TREATMENT WITH THE MICRORNA-21 INHIBITOR RG-012 GIVEN WITH AND WITHOUT RAMIPRIL DELAYS RENAL IMPAIRMENT PROGRESSION AND PROLONGS SURVIVAL WHEN INITIATED UP TO CHRONIC KIDNEY DISEASE (CKD) STAGE 3 IN A MOUSE MODEL OF ALPORT SYNDROME

John Grundy[1], Cynthia Heinlein[1], Kara Kersjes[1], Kai Liu[1], Tate Owen[1], Shweta Pandya[1], Robb Pagarigan[1], and Shiguang Liu[2]
[1]Regulus Therapeutics, San Diego, CA, USA; [2]Sanofi-Genzyme R&D Center, Framingham, MA, USA

Introduction and Aims

RG-012, an anti-miR-21 oligonucleotide in early clinical development, has previously been shown to significantly prolong survival, by an additional ~2–4 weeks, in a rapidly progressing Col4a3 deficient mouse model of Alport syndrome (Col4a3 mutant mice on the 129X1/SvJ background) when administered early in renal disease progression (CKD stages 1–2). Col4a3 mutant mice have increased kidney expression of miR-21 leading to progressive renal fibrosis. Hence, untreated Col4a3 mutant mice begin to develop proteinuria by 4 weeks of age, reach CKD stages 3 and 4 by ~6 and 8 weeks of age, respectively, and have a typical reduced lifespan of ~9–10 weeks of age. RG-012 is a single-stranded synthetic oligonucleotide that specifically sequesters miR-21 in diseased cells, forming an inactive heteroduplex. In this study, we examined the ability of RG-012 to enhance survival when treatment was initiated at CKD stages 2–4. RG-012 was examined as a single agent, as well as in combination with ramipril (which when given alone has been previously characterized to delay renal impairment progression in this animal model). Due to rapid disease progression in this animal model, in addition to RG-012 given once weekly, we also evaluated a loading regimen plus weekly maintenance dosing of RG-012 that was designed to more rapidly achieve and maintain effective kidney concentrations.

Methods

Table 1: Col4a3$^{-/-}$ Mice Survival Study Design

Treatment Group	# Animals	Age at Start of Ramilpril Treatment (10mg/kg daily in drinking water)	Age at Start of RG-012 Treatment (60mg/kg SC)	RG-012 Dose Schedule
PBS	31	—	—	—
RG012 QW start 6wk	24	—	6 weeks (~CKD3)	weekly
RG012 TIW,QW start 6 wk	27	—	6 weeks (~CKD3)	loading dose then weekly maintenance
RG012 TIW,QW start 7 wk	19	—	7 weeks (~CKD3-4)	loading dose then weekly maintenance
RG012 TIW,QW start 8 wk	23	—	8 weeks (~CKD4)	loading dose then weekly maintenance
Ramilpril (QD start 4 wk)	26	4 weeks (~CKD2)	—	—
Combo, RG012 start 6wk	26	4 weeks (~CKD2)	6 weeks (~CKD1)	loading dose then weekly maintenance
Combo, RG012 start 8wk	22	4 weeks (~CKD2)	8 weeks (~CKD3)	loading dose then weekly maintenance
Combo, RG012 start 9wk	24	4 weeks (~CKD2)	9 weeks (~CKD3)	loading dose then weekly maintenance
Combo, RG012 start 10wk	27	4 weeks (~CKD2)	10 weeks (~CKD3-4)	loading dose then weekly maintenance

- RG-012 loading/maintenance dosing: 3x per week for 2 weeks, followed by 1x weekly
- Renal function status assessed by serum BUN at weeks 6 and 8 (ramipril monotherapy group measured at weeks 6,7,9, and 10)
- Genotype for all study animals was confirmed

Results

Figure 1. Col4a3$^{-/-}$ Mice Survival Curves: RG-012 Monotherapy vs. PBS Treatment Groups

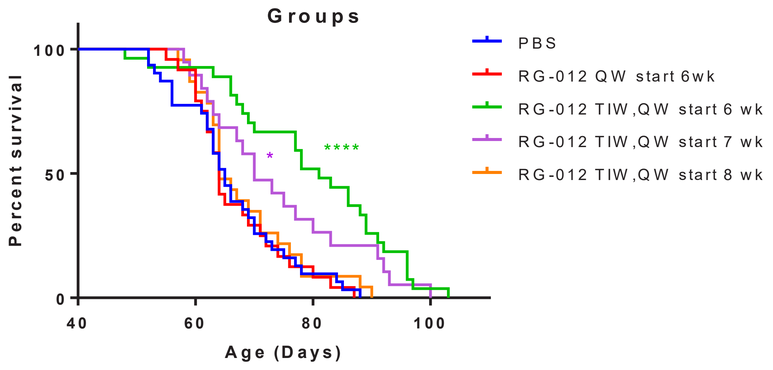


Fig 1 stats analysis: Log rank test comparing RG-012 monotherapy treatment groups to PBS. * p<0.05, ****p<0.0001

Figure 2. Col4a3$^{-/-}$ Mice Survival Curves: RG-012 + Ramipril Combination vs. Ramipril Monotherapy Treatment Groups

