

Standardizing Immunomodulatory Dosing of Sirolimus in Nonhuman Primates for Gene Therapy: A Data-Driven Review

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ABSTRACT

Gene therapy platforms such as viral vectors and lipid nanoparticles can trigger substantial immunogenic responses, necessitating the use of immunomodulatory agents to improve tolerability. Sirolimus reduces T cell-driven inflammation and is increasingly incorporated into nonhuman primate studies; however, standardized preclinical dosing guidance remains limited.

The purpose of this abstract is to establish a nonclinical reference range for Sirolimus dosing in mg/kg/day to facilitate therapeutic dose calculations in nonhuman primates.

Sirolimus dosing regimens from recent nonclinical studies were reviewed to derive practical mg/kg/day dosing recommendations aligned with therapeutic blood concentration targets.

The absence of a well-defined reference dosing range necessitates more frequent blood sampling—often on a weekly basis—to confirm or adjust trough whole blood concentrations and ensure they remain within the intended therapeutic target range. This increased sampling frequency may heighten animal handling and procedural stress, with potential implications for animal welfare, while also adding operational complexity to study conduct.

Among animals treated with sirolimus, 21 cynomolgus monkeys were assessed for relationships between mg/kg/day dose and trough whole blood concentrations. Sirolimus exposure generally increased with dose, although notable inter-animal variability was observed. Measured sirolimus trough concentrations ranged from 1.2 to 8.5 ng/mL across dose levels of 0.15 to 0.57 mg/kg/day, supporting an intended target therapeutic range of 2 to 10 ng/mL. Based on these cumulative data, a dosing range of 0.3 to 0.5 mg/kg/day is proposed to more reliably achieve target trough concentrations, enhance cross-study reproducibility, and potentially reduce the need for frequent blood sampling to confirm exposure.

INTRODUCTION

Gene therapy continues to progress as a viable therapeutic intervention for genetic disorders. These therapeutic interventions often use viral vectors (e.g., adeno-associated virus) and lipid nanoparticles as a platform, which may elicit severe immunogenic responses. Immunomodulatory agents such as sirolimus, an mTOR inhibitor, improve tolerability due to the immunosuppressive effects, including modulation of T-cell activity and reduction of inflammatory responses.

Table 1. Test System

Species/strain	Macaca fascicularis/Cynomolgus monkeys
Age	2.0 to 3.8 years
Body weight	2.00 to 3.13 kg
Total Animals Selected	9 Males, 12 Females

Clinically, sirolimus is routinely titrated to trough whole blood concentrations rather than fixed doses, reflecting its narrow therapeutic window and inter-individual pharmacokinetic variability. Across approved human indications, sustained trough concentrations in the low to mid single-digit ng/mL range are sufficient to achieve meaningful immunomodulatory effects, particularly suppression of T cell proliferation and cytokine-driven inflammation, while minimizing dose-limiting toxicities such as dyslipidemia, impaired wound healing, and myelosuppression.

MATERIALS AND METHODS

Table 2. Blood Collection and Dose Administration Details

Blood Collection	Weekly
Dose Frequency	Once Daily (Beginning on Day -3)
Starting Dose	2-3 mg/m ² /day
Maintenance Dose	0.5 to 8 mg/m ² /day
Total Number of Data Point Selected	138 occasions

Across multiple safety assessment studies conducted over the past two years, a subset was identified in which cynomolgus monkeys received sirolimus as an immunomodulatory agent as part of the study design, either as monotherapy or in combination with other immunosuppressive agents. Dosing regimens included once or twice daily administration, implemented with or without weekly dose adjustments. From this overall population, 21 animals were selected for focused evaluation of the relationship between administered target dose levels and corresponding whole blood sirolimus concentrations, with a total of 138 data points.

Whole blood samples were collected weekly, using K₂EDTA as the anticoagulant, and Sirolimus concentrations were quantified via high-performance liquid chromatography with tandem mass spectrometry (LC-MS/MS). For each animal, the sirolimus dose was calculated based on initial level (mg/m²), most recent body weight, and body surface area (BSA) conversion methodology. This value was then converted into a calculated dosing unit of mg/kg.

$$BSA (m^2) = \frac{969 \cdot \text{Weight (kg)}^{0.67}}{10,000}$$

RESULTS AND DISCUSSION

While dose levels of approximately 0.15 to 0.4 mg/kg/day generally achieved sirolimus concentrations within the intended target range, a slight, dose-dependent increase in whole-blood sirolimus concentrations was observed with increasing dose levels. Despite this trend, exposures were frequently clustered toward the lower end of the 2 to 10 ng/mL target window.

In addition, multiple weekly dose adjustments were required in several animals to maintain target trough concentrations, with this need observed predominantly in female animals, underscoring the degree of inter-animal variability in sirolimus pharmacokinetics. This variability introduced the potential for transient sub-therapeutic exposures between dose adjustments.

