

respect to complement function has not been standardized. The aim of this study was to evaluate novel complement functional assays for their application in routine follow-up of eculizumab-treated patients.

**Methods:** Complement activity in serum samples was analyzed using Wieslab® complement screen assay. The presence of eculizumab-C5 complexes in serum, EDTA plasma samples and in urine was measured using ELISA. Levels of sC5b-9 in urine were measured using electroluminescent epitope assay.

**Results:** First, we documented that the Wieslab® complement screen assay showed a sensitivity of 1-2% of C5 activity by adding purified C5 or normal human serum to a C5 deficient serum. Second, we found that all the patient samples obtained during the standard treatment course, were completely blocked for terminal complement pathway activity. Moreover, complement remained fully blocked when intervals between the eculizumab infusions were extended to four weeks. Levels of complexes between eculizumab and C5 were inversely correlated to the complement activity ( $p=0.01$ ). Third, titrating serum from eculizumab-treated patients into normal serum, revealed that eculizumab was present in excess up to four weeks after infusion. Finally, we showed that increased urine sC5b-9 disappeared after eculizumab treatment.

**Conclusions:** We demonstrate sensitive, reliable and easy-performed assays to monitor eculizumab-treated patients, which can be used to design individual dosage regimens.

**Funding:** Government Support - Non-U.S.

## SA-PO537

### Dose Exposure Relationship of AKB-6548 Is Independent of the Level of Renal Function Akshay Buch, Bradley J. Maroni, Charlotte S. Hartman. Akebia Therapeutics, Inc., Cambridge, MA.

**Background:** AKB-6548 is a novel, once-daily, oral hypoxia-inducible factor prolyl-hydroxylase inhibitor (HIF-PHI) in development for the treatment of anemia in non-dialysis dependent (NDD) and dialysis dependent (DD) chronic kidney disease (CKD). Studies in NDD-CKD patients have shown AKB-6548 produces physiologic increases in erythropoietin, enhances iron mobilization, and produces a dose dependent increase in hemoglobin levels.

**Methods:** The pharmacokinetics (PK) of AKB-6548 have been evaluated in single and multiple dose studies in healthy volunteers and CKD patients (NDD-CKD and DD-CKD) over a broad range of dose levels. An analysis of results across studies was conducted to assess the potential impact of renal function on selecting the dose levels and dosing regimen in CKD patients. The analyses evaluated dose-linearity in drug exposure, clearance routes of AKB-6548 and its metabolites, as well as the impact of CKD severity and the hemodialysis procedure on drug disposition.

**Results:** In healthy volunteers, AKB-6548 demonstrated dose linearity and proportionality over single doses of 80-1200 mg and multiple doses of 500-900 mg/day. The PK parameters of AKB-6548 in healthy volunteers, NDD-CKD and DD-CKD patients were similar except for a slightly longer half-life with declining renal function (4.7 hr, 7.9 hr. and 9.1 hr., respectively). A mass balance study (using  $^{14}\text{C}$ -labeled drug) in healthy volunteers demonstrated both renal and fecal routes of parent drug and metabolite clearance, supporting use of AKB-6548 in CKD patients without significant differences in drug exposure. Moreover, AKB-6548 was minimally cleared by the hemodialysis procedure and the PK parameters were similar whether AKB-6548 was administered prior to or following dialysis.

**Conclusions:** The results demonstrate a linear dose-exposure relationship for AKB-6548 over a wide range of doses and support the utility of once-daily dosing and similar dose levels across the spectrum of CKD severity.

**Funding:** Pharmaceutical Company Support - Akebia Therapeutics

## SA-PO538

### Nonclinical Pharmacokinetics and Toxicokinetics of RG-012, an Inhibitor of MicroRNA-21 Being Investigated for Treatment of Alport Syndrome John Stewart Grundy,<sup>1</sup> Kai Liu,<sup>1</sup> Steven Neben,<sup>1</sup> Cindy L. Berman,<sup>2</sup> Deidre Mackenna,<sup>1</sup> Neil W. Gibson.<sup>1</sup> <sup>1</sup>Regulus Therapeutics, San Diego, CA; <sup>2</sup>Berman Consulting, Wayland, MA.

**Background:** RG-012 is a single-stranded chemically modified oligonucleotide being developed to treat patients with Alport syndrome, which is characterized by loss of renal function associated with defects in specific collagen genes expressed in the kidney glomerular basement membrane. RG-012 inhibits miR-21, a microRNA target known to have increased expression in context of kidney stress and associated with renal dysfunction.

**Methods:** In vitro pharmacokinetic (PK) evaluations of RG-012 conducted during preclinical development included: plasma protein binding, metabolic stability in whole blood and liver lysates, and CYP450 inhibition/induction potential in cryopreserved human hepatocytes. In vivo PK and toxicokinetic (TK) properties of RG-012, and its major active metabolite (RG0005), were determined upon subcutaneous dosing of RG-012 in a set of nonclinical PK, pharmacology, and safety studies in CD-1, SV129, and COL4A3 mice (6.25–450 mg/kg/week) and cynomolgus monkeys (10–225 mg/kg/week).

