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

M.H. van der Ree[1, 2], F. Stelma[1, 2], J.M.L. de Vree[3], S.B. Willemse[1], M. van der Valk[1], A.C. van Nuenen[2], A. K. Patick[5], P. Grint[5], S. Neben[5], A. Pavlicek[5], E. van der Veer[6], N.A. Kootstra[2], H.W. Reesink[1, 2]

Department of Gastroenterology and Hepatology (1) and Experimental Immunology (2), Academic Medical Center, Amsterdam, The Netherlands, 3. Department of Gastroenterology and Hepatology, University Medical Center Groningen, Groningen, The Netherlands 4. PRA Healthsciences, Zuidlaren, The Netherlands, 5. Regulus Therapeutics, San Diego, CA, USA, 6. DDL Diagnostic Laboratories, Rijswijk, The Netherlands

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.

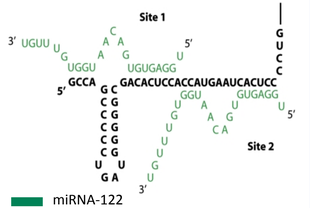


Figure adapted from Machlin et al, PNAS 2011

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

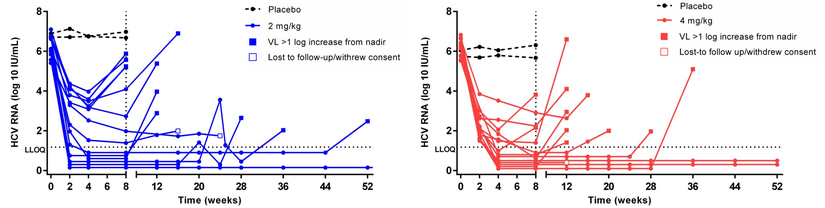
- Patients received a single injection of 2 (n=14) or 4 mg/kg (n=14) RG-101 or placebo (n=4) and were followed from 8 up to 52 weeks after dosing. Follow-up ended if patients experienced a viral rebound (>1 log increase in HCV RNA from nadir).
- Sequence analysis of the HCV 5'UTR (nucleotide 1-270) was 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.

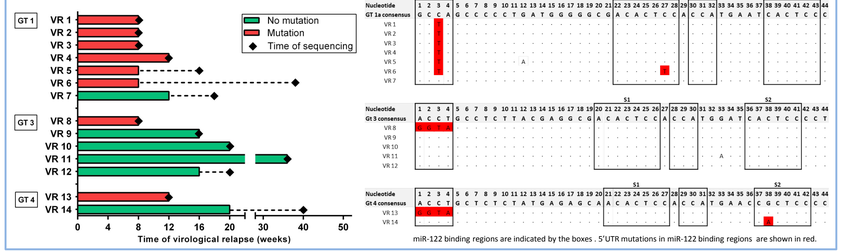
Figure 1. Change in HCV RNA levels of patients dosed with RG-101 and placebo



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.

Figure 2. Sequence analysis of the HCV 5'UTR in patients with viral rebound



miR-122 binding regions are indicated by the boxes . 5'UTR mutations in miR-122 binding regions are shown in red.

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

- HCV replicons containing C2G, C3U and C2G/C3U mutations demonstrated reduced viral fitness[a] with mean (± SD) replication levels compared to wild-type virus of 2.5 ± 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.

Table 1. Susceptibility of viral mutants to various compounds

Compound	Target	Fold change [b.]		
		5'UTR mutation		
		C2G	C3U	C2G/C3U
RG-101	miR-122	0.2	>8.9	>8.9
RG-1649	miR-122	0.3	>20.8	>20.8
Ledipasvir	NS5A	0.5	0.5	0.6
Sofosbuvir	NS5B NPI	2.4	1.3	0.8
Simeprevir	NS3/NS4A	1.0	1.2	0.9
Daclatasvir	NS5A	0.7	0.7	0.8
Paritaprevir	NS3/NS4A	0.9	1.5	1.4
Ombitasvir	NS5A	0.8	0.6	0.5
Dasabuvir	NNPI	0.5	0.9	1.1

a. Viral fitness was calculated as the ratio of the normalized RLU signal for the mutant HCV Gt1b replicon to the normalized RLU signal for the wild-type HCV Gt1b replicon.

b. Fold change expressed as the average ratio of the EC_{50} for the mutant HCV GT1b replicon to the EC_{50} for the wild-type HCV GT1b replicon based on two experiments.

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. Both C3U and C2G/C3U HCV variants demonstrated reduced susceptibility to RG-101 but were fully susceptible to DAAs.

contact: meike.vanderree@amc.nl