Sequence Analysis for Resistance Monitoring Following a Single Dose of RG-101, an anti-miR Targeting microRNA-122, in Chronic Hepatitis C Patients


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BACKGROUND

• MicroRNA-122 binding to the 5'UTR of the viral RNA promotes HCV RNA stability and accumulation, and protects the HCV genome from degradation.

• RG-101 is an oligonucleotide inhibitor of miR-122 that is linked to GalNAc-carbohydrate which binds to the asialoglycoprotein receptor on the hepatocyte leading to ~20-fold increased potency.

OBJECTIVES

• The aim of this study was to assess if nucleotide changes in the 5'UTR of the HCV genome appeared in patients with viral rebound following RG-101 dosing.

MATERIALS & METHODS

• Patients received a single injection of 2 (n=14) or 4 mg/kg (n=14) RG-101 or placebo (n=4) and were followed from 8 up to 52 weeks after dosing. Follow-up ended if patients experienced a viral rebound (>1 log increase in HCV RNA from nadir).

• Sequence analysis of the HCV 5'UTR (nucleotide 1-270) was done at baseline and at time of viral rebound with HCV RNA >20,000 IU/mL.

• cDNA was synthesized by 5' rapid amplification of cDNA ends (5' RACE System, Life Technologies), followed by population-based sequencing (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems TM) and deep sequencing (Illumina MiSeq).

• Susceptibility of HCV genotype 1b replicons constructed to contain C2G, C3U, and C2G/C3U mutations to RG-101 and other anti-HCV compounds were evaluated in transient transfection assays in HuH7 cells.

RESULTS

1. Undetectable HCV RNA levels 52 weeks after a single dose of RG-101

• 22/28 patients dosed with RG-101 experienced a viral rebound up to 52 weeks of follow-up. 3/28 patients were lost to follow-up. 3/28 patients (HCV genotype 3 (n=1) and genotype 4 (n=2)) had undetectable HCV RNA levels at week 52.

2. Mutants associated with virological rebound in patients dosed with RG-101

• In 14/22 patients with viral rebound, a matched sample was available for analysis: 6/14 patients with rebound (week 12-36) had no 5'UTR mutations; 8/14 patients with rebound (week 8-12) had C3U (HCV genotype 1a) or C2G/C3U mutations and G1A and U4A polymorphisms (HCV genotype 3 and 4) in the 5'UTR miR-122 binding regions.

3. In vitro viral fitness of mutants and susceptibility to DAAs

• HCV replicons containing C2G, C3U and C2G/C3U mutations demonstrated reduced viral fitness with mean (± SD) replication levels compared to wild-type virus of 2.1 ± 0.5, 11.4 ± 4.0 and 14.3 ± 4.9%, respectively.

• HCV C3U and C2G/C3U variants demonstrated reduced susceptibility to RG-101 but were fully susceptible to all other compounds tested.

CONCLUSIONS

1. C3U (HCV genotype 1) and C2G/C3U (HCV genotype 3 and 4) HCV variants were associated with viral rebound in patients dosed with RG-101.

2. Mutants associated with virological rebound in patients dosed with RG-101

• In vitro viral fitness of mutants and susceptibility to DAAs

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