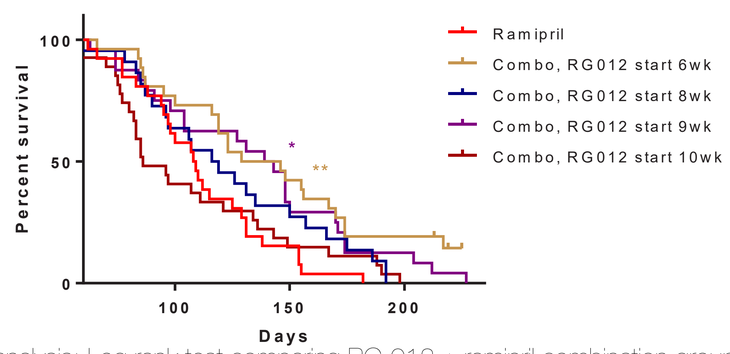


Fig 2 stats analysis: Log rank test comparing RG-012 + ramipril combination groups to ramipril monotherapy. * p<0.05, ** p<0.01

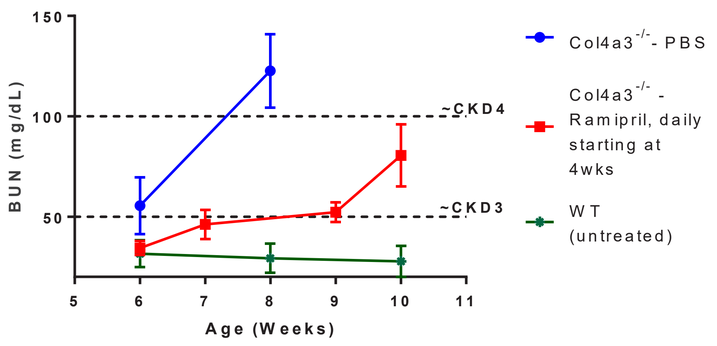
Table 2. Comparison of Col4a3$^{-/-}$ Mice Survival Times (Days) Between Treatment Groups

Treatment Group	50th percentile (MST)	75th percentile	90th percentile
PBS	65	70	78
RG012 QW start 6wk	64	71	80
RG012 TIW,QW start 6 wk	81	89	96
RG012 TIW,QW start 7 wk	70	80	92
RG012 TIW,QW start 8 wk	64	71	78
Ramilpril (QD start 4 wk)	108.5	131	154
Combo, RG012 start 6wk	137.5	174	NYD (>227)
Combo, RG012 start 8wk	117.5	157	186
Combo, RG012 start 9wk	141	170	204
Combo, RG012 start 10wk	86	134	167

Abbreviations: MST, median survival time; NYD, not yet determined (still surviving animals)

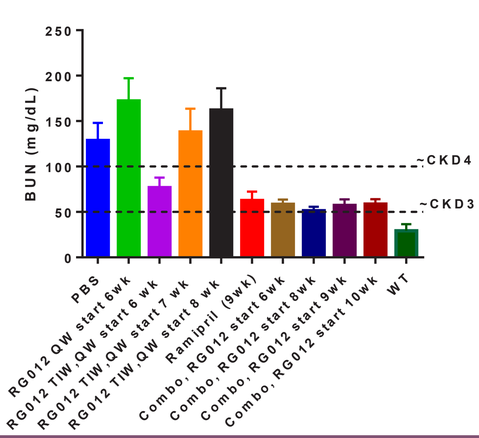
- Compared to PBS, RG-012 monotherapy showed significant increase in survival when loading/maintenance treatment started at 6 (p<0.0001; ~CKD 3) and 7 weeks (p=0.02; ~CKD 3) (Fig. 1)
- No increased survival when RG-012 monotherapy loading/maintenance treatment started at week 8 (p=ns; ~CKD 4) and weekly maintenance only treatment started at week 6 (p=ns; ~CKD 3) (Fig. 1)
- A significant survival advantage over ramipril monotherapy was seen when combination treatment begun at weeks 6 or 9 (p=0.003 and p=0.02, respectively; ~CKD 2–3), but not week 10 (p=ns; ~CKD 3–4) (Fig. 2)
- A clear trend for survival advantage was also seen when combination treatment begun at 8 weeks of age, but did not reach statistical significance (p=0.11; ~CKD 3) (Fig. 2 and Table 2)

Figure 3. Effect of Ramipril Monotherapy on BUN in Col4a3-/- Mice



- Ramipril monotherapy started at 4 weeks of age in Col4a3$^{-/-}$ mice delays renal impairment progression (Fig. 3)

Figure 4. Comparison of Serum BUN at 8-9 Weeks of Age Between Treatment Groups



- Mean serum BUN typically lower in groups showing survival advantage (Fig. 4)

Conclusions

In a rapidly progressing mouse model of Alport syndrome, delayed renal impairment and prolonged survival was seen when a loading plus maintenance treatment regimen of RG-012 (given with or without ramipril) was initiated up to CKD stage 3, but not CKD stage 4. These findings provide nonclinical support for evaluation of RG-012 in the clinic involving treatment of Alport syndrome patients, whose disease currently has limited therapeutic options.

53 ERA 257-SP
Chronic Kidney Disease. Pathophysiology, progression & risk factors.
John Grundy
DOI: 10.3252/pso.eu.53era.2016
ePosters supported by F. Hoffmann- La Roche Ltd.
53rd CONGRESS ERA-EDTA VIENNA, MAY 21st-24th, 2016 AUSTRIA
Held jointly with Österreichische Gesellschaft für Nephrologie
PosterSessionOnline.com