PHARMACOKINETICS, PHARMACODYNAMICS, AND TOXICITY PROFILE OF RG-101, A NOVEL GALNAC-CONJUGATED HEPATOCYTE-TARGETING INHIBITOR OF MICRORNA-122, IN RODENTS AND CYNOMOLGUS MONKEYS

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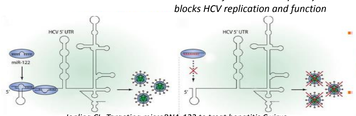
REGULUS THERAPEUTICS™

BACKGROUND

miR-122 is a critical host factor for HCV replication

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

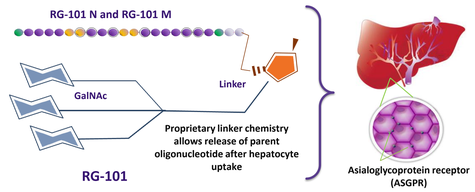


- miR-122 binds to **two seed sequences within IRES that enable HCV replication and function**
- miR-122 seed sequences are **highly conserved across all HCV genotypes**
- Development of escape mutants is highly unlikely

Jopling CL. Targeting microRNA-122 to treat hepatitis C virus infection. Viruses 2010; 2: 1382-1393

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 ASGPR

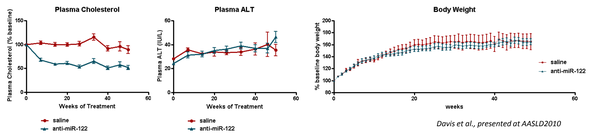


- RG-101 is a phosphorothioate backbone oligonucleotide complimentary to miR-122, conjugated to a trientinary N-acetylgalactosamine (GalNAc) moiety through a linker that is sensitive to endonuclease cleavage
- RG-101 oligonucleotide component is a mixture of DNA, methoxyelthyl (MOE), and constrained 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

Long term pharmacologic inhibition of miR-122 is well tolerated in normal mice

- miR-122 is a key regulator of metabolic function in the liver, although it is not essential for hepatocyte development
- Inhibition of miR-122 results in changes in expression of genes involved in cholesterol homeostatis, fatty acid, and lipid metabolism (Esau, CellMetab 2006)
- In miR-122 knockout mice, hepatosteatosis develops early in life, followed by inflammation, fibrosis, and HCC after about a year (Hsu, JCI 2012); inhibition of miR-122 in liver for 6 months or less did not result in increased risk of HCC (Xie, Mol Ther 2013)

Lack of Obvious Adverse Findings after Long-term miR-122 Inhibition in Mice

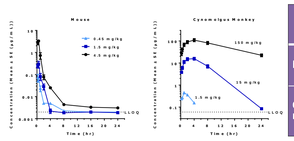


Davis et al., presented at AASLD2010

- Normal CD1 Mice (n=8/group) were treated weekly with saline or 75 mg/kg anti-miR-122 oligonucleotide for 50 weeks
- Long-term anti-miR-122 treatment resulted in a significant and sustained decrease in total plasma cholesterol consistent with pharmacodynamic effects of miR-122 inhibition, with no obvious deleterious effects based on body weight, clinical chemistry, and histopathologic evaluation

PHARMACOKINETICS & METABOLISM

Plasma concentration profile of RG-101 after single subcutaneous administration in mouse and monkey



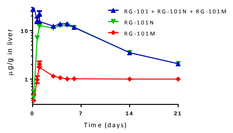
Species	Dose (mg/kg)	T_{max} (hr)	C_{max} (µg/mL)	$AUC_{0\text{-}24\ hr}$ (µg*hr/mL)
Mouse	0.45	0.25	0.05	0.1
	1.5	0.5	0.2	0.23
	4.5	0.5	3.0	2.55
Cynomolgus monkey	1.5	1	0.5	1.2
	15	4	22.0	208.4
	150	4	157.5	2174.3

- RG-101 has rapid absorption and clearance in both mouse and monkey
  - T_{max} is less than 4 hours and clearance within 24 hours at pharmacologic doses
  - RG-101 is completely stable in plasma with no significant metabolism detected (data not shown)

Tissue distribution and metabolism of RG-101 after single and monthly subcutaneous administration in mouse and monkey

