RG-101 DEMONSTRATES FAVORABLE IN VITRO ANTIVIRAL ACTIVITY AND CROSS RESISTANCE PROFILE TO SUPPORT CLINICAL COMBINATION STUDIES IN HCV PATIENTS

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THE INTERNATIONAL LIVER CONGRESS™ 2016 APRIL 13-17, BARCELONA, SPAIN

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Abstract

Background and aims: RG-101 is a GalNAc-conjugated antisense oligonucleotide inhibitor of the liver-expressed host cell factor microRNA-122 (miR-122), which has been shown to be required for replication of the hepatitis C virus (HCV). In preclinical studies, RG-101 dose dependently reduced viral load in genotype (GT) 1a and 3a HCV infected human liver chimeric mice. In a Phase 1 clinical trial, a single administration of RG-101 produced mean viral load reductions of 4.8 log (4 mg/kg) and 4.1 log (2 mg/kg) in patients infected with either HCV GT 1, 3, or 4 at day 29. To support additional clinical activities, we evaluated the *in vitro* antiviral activity of RG-101 against all HCV GTs, against HCV replicons resistant to NS3, NS5A and NS5B inhibitors, and in combination with direct acting antiviral (DAA) drugs.

Methods: *In vitro* antiviral activity of RG-101 alone or in combination with other anti-HCV agents was performed in the GT 1b HCV replicon system. Other antiviral studies were performed using recombinant HCV strains that contained the 5' UTR from HCV GTs 1 to 6, or HCV GT1b replicons constructed to contain DAA-resistance associated mutations. All studies with RG-101 were performed without the use of a transfection agent.

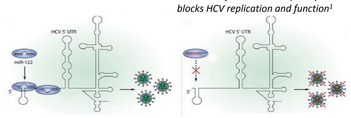
Results: RG-101 demonstrated robust antiviral activity against HCV GT1b with mean EC_{50} and EC_{90} values of 0.23 µM and 9.0 µM, respectively. No cytotoxicity of RG-101 was observed up to the highest concentration tested (320 µM) indicating a favorable *in vitro* therapeutic index of >821. RG-101 also demonstrated antiviral activity against all HCV GTs tested (HCV GT 1a, 1b, 2a, 3a, 4a, 5a, or 6a). Combination studies of RG-101 with non-nucleoside and nucleoside inhibitors of NS5B (dasabuvir, sofosbuvir), NS5A (daclatasvir, ledipasvir, ombitasvir), or NS3 (simeprevir) indicated additive interactions. In addition, RG-101 demonstrated broad antiviral activity when tested against HCV replicons resistant to NS3, NS5A and NS5B inhibitors with less than 2-fold reductions in activity when compared to a wild-type HCV replicon.

Conclusions: RG-101 has demonstrated robust *in vitro* antiviral activity against all HCV GTs, against HCV replicons resistant to NS3, NS5A and NS5B inhibitors, and in combination with other DAA drugs with little to no cytotoxicity. This data set supports the continued clinical development of RG-101 in HCV infected individuals.

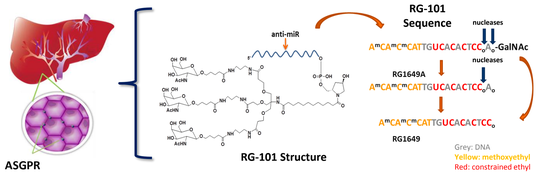
miR-122 is essential for HCV replication

- miR-122 is the most abundant microRNA in hepatocytes
- miR-122 binds to regions in the 5'UTR of HCV that include miR-122 "seed" sites
- miR-122 seed sites are highly conserved across all HCV genotypes

Inhibition of miR-122 in hepatocytes blocks HCV replication and function[1]



RG-101 is a GalNAc-conjugated phosphorothioated oligonucleotide inhibitor of miR-122 that is taken up into hepatocytes by the asialoglycoprotein receptor (ASGPR)

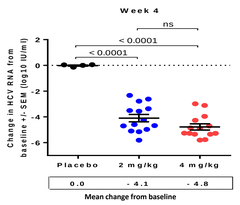


- RG-101 is a chemically modified anti-miR-122 oligonucleotide conjugated to a triantennary N-acetylgalactosamine (GalNAc) moiety through a linker that is sensitive to nuclease cleavage
- RG1649A (unconjugated metabolite of RG-101) and RG1649 (n-1 metabolite of RG1649A) are active metabolites generated in the liver following uptake of RG-101 into hepatocytes.[2]

RG-101 demonstrates antiviral efficacy in HCV infected patients

In HCV infected individuals, a single SC dose of RG-101 demonstrated:

