RENAL IMPAIRMENT EFFECTS ON PLASMA AND TISSUE EXPOSURE OF UNCONJUGATED AND GALNAC-CONJUGATED ANTIMIRS IN A CHRONIC KIDNEY DISEASE (CKD) MOUSE MODEL

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Introduction and Aims

MicroRNAs (miRs) are an important class of small translational regulatory RNAs that don't encode proteins but rather regulate expression of multiple target messenger RNAs, and represent attractive therapeutic targets. Specific miRs can be dysregulated or serve as host factors impacting disease susceptibility, and are implicated in a wide range of diseases such as: miR-21 upregulation in Alport syndrome (characterized by progressive loss of renal function), and interaction of miR-122 with hepatitis C virus (HCV) facilitating viral replication in infected livers. AntimiRs (microRNA antagonists) are single-stranded synthetic oligonucleotides that sequester a target miR in diseased cells, forming an inactive heteroduplex. RG-012, an unconjugated anti-miR-21, is planned to be extensively evaluated in patients with Alport Syndrome, a rare genetic disease in which patients experience a progressive loss of kidney function which may lead to end stage renal disease (ESRD) requiring dialysis or transplantation. RG-101, a GalNAc-conjugated anti-miR-122 (providing enhanced delivery to hepatocytes), also has some planned clinical testing in a CKD population (non-HCV and HCV-infected subjects). Since no or little clinical and nonclinical information exists regarding the impact of CKD on the plasma and tissue exposure of unconjugated or conjugated antimiRs (or other types of oligonucleotides), we examined the pharmacokinetic disposition of RG-012 and RG-101 using a Col4a3 mutant mouse model in comparison to healthy wild type littermate control mice. Col4a3 mutant mice lack type IV collagen α3 and have increased kidney expression of miR-21 leading to progressive renal fibrosis; resulting in development of proteinuria by ~4 weeks of age, reaching CKD stages 3 and 4 by ~6 and 8 weeks of age, respectively, and a reduced lifespan of ~9–11 weeks.

Methods

Plasma (C_{max} and AUC) and tissue (liver and kidney) drug exposure of RG-101 and RG-012 were compared between mutant (Col4A3$^{(-/-)}$) and wild type mice (SV129 WT) after single or repeated (RG-012 only) weekly SC injections at various dose levels. Influence of renal impairment progression was examined using male and female mutant mice at ~CKD stage 2-3 (mean BUN ~33 mg/dL, age 6 weeks) or ~CKD stages 4–5 (mean BUN ≥100 mg/dL, age 9–11 weeks) versus age paired healthy mice (mean BUN <19 mg/dL, age 6–11 weeks). AntimiRs and related major metabolites were extracted from evaluated matrices and quantitated using HPLC-MS methodologies. Plasma and tissue exposures of total antimiRs (parent + major metabolites) were determined from composite group mean data.

Summary of In-Life Portion Study Design

- RG-012 Single & Repeat Dose at CKD stages 1-3
  - Age: 24 days (~3 weeks) at the first dose
  - Regimen: Single dose or QW x 6
  - Plasma collection: 0.25, 0.5, 1, 2, 4, 8, and 24 hrs, N=2-4
  - Tissue collection: 48 hrs post dose, N=10-14
- RG-012 Single Dose at CKD stage 4
  - Age: 59 days (~8 weeks)
  - Plasma collection: 0.5, 1, 2, 4, 8, and 24 hrs, N=2-4
  - Tissue collection: 24 hours post dose, N=2-4
- RG-101 Single Dose at CKD stages 2-5
  - Age: 6 or 9-11 weeks
  - Plasma collection: 0.5, 1, 2, 4, 6, 8, 24, 48, 168, and 336 hrs, N=2-4
  - Tissue collection: 48 hrs post dose, N=2-4

Results

RG-012 (Unconjugated AntimiR)

CKD Stages 1-3:

RG-012 Single and Repeat Dose: Plasma and Tissue Exposures of Sum (RG-012+RG0005)

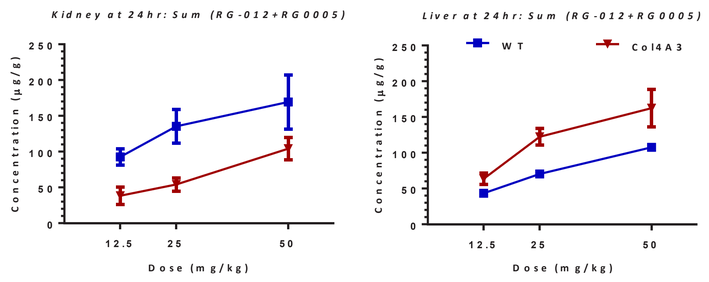
Dose (mg/kg)	Regimen	Age	Col4A3$^{(-/-)}$, *CKD: 1-3*				SV129 WT, Normal Renal Function				Fold-Change (Col4A3:WT)			
			AUC_{last} (µg*hr/mL)	C_{max} (µg/mL)	Kidney (C_{48hr}, µg/g)	Liver (C_{48hr}, µg/g)	AUC_{last} (µg*hr/mL)	C_{max} (µg/mL)	Kidney (C_{48hr}, µg/g)	Liver (C_{48hr}, µg/g)	AUC_{last}	C_{max}	Kidney	Liver
25	Single Dose	24 Days (~3 wks; ~CKD 1)	81.6	46.3			80.4	37.6			1.01	1.23		
50			178.2	86.1			137.8	66.2			1.29	1.30		
25	QW x 6	61 Days (~8 wks; ~CKD 3)	90.9	42.9	87.2	173.9	64.6	33.5	99.9	97.1	1.41	1.28	0.87	1.79
50			229.5	74.0	141	324.8	130.5	46.9	132.2	185.5	1.76	1.58	1.07	1.75

