
- 1a+1b = 20% (1-164%)
- 1a = 11% (1-56%)
- 1b = 57% (19-164%)

N=109
N=71
N=38
Susceptibility of replicons containing patient virus derived NS5A sequences to NS5A inhibitors

Samples with NS5A DRMs (red)
1 (1a). M28T
2 (1a). Q30H
3 (1a). Q30H
4 (1a). Q30H, Y93H
5 (1a) Q30Q/H, Y93Y/H/N
6 (1a). L31L/M
7 (1b). L31L/M, Y93Y/H
8 (1b). Y93Y/H

Median FC (range) relative to Con1
1a IC50 FC = 1.38 (0.52->200)
1a IC95 FC = 2.90 (1.12->200)
1b IC50 = 0.71 (0.49-2.50)
1b IC95 = 0.95 (0.54->200)
Inhibition Profiles of Virus Populations and Clones that Contain or Lack NS5A Inhibitor Resistance Mutations

Sample 7 pool (1b): L31M + Y93Y/H

Sample 7 clone-1: L31M + Y93 (30/39)

Sample 7 clone-2: L31M + Y93H (9/39)

Sample 8 pool (1b): Y93Y/H

Sample 8 clone-1: Y93 (24/41)

Sample 8 clone-2: Y93H (17/41)
NS5B polymerase inhibitors

(n=48, 1a=26, 1b=22)
Replication capacity of replicons containing patient-derived NS5B sequences

Median (range) RC (% of Con1)

1a+1b = 34% (1-174%)
1a = 16% (1-78%)
1b = 73% (21-174%)
NS5B NI susceptibility of replicons containing patient-derived NS5B sequences

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<tr>
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<tr>
<td>INF</td>
<td>INF IC50 = 0.94 (0.71-2.33)</td>
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<td>INF IC95 = 0.98 (0.67-1.66)</td>
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<td>NI</td>
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<td>NI IC95 = 0.87 (0.54-1.60)</td>
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NS5B NNI susceptibility of replicons containing patient-derived NS5B sequences

Samples with NNI DRMs (red)
NNI-A: V499A/T
NNI-D: C316H

Median FC (range) relative to Con1

NNI-A IC50 = 1.23 (0.11-5.86)
NNI-A IC95 = 1.83 (0.15-6.05)

NNI-D IC50 = 0.64 (0.16-210.56)
NNI-D IC95 = 0.77 (0.23-35.03)
Genetic determinants of variation in NS5B NNI-A Susceptibility

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Median FC: 1.23 1.64 0.85 0.45 1.90 0.64
Max. FC: 5.86 3.96 5.86 0.56 5.86 4.41
Min. FC: 0.11 0.30 0.11 0.39 0.26 0.11
HCV replicons containing patient derived NS3 protease, NS5A and NS5B polymerase sequences, as well as site directed mutations (see poster O-10), were used to characterize variations in the susceptibility of GT1a and GT1b viruses to NS3/4A protease, NS5A and NS5B polymerase inhibitors, respectively.

In general, GT1b reporter replicons containing GT1b patient virus derived sequences (NS3, NS5A, NS5B) replicated better than GT1b reporter replicons containing GT1a patient virus derived sequences.

Variations in susceptibility were comparable among inhibitors of NS3/4A, NS5A and NS5B (mostly within 10-50 fold) with the exception of an NS5B nucleoside inhibitor, which exhibited notably less variation (3-4 fold).

Broader variations in susceptibility were observed among replicons derived from viruses with substitutions at resistance associated positions.

Amino acid substitutions at acquired DAA resistance associated positions were observed in a minority of isolates and accounted for some, but not all, of the variations in NS3/4A, NS5A and NS5B inhibitor susceptibility.

Whether or not these observed variations in DAA susceptibility are relevant to routine clinical practice, either now or in the future, is uncertain and will require further investigation.
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