Given the observed variability and the risk of failing to consistently maintain pharmacologically relevant trough levels in some animals, exploration of modestly higher dose levels is justified to more reliably achieve mid-range target exposures. Slight upward adjustment of the dose is expected to improve the consistency of attaining sirolimus concentrations associated with sustained immunomodulatory activity, while remaining within exposure ranges that are clinically supported and generally well tolerated. Based on cumulative exposure data across studies, an upper dose limit of 0.5 mg/kg/day is proposed. This upper bound is anticipated to reduce the frequency of dose adjustments required to maintain target trough levels, improve exposure consistency across animals, and support more reproducible interpretation of immunomodulatory effects.

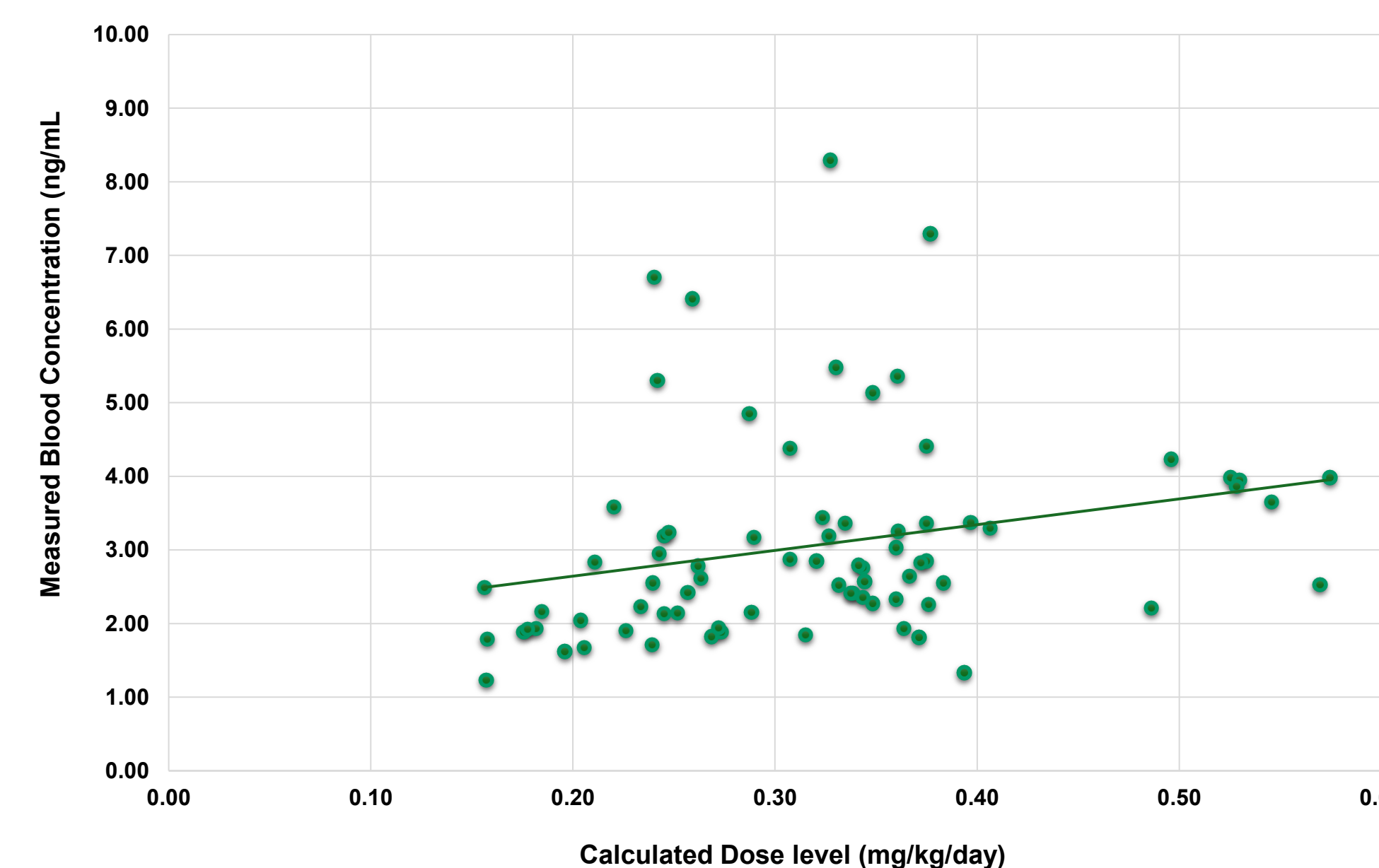


Figure 1. Female - Blood sirolimus concentrations (ng/mL) vs. calculated