CKD Stage 4:

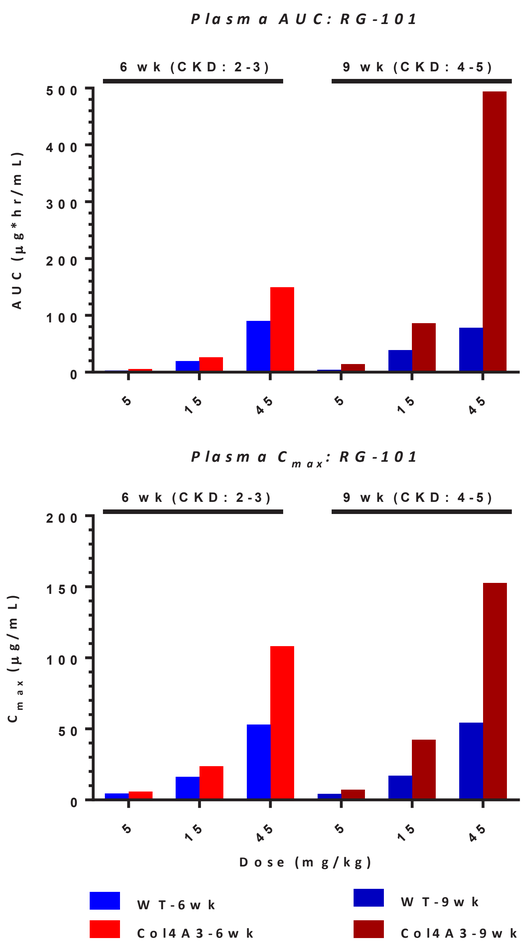
RG-012 Single Dose:
Plasma Exposure of Sum (RG-012+RG0005)

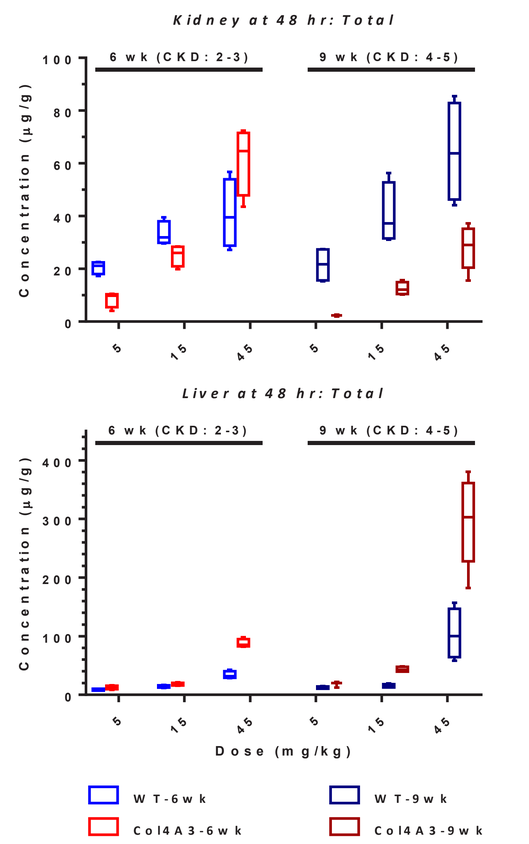
Dose (mg/kg)	Col4A3$^{(-/-)}$ Age: 59 days ~*CKD 4*		SV129 WT Age: 59 days Normal Renal Function		Fold-Change (Col4A3:WT)	
	AUC_{last} (µg*hr/mL)	C_{max} (µg/mL)	AUC_{last} (µg*hr/mL)	C_{max} (µg/mL)	AUC_{last}	C_{max}
12.5	62.1	39.1	27.3	17.3	2.27	2.26
25	177.8	70.2	64.2	39.4	2.77	1.78
50	350	125.4	149.5	84.4	2.34	1.49

RG0005: major active metabolite



RG-101 (GalNAc-Conjugated AntimiR)





Total = Combination of all full length RG-101 related moieties

RG-101 Single Dose: Plasma and Tissue Exposure Fold-Changes (Col4A3:WT)

Age (week)	Mice Strains (CKD Stage)	Dose (mg/kg)	Plasma AUC Ratio	Plasma C_{max} Ratio	Liver C_{48hr} Ratio	Kidney C_{48hr} Ratio
6	Col4A3$^{(-/-)}$ *(CKD: 2-3)* *vs.* WT SV129 (normal renal function)	5	1.77	1.30	1.46	0.42
		15	1.34	1.47	1.22	0.76
		45	1.65	2.04	2.60	1.51
~9–11	Col4A3$^{(-/-)}$ *(CKD: 4-5)* *vs.* WT SV129 (normal renal function)	5	3.24	1.68	1.50	0.11
		15	2.22	2.51	2.94	0.31
		45	6.31	2.81	2.86	0.44

Conclusions

These findings indicate that ~CKD stage 3 in mice has only moderate effects (≤2-fold at dose levels of 12.5-50 mg/kg for RG-012 and 5-15 mg/kg for RG-101) on the plasma and tissue disposition of both unconjugated and GalNAc-conjugated antimiRs. However, ~CKD stages 4–5 in mice produces somewhat greater magnitude effects (exposure in liver increased ~2-4 fold, decreased in kidney ~2-8 fold). In general, the nature of the effects observed appear consistent with expectations. These findings will require clinical confirmation in trials with renal impaired subjects. If clinically translatable, the impact of such observed exposure effects will depend upon the target organ, therapeutic index of the antimiR, and duration of treatment, and may not necessarily warrant potential dosage adjustment even in patients with severe impairment or ESRD.

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