Pharmacologic Doses

PK of RG-101 in Mouse Liver Following Single SC Dose of 4.5 mg/kg

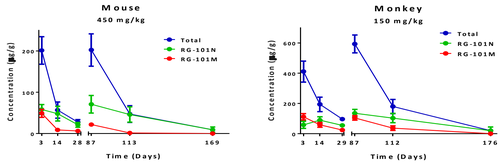


Tissue Concentrations in mouse 24 hours after single dose

RG-101 Dose (mg/kg)	Analyte Measured	Kidney (µg/g)	Liver (µg/g)	Kidney/Liver ratio
0.45	RG-101N	<0.5	1.6	<0.3
	RG-101M	<0.5	<0.5	NA
	Total	<0.5	1.6	<0.3
1.5	RG-101N	1.1	4.9	0.22
	RG-101M	<0.5	0.8	<0.6
	Total	1.1	7.4	0.15
4.5	RG-101N	7.1	13.3	0.53
	RG-101M	1.1	2.5	0.44
	Total	16.8	27.7	0.60

Toxicologic Doses

Kinetics of RG-101 metabolism in mouse and monkey after single or multiple monthly doses

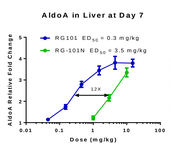


Liver concentrations of total oligo (Total), RG-101N, and RG-101M after a single SC dose, or repeat SC doses, of 450 mg/kg in CD-1 mice and 150 mg/kg in cynomolgus monkeys. In the single dose study, drug was administered on Day 1 and tissue concentrations were measured on Day 3, 14 and 28 or 29. In the repeat dose studies drug was administered on Day 1, 29, 57 and 85 and tissue concentrations were measured on Day 87, 112/113 and 169/176.

- RG-101 is rapidly metabolized to naked oligonucleotide species in tissue and active metabolites have half-life of about 14 days in the liver
  - Metabolism is rapid and fairly complete in mouse liver, less efficient in monkey liver
  - Saturation of cleavage mechanism is seen at higher doses and higher tissue concentrations
  - Minimal accumulation of parent or active metabolites observed after four monthly doses
  - Kidney to liver (K/L) ratio is substantially lower than a typical oligonucleotide (5-10 fold range) which suggests good therapeutic index

PHARMACODYNAMICS

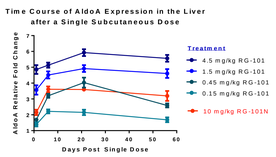
High potency, rapid onset and sustained activity of RG-101 after single subcutaneous administration in mouse



Liver and kidney concentration at 24 hours near ED90 dose in mouse

Treatment	Dose (mg/kg)	Analyte Measured	Liver (µg/g)	Kidney (µg/g)
RG-101N	10	RG-101N	30	153
		RG-101M	NA	NA
		Total	NA	NA
RG-101	1.5	RG-101N	4.9	1.1
		RG-101M	0.8	<0.5
		Total	7.4	1.1

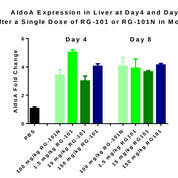
NA: Not applicable

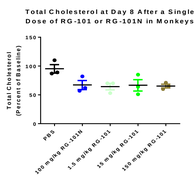


- Pharmacology of RG-101 and RG-101N in mice:
  - A single dose of GalNAc conjugate RG-101 is 12-30-fold more potent than unconjugated RG-101N based on AldoA gene de-repression and serum cholesterol lowering
  - GalNAc conjugate RG-101 results in 6-fold lower drug levels in liver and 140-fold lower drug levels in kidney at ED_{90} compared to RG-101N
  - Onset of action is within 24-hours and > 2 months duration of action after a single dose

High potency, rapid onset of action, and sustained activity of RG-101 after single and repeated subcutaneous administration in cynomolgus monkeys

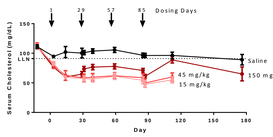
Maximal pharmacologic activity is seen at doses as low as 1.5 mg/kg in monkeys

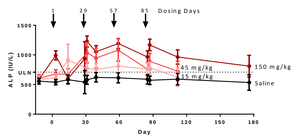




- After a single dose of RG-101 in monkeys:
  - Maximal miR-122 target gene activation is seen in liver by Day 4 and at lowest dose tested of 1.5 mg/kg
  - Maximal cholesterol lowering is also seen at 1.5 mg/kg

Cholesterol reduction and Alkaline Phosphatase elevation as pharmacodynamic markers