- Mean viral load reductions of **4.1 $\log_{10}$** (2 mg/kg) and **4.8 $\log_{10}$** (4 mg/kg) at **29 days**
- Mean viral load reductions of **3.6 $\log_{10}$** (2 mg/kg) and **4.7 $\log_{10}$** (4 mg/kg) at **57 days**
- Activity against HCV GT1, GT3 and GT4
- See Poster THU-232 for additional clinical results



RG-101 is a potent and specific inhibitor of miR-122 in Huh7 cells

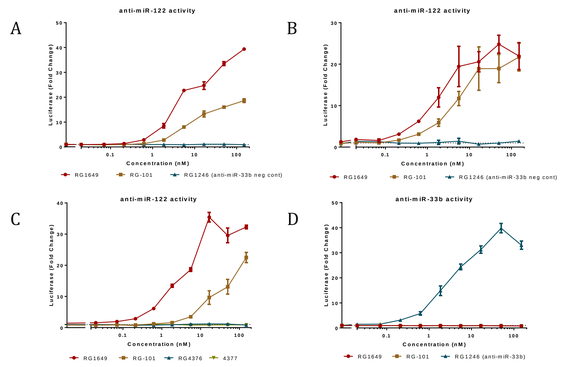


Figure 1. RG-101, RG1649, or negative controls RG1246 (anti-miR-33), RG4376, or RG4377 (two anti-miR-122 mismatches) were transfected into Huh7 cells expressing a miR-122 luciferase reporter with (panels A & C) or without (panel B) a plasmid expressing exogenous miR-122, or transfected into Huh7 cells expressing a miR-33 luciferase reporter along with a plasmid expressing miR-33 (panel D). Inhibitory activity of the anti-miRs is shown by a positive luciferase signal.

- RG-101 and RG1649, but not anti-miR-33, were active with low nM potency against either transfected miR-122 or endogenous miR-122
- Anti-miRs with several mismatches to miR-122 showed no activity against miR-122; neither RG-101 nor RG1649 showed activity against miR-33
- These results demonstrate the exquisite specificity of RG-101 and the active metabolite RG1649 for miR-122

RG-101 shows antiviral activity against GT1b HCV replicon in Huh7 cells

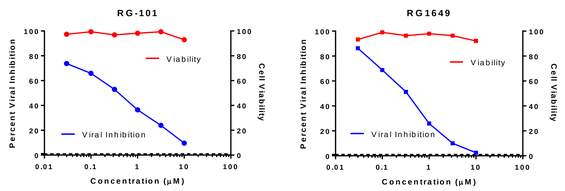


Figure 2. Six half-log serial dilutions of RG-101 or RG1649 were added without transfection reagents (unassisted uptake) to sub-confluent cultures of the Huh-7 GT1b replicon-containing cell line ET (luc-ubi-neo-ET). After 72 hours of incubation, antiviral activity was determined by luciferase activity using the Britelite plus luminescence reporter gene kit (Perkin Elmer, Shelton, CT) and cytotoxicity was assessed using the CytoTox-1 cell proliferation assay (Promega).

Table 1. In vitro antiviral activity of RG-101 against HCV genotype 1b

Experiment	EC_{50} (µM)	EC_{90} (µM)
1	0.20	8.30
2	0.39	9.71
3	0.09	>10.0
Mean	0.23	9.0

- RG-101 inhibited the replication of HCV genotype 1b with a mean EC_{50} of 0.23 µM
  - RG1649 EC_{50} = 0.33 µM in the same assay
- In a separate experiment (not shown), no cytotoxicity was observed up to the highest concentration of RG-101 tested (320 µM) yielding a therapeutic index of > 821 for RG-101

RG-101 is a pan-genotypic HCV inhibitor with activity against chimeric replicons containing 5'UTRs of genotypes 1 through 6

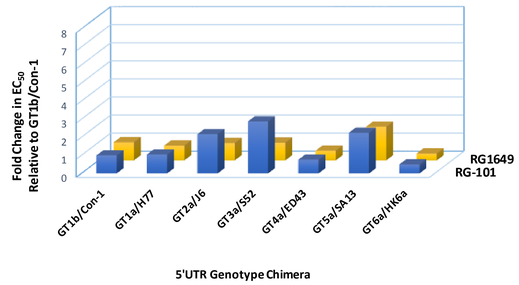


Figure 3. Antiviral activity was evaluated by unassisted uptake of RG-101 or RG1649 in Huh7 cells transfected with chimeric HCV replicon RNAs. Chimeric replicons were constructed containing the 5'UTR from genotypes 1a to 6a on GT1b/Con-1 backbone as described by Li, Y.P. et al. (ref 3). Relative fitness of the chimeric replicons ranged from 40 to 207% with RLU signals > 50,000. Broad antiviral activity was observed for sofosbuvir as a positive control (not shown). RG-101 and RG1649 were evaluated over eight, four-fold serial dilutions. Results are mean values from two independent experiments.

