PHARMACOKINETICS AND PHARMACOLOGY OF RG-101, A NOVEL GALNAC-CONJUGATED OLIGONUCLEOTIDE TARGETING MICRORNA-122, IN HEALTHY VOLUNTEERS

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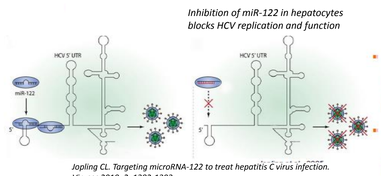
BACKGROUND

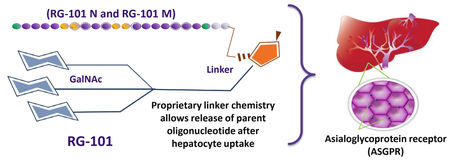
miR-122 is a critical host factor for HCV replication



- miR-122 binds to **two seed sequences within IRES that enable HCV replication and function**
- miR-122 seed sequences are **highly conserved across all HCV genotypes**
- Development of escape mutants is highly unlikely

Regulus Controls Key IP Associated with miR-122
US Patent No. 7,307,067, 7,838,504, 8,217,020
EP Patent No. 1,747, 023
Japan Patent No. 4,943,322

RG-101 is a GalNAc-conjugated phosphorothiated oligonucleotide inhibitor of miR-122 that targets hepatocytes through asialoglycoprotein receptor (ASGPR)



- RG-101 is a phosphorothioate backbone oligonucleotide complimentary to miR-122, conjugated to a trientinary N-acetylgalactosamine (GalNAc) moiety through a linker that is sensitive to endonuclease cleavage
- RG-101 oligonucleotide component is a mixture of DNA, methoxyelthyl (MOE), and constrained ethyl (cEt) nucleosides
- RG-101N and RG-101M are active metabolites generated in the liver following uptake of RG-101 into hepatocytes mediated by binding to Asialoglycoprotein receptor (ASGPR).
- RG-101M is the unconjugated metabolite of RG-101; RG-101N is the n-1 metabolite of RG-101M
- RG-101 is about 20-fold more potent than RG-101N when tested in animal models (see Poster P0907)

STUDY DESIGN and METHODS

Study Design: Randomized, double-blind, single and multiple ascending dose study to investigate safety, tolerability, pharmacokinetics, and pharmacodynamics of RG-101 in healthy volunteers, including a single dose proof-of-concept part in patients infected with hepatitis C virus, and a drug-drug interaction (DDI) part with simeprevir in healthy volunteers. The study consisted of 4 parts, summarized in the table below:

Table 1: Study Design

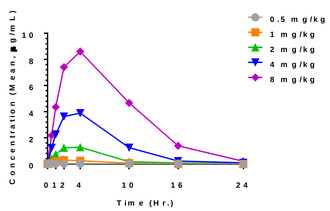
Part	Design	Dose (mg/kg)	Regimen	Cohort
A	SAD in HV	0.5, 1, 2, 4, 8 (SC)	single dose	8 subjects/group (6 treatment, 2 placebo)
B	MAD in HV	2 (SC)	*q4w* x 4 (once in every 4 weeks, 4 repeat doses)	10 subjects/group (8 treatment, 2 placebo)
C	DDI in HV	RG-101: 2 (SC) SMV: 150 mg (PO)	RG-101: single dose on Day 5 SMV: *qd* on Days 1, 5-14	8 subjects/group (8 treatment)
D	SAD POC in HCV	2, 4 (SC)	single dose	16 subjects/group (14 treatment, 2 placebo)

SAD: Single ascending dose; MAD: multiple-ascending dose; HV: healthy volunteer, HCV: HCV patients; POC: proof-of-concept; *qd*: once per day; *q4w*: once every 4 weeks; SC: subcutaneous; PO: per oral; SMV: simeprevir

Bioanalytical Methodology: Concentrations of RG-101, metabolites RG-101M and/or RG-101N in plasma or urine were measured using high performance liquid chromatography with fluorescence detection (HPLC-FL) method, where analytes were hybridized with a fluorescent-labeled probe followed by HPLC separation and detection by fluorescence.

RESULTS (Part A, SAD in HV)

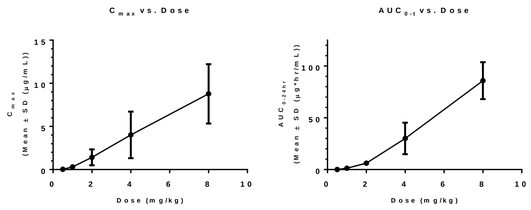
Figure 1. RG-101 plasma profile over time



- **RG-101 was rapidly absorbed into and cleared out of plasma during the first 24 hours post dose**

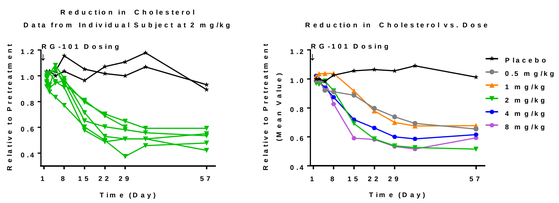
- **During the first 24 hours post dose, metabolites (RG-101N and RG-101M) were Below Limit of Quantitation (BLOQ) in either plasma or urine with <4% of the total dose eliminated through urinary excretion. This suggests RG-101 was efficiently distributed intact into tissues**

Figure 2. Plasma C_{max} and AUC plotted versus dose



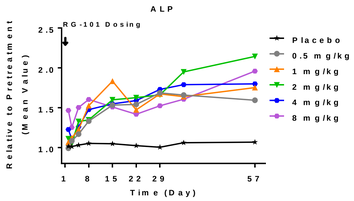
- **Plasma exposure (C_{max} and AUC) increased in greater than dose-proportional fashion**

Figure 3. Reduction in serum cholesterol over time



- **Onset of cholesterol lowering was evident 1 week after a single dose of RG-101 and persisted through the end of the study (up to ~2 months post dose)**
- **Reduction in cholesterol appeared to be dose-dependent between 0.5 to 2 mg/kg, but comparable between 2 and 8 mg/kg**
- **At 2 mg/kg, the mean plasma C_{max} was 1.4 µg/mL and $AUC_{0\text{-}24hr}$ was 6.2 µg*hr/mL, approximately 70x and 170x, respectively, below exposure achieved in monkey at NOAEL**

Figure 4. Increase in alkaline phosphatase (ALP) over time

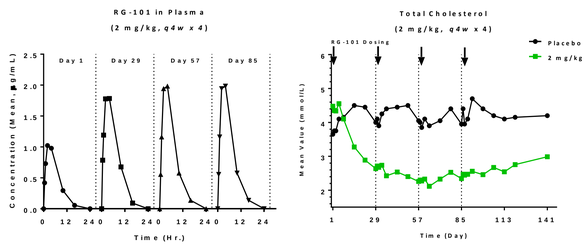


- **ALP is a putative direct target of miR-122**
- **Onset of ALP increase was evident 1 week after a single dose of RG-101 and persisted through the end of the study (up to ~2 months post dose)**

- **Increase in ALP appeared to be comparable among all dose groups**

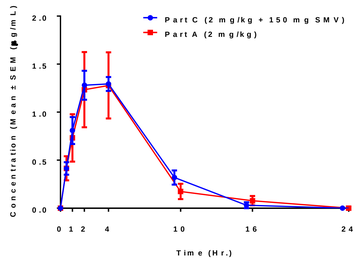
RESULTS (Parts B, C, and D)

Figure 5. Plasma PK profile and serum cholesterol modulation after repeat dosing of RG-101



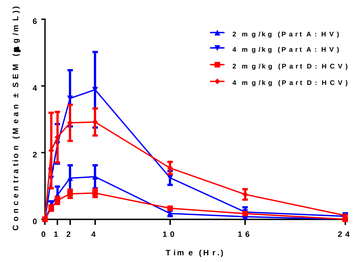
- **Similar plasma profiles after a single or multiple doses**
- **Plasma exposure of RG-101 appeared to be somewhat higher after the 2nd dose than 1st, but comparable between 2nd, 3rd and 4th doses**
- **Cholesterol lowering is maintained throughout the treatment period and persisted for 8 weeks after the last dose**