- In monthly repeat dose toxicity studies of RG-101 in monkeys:
  - Maximal cholesterol reduction is observed by Day 14 after the first dose. No further decrease is seen with subsequent repeat doses.
  - Increase in serum alkaline phosphatase is observed and may be a pharmacologic effect since ALP is a direct target of miR-122 (Persson R, OTS Mtg, 2012; data not shown). Further increases with subsequent dosing may be a combination of target gene de-repression and enzyme release

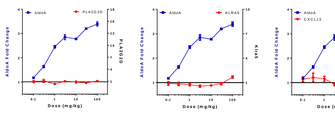
TOXICITY PROFILE

Toxicology and Safety Pharmacology Studies Performed to Support RG-101 Clinical Trials in Humans

Study title		Significant observations
Comparison of Toxicity Profiles of miR-122 Candidates in CD-1 Mice After 6 Weekly Administrations	Non-GLP	No adverse effects including histopath up to 30 mg/kg weekly dose
Comparison of Acute Toxicity Profiles of miR-122 Candidates in 129X1/SvJ Mice After Single Bolus Administration	Non-GLP	No adverse effects up to 300 mg/kg single dose
Toxicity Profile of miR-122 Candidate RG-101 in CD-1 Mice After 4 Weekly Administrations	Non-GLP	No significant adverse effects up to 300 mg/kg weekly dose
Comparison of Toxicity Profiles of miR-122 Candidates in Cynomolgus Monkeys	Non-GLP	No adverse findings up to 100 mg/kg single dose; typical Cmax-related changes*
Single and Repeat Dose Toxicity Study of RG-101 by Subcutaneous Injection in Cynomolgus Monkeys with a 3-Month Recovery Period	GLP	No adverse clinical or histopathological findings; mild LFT elevations and single cell necrosis in liver at 150 mg/kg. NOAEL = 45 mg/kg (C_{max} = 92-100 µg/ml)
A Single-Dose (Subcutaneous) Toxicity and Toxicokinetic Study of RG-101 in the Mouse with a 4-Week Recovery Period (including CNS safety)	GLP	No adverse clinical or histopathological findings up to 450 mg/kg (C_{max} = 208 µg/ml); no effect on CNS
A Multiple-Dose (Subcutaneous) Subchronic Toxicity and Toxicokinetic Study of RG-101 in the Mouse with a Three-Month Recovery	GLP	No adverse clinical or histopathological findings up to 450 mg/kg (C_{max} = 193-299 µg/ml)
RG-101: Cardiovascular, Respiratory Parameters, and Body Temperature Effects in Conscious, Telemetered, Cynomolgus Monkeys	GLP	No effect on CV or respiratory parameters or body temp up to 150 mg/kg (C_{max} = 242 µg/ml)
Bacterial Reverse Mutation (Ames) Assay	GLP	Negative
In Vivo Micronucleus and Comet Assay in Mice	GLP	Negative at 2000 mg/kg/day

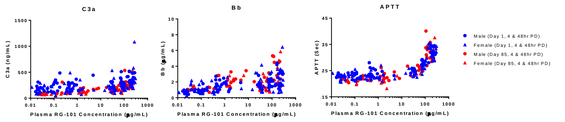
*C_{max} related transient increases in complement (C3a, Bb) and coagulation parameters (APTT, PT), typically observed with oligonucleotide therapies (Henry, SP et al., 2008), were seen in all monkey studies; LFT, Liver Function Tests; NOAEL, No Observed Adverse Effect Level

RG-101 is potent (AldoA modulation) and does not induce inflammatory genes in rats



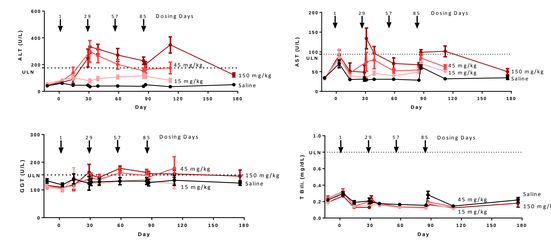
RG-101 was dosed SC in rats over a range of 0.15-150 mg/kg. All panels show potency against AldoA relative to the potency against three specific off-target interferon responsive liver mRNAs: PLA2G2D, Klra5, and Cxcl13

Mild oligonucleotide "class " effects observed with single and repeated dosing of RG-101 in monkeys