- RG-101 and RG1649 were active against all HCV genotypes with no more than 3-fold reduced activity compared to wild-type GT1b and no more than 6-fold variability in activity among all HCV genotypes tested

RG-101 has additive activity in combination with DAAs of various target classes on HCV GT1b replication

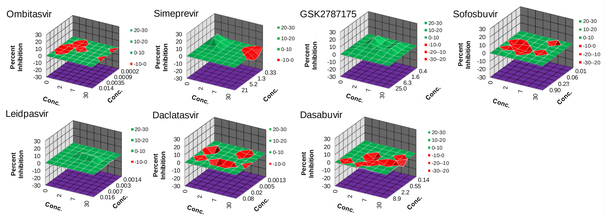


Figure 4. Analysis of the interaction of RG-101 with various DAAs covering multiple target classes was performed by the method of Prichard and Shipman (ref 4). Seven 2-fold dilutions of RG-101 were evaluated alone or in all possible combinations with seven 2-fold dilutions of each DAA bracketing the known EC_{50}s. RG-101 was added without transfection reagents. Calculated independent effects were subtracted from the observed combined effects. Volumes with positive values at the 95% confidence interval indicate synergy, while volumes with negative values indicate antagonism. Cytotoxicity was determined in parallel (not shown). Data are from one representative experiment.

Table 2. Antiviral activity of RG-101 in combination with various DAAs

Second Drug (Target)	Volume (µM²%)		Interaction
	Synergy	Antagonism	
Ledipasvir (NS5A)	0.00; 19.91	-3.17; 0.00	Additive
Daclatasvir (NS5A)	0.00; 0.82	-32.36; -37.87	Additive
Ombitasvir (NS5A)	0.74; 0.20	-24.96; -4.15	Additive
GSK2878175 (NS5B)	0.11; 46.74	-35.44; 0.00	Additive
Simeprevir (NS3)	0.32; 25.91	-27.69; -21.74	Additive
Sofosbuvir (NS5B)	1.34; 0.00	-16.69; -27.21	Additive
Dasabuvir (NS5B)	2.53; 0.00	-26.34; -26.02	Additive

- No cytotoxicity was observed with any of the DAAs or RG-101 alone or in combination
- RG-101 demonstrated additive effects in combination with each DAA tested

RG-101 is active against DAA Resistance-Associated HCV Variants (RAVs)

Compound	Fold Change						
	NS3			NS5B		NS5A	
	R155K	A156T	D168A	S282T	M414T	L31V	Y93H
RG-101	0.7	0.3	0.5	2.5	1.1	0.9	0.8
RG1649	1.1	0.3	0.5	1.3	1.2	1.1	1.1
Daclatasvir (NS5A)	NT	NT	NT	0.5	NT	12.3	24.9
Ombitasvir (NS5A)	NT	NT	NT	0.5	NT	55.8	477.0
Paritaprevir (NS3)	104.0	20.1	123.8	1.3	NT	NT	NT
Sofosbuvir (NS5B)	0.7	0.7	1.0	12.1	0.8	0.8	0.8
Dasabuvir (NS5B)	NT	NT	NT	0.9	69.5	NT	NT

Table 3. Antiviral activity of RG-101 and RG1649 were determined against HCV GT1b replicons containing amino acid substitutions that confer resistance to various DAA classes. DAAs themselves served as positive controls. Fold change is expressed as the average ratio of the EC_{50} for the mutant replicon to the EC_{50} for the wild-type GT1b replicon based on one or two experiments; NT = not tested.

- RG-101 and RG1649 demonstrated broad antiviral activity against all DAA-resistant HCV variants with less than 3-fold reductions in susceptibility

Summary and Conclusions

- RG-101 and its active metabolite RG1649 demonstrate robust antiviral activity *in vitro* with low µM potency and no observed cytotoxicity up to 320 µM
- RG-101 and RG1649 have pan-genotypic activity (GT1a through GT6a) with ≤ 3-fold reduced activity across HCV genotypes compared to HCV GT1b
- Combinations of RG-101 with a variety of HCV NS3, NS5B, and NS5A inhibitors shows additive effects
- RG-101 and RG1649 were fully active (within 3-fold) against GT1b HCV replicons containing known DAA RAVs
- RG-101 is a pan-genotypic inhibitor of HCV replication with additive activity when combined with other HCV drugs and no resistance to tested DAA RAVs
- These studies support the continued testing of RG-101 in combination with DAAs in the clinic

References

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Acknowledgements

- We thank Robert Hamatake (GSK) for supplying the GSK2878175 investigational agent and advice under the Regulus/GSK clinical trial collaboration agreement

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