Figure 6. RG-101 plasma profile ± simeprevir (SMV)



- **No DDI observed between RG-101 and simeprevir**
  1) **simeprevir plasma exposure following a single dose of 150 mg was similar when given alone or combined with a single dose of 2 mg/kg RG-101 (Data not shown here)**
  2) **RG-101 plasma exposure following a single 2 mg/kg SC injection was similar when given alone or combined with multiple doses of simeprevir 150 mg *qd***

Figure 7. RG-101 plasma profile in health volunteers (HV) vs. HCV patients



- **Mean plasma profiles appeared generally comparable between HCV patients (N=14) and healthy volunteers (N=6) after a single SC injection of 2 or 4 mg/kg RG-101**
- **Metabolites were BLOQ in HCV patients in either plasma or urine**
- **Elimination of RG-101 through urine excretion during the first 24 hours post dose was < 4% of the total dose**

RESULTS (Cross Comparisons)

Table 2: RG-101 (2 or 4 mg/kg) plasma exposure across treatment groups: Geometric mean (geometric coefficient of variation (CV%))

Treatment	Comment	N	C_{max} (µg/mL)	$AUC_{0\text{-}t}$ (µg*hr/mL)
2 mg/kg	A (SAD)	6	1.19 (72%)	5.68 (46%)
	B (MAD, 1st Dose)	8	0.914 (55%)	6.20 (30%)
	C (DDI, w/SMV)	8	1.41 (24%)	7.92 (24%)
	D (POC in HCV)	14	0.733 (58%)	6.52 (28%)
4 mg/kg	A (SAD)	6	3.40 (70%)	25.1 (55%)
	D (POC in HCV)	14	3.03 (86%)	28.5 (35%)

- **RG-101 plasma exposure was comparable at the same dose levels across different cohorts**

Figure 8. RG-101 plasma profile in different species

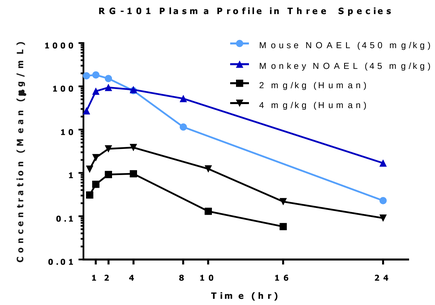


Table 3: RG-101 Plasma exposure in different species

Species	Dose (mg/kg)	Mean C_{max} (µg/mL)	Mean $AUC_{0\text{-}24}$ (µg*hr/mL)
Mouse	450 (NOAEL)	185	712
Monkey	45 (NOAEL)	95.6	1016
Human	2	1.4	6.2
	4	4.0	30.0

- **Pharmacokinetic parameters of RG-101 (2 mg/kg) in humans suggests a wide safety margin: C_{max} in humans is approximately 70 fold less than that achieved in monkey at NOAEL; $AUC_{0\text{-}24hr}$ in humans is approximately 170 fold less than that achieved in monkey at NOAEL**

CONCLUSIONS

- **RG-101 was stable in plasma**
- **RG-101 was rapidly absorbed into and cleared out of plasma presumably reflecting extensive distribution into tissues**
- **Surrogate markers of miR-122 inhibition (cholesterol and alkaline phosphatase) show maximal changes at 2 mg/kg. Changes in surrogate markers were maintained for at least 57 days after a single injection**
- **Comparable plasma PK profiles of RG-101 were observed after repeat dosing when compared to the first dose**
- **Repeat dosing of RG-101 reduced cholesterol throughout the treatment period**
- **No drug-drug interaction was observed between RG-101 and simeprevir**
- **Comparable plasma exposure was observed in HV and HCV subjects**
- **Pharmacokinetic parameters of RG-101 in humans suggests a wide safety margin. At 2 mg/kg the C_{max} and $AUC_{0\text{-}24hr}$ in humans are ~ 70 and ~ 170 fold less, respectively than those achieved in monkey at NOAEL**

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