- Complement (C3a and Bb) activation with RG-101 is C_{max} related but mild
- Effects on coagulation parameters (PT, APTT, fibrinogen) are Cmax related with large safety margin

Mild and reversible effects of RG-101 on liver enzymes at higher doses in monkeys

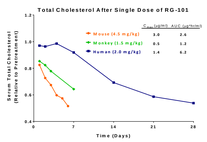


- ALT values are highest after 2nd dose administration.
  - No elevation seen after 3rd or 4th dose at 15 and 45 mg/kg which suggests an adaptive response by the liver
- ALT levels at low dose (15 mg/kg) remain around the normal range during the time course of dosing

SUMMARY and CONCLUSIONS

- RG-101, a GalNAc conjugated anti-miR oligonucleotide targeting miR-122, was extensively characterized in rodents and monkeys
  - In mice and monkeys, RG-101 is rapidly cleared from plasma and rapidly and efficiently absorbed in tissues with 5-10-fold lower kidney-to-liver ratio than typical oligonucleotide therapeutics
  - Once in liver, RG-101 is rapidly converted to naked oligonucleotide with about 14 day tissue half-life
  - Minimal accumulation of RG-101 or its metabolites occurs with monthly dosing
  - Due to rapid uptake in liver, onset of action occurs within 24 hours based on modulation of endogenous miR-122 target genes and maximal activation is seen within 14 days based on cholesterol lowering in mice and monkeys
  - Duration of RG-101 action is more than 2 months based on modulation of endogenous miR-122 target genes
  - RG-101 is well-tolerated in mice with no adverse findings after single or four monthly doses up to 450 mg/kg
  - RG-101 is well-tolerated in monkeys up to 150 mg/kg with no adverse findings at 45 mg/kg.
- PK, PD, and tox findings for RG-101 suggested a very favorable profile for advancement to clinical studies in healthy volunteers and for treatment of HCV infected patients

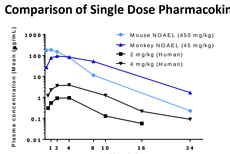
Comparison of Pharmacodynamics of RG-101 in Mouse, Monkey, and Human



Total serum cholesterol was measured after a single subcutaneous dose of RG-101 given to CD1 mice, cynomolgus monkeys, or in healthy human volunteers in a Single Ascending Dose study (see Poster P1352 and Late-Breaker Talk, Saturday 17:30). Cholesterol reduction is an indirect pharmacodynamic marker of miR-122 inhibition

- Pharmacodynamic profiling of RG-101 in multiple species demonstrates:
  - Similar rapid pharmacodynamic effects in all species after a single dose
  - Prolonged effect in all species (mouse not shown, see above for monkey at higher doses, human sustained at maximum reduction through Day 56 not shown)
- Effect on cholesterol lowering in humans suggests maximal effect of RG-101 at 2 mg/kg (see poster P1352)

Comparison of Single Dose Pharmacokinetics of RG-101 in Mouse, Monkey, and Human



		RG-101	
Species	Dose (mg/kg)	C_{max} (µg/mL)	$AUC_{0\text{-}24}$ (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

- **RG-101 presentations at EASL2015:**
  - **Sat 17:30 Late-Breaker Talk: A SINGLE SUBCUTANEOUS DOSE OF 2 MG/KG OR 4 MG/KG OF RG-101, A GALNAC-CONJUGATED OLIGONUCLEOTIDE WITH ANTAGONIST ACTIVITY AGAINST MIR-122, RESULTS IN SIGNIFICANT VIRAL LOAD REDUCTIONS IN CHRONIC HEPATITIS C PATIENTS**
  - **Hall B ePoster P1352: PHARMACOKINETICS AND PHARMACOLOGY OF RG-101, A NOVEL GALNAC-CONJUGATED OLIGONUCLEOTIDE TARGETING MICRORNA-122, IN HEALTHY VOLUNTEERS**
  - **Hall B ePoster P0904: RG-101, A NOVEL GALNAC-CONJUGATED INHIBITOR OF MICRORNA-122, DEMONSTRATES SIGNIFICANT VIRAL LOAD REDUCTION AND REDUCES LIVER STEATOSIS IN HUMAN HEPATOCYTE CHIMERIC MICE INFECTED WITH GENOTYPE 1A OR HARD-TO-TREAT GENOTYPE 3A HEPATITIS C VIRUS (HCV)**

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