**Results:** Both RG-012 and RG0005 were highly bound ( $\geq 98\%$ ) to proteins in plasma from mice, monkeys, and humans. RG0005 was the only major degradation product seen in whole blood and liver lysates from all three species. RG-012 exhibited little to no potential to inhibit or induce CYP450 enzymes. Upon administration of RG-012, PK and TK properties of RG-012 and RG0005 were consistent across all nonclinical species and were characterized by rapid absorption, dose-dependent plasma exposure, rapid plasma clearance due mainly to extensive tissue distribution, and subsequent slow tissue elimination (half-lives in liver and kidney of ~2–3 weeks). Comparison of plasma and tissue exposure levels (RG-012+RG0005 combined) seen at the NOAEL in cynomolgus monkeys, the most

clinically relevant toxicological species, with those achieved at pharmacologically active dose regimens ( $\text{ED}_{50}$  and  $\text{ED}_{90}$ ) in a COL4A3 mutant mouse model, indicates a therapeutic index (TI) ranging from 3.4 to 25.4.

**Conclusions:** In conclusion, favorable nonclinical PK/TK properties and TI of RG-012 support initial clinical testing.

**Funding:** Pharmaceutical Company Support - Regulus Therapeutics

## SA-PO539

### Novel Methodology for Assessing Inhibition of MicroRNA-21 by RG-012, a MicroRNA Therapeutic in Development for the Treatment of Kidney Dysfunction in Patients with Alport Syndrome John Rolf Androsavich, Xueqing Liu, Shweta Pandya, Deidre Mackenna. Regulus Therapeutics, San Diego, CA.

**Background:** microRNA-21 (miR-21) is upregulated in animals models with kidney dysfunction and also in patients with chronic kidney disease (CKD). RG-012 is a miR-21 inhibitor entering clinical development for treatment of CKD in Alport syndrome (AS) patients. RG-012 demonstrates activity both as a monotherapy and in combination with the ACE inhibitor ramipril in the Col4A3 deficient mutant mouse model of AS.

**Methods:** Two distinct methods have been developed to evaluate the ability of RG-012 to inhibit miR-21 in preclinical studies – the polysome shift assay (PSA) and alterations in the expression of miR-21 target mRNAs. The PSA measures direct target inhibition at the level of the physical interaction between microRNA and messenger RNA targets. C57Bl/6 mice were treated subcutaneously with RG-012 at doses ranging from 0.1 to 100 mg/kg. Tissue homogenates were separated using sucrose gradient ultracentrifugation, with microRNAs associating with messenger RNA targets in the heavier polysome fraction. The ability of RG-012 to displace the target miR-21 from the polysome containing fraction was used to assess direct target engagement.

**Results:** RG-012 demonstrated dose dependent displacement of miR-21 from polysomes in both liver and kidney with a maximum effect reached at dose levels that are efficacious in the Col4A3 mouse models. Loss of miR-21 from the polysomes was specific as levels of Let-7a, a control microRNA to which RG-012 has no complementarity, were unaffected. In the liver, target engagement was also assessed using mRNA derepression of a set of confirmed miR-21 target genes. Here, target gene derepression strongly correlated with polysome displacement. This comparison was not possible in kidney, however, because target genes are not regulated in kidneys in the absence of stress.

**Conclusions:** RG-012 directly and specifically inhibits miR-21 resulting in its displacement from actively translating polysome complexes and subsequent derepression of messenger RNA targets.

**Funding:** Pharmaceutical Company Support - Regulus Therapeutics

## SA-PO540

### Tacrolimus Pharmacokinetics in Nephrotic Stage Mara Medeiros,<sup>1</sup> Saul Valverde,<sup>1</sup> Luis Velasquez-Jones,<sup>1</sup> Ana M. Hernández,<sup>1</sup> Gilberto Castañeda-Hernández,<sup>2</sup> Guido Filler.<sup>3</sup> <sup>1</sup>Hospital Infantil de México Federico Gómez, Mexico; <sup>2</sup>CINVESTAV, IPN, Mexico; <sup>3</sup>Children's Hospital, Univ of Western Ontario, Canada.

**Background:** While tacrolimus (Tac) therapy is not first-line therapy for childhood nephrotic syndrome, it is often used instead of cyclosporine to ameliorate the side effects. The pharmacokinetics of Tac can be influenced by many conditions, and it has a high plasma protein binding. The Tac pharmacokinetics during relapse and remission of childhood nephrotic syndrome have not been well described.

**Methods:** We performed 14 pharmacokinetic (PK) profiles (with measurements before and 0.5, 1, 2, 4 and 12 hours post intake) in 7 children with steroid-resistant nephrotic syndrome (SRNS) during relapse and in remission. These data were compared with historical PK data of 161 PK profiles in 87 pediatric renal transplant recipients with measurements before, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 12 hours post intake. Tac levels were measured using the Abbott Tacro II assay. We used descriptive statistics to generate percentiles and compared these with the SRNS patients. We also compared the PK profiles during relapse and remission.

**Results:** Median age of SRNS patients was 3.2 years. Tacrolimus dose, biochemical values and pharmacokinetics parameters are shown in Table 1. Values as median and interquartile range.

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#### Contact ASN

American Society of Nephrology  
1510 H Street, NW, Suite 800  
Washington, DC 20005  
202-640-4660 phone, 202-637-9793 fax  
[email@asn-online.org](mailto:email@asn-online.org), [www.asn-online.org](http://www.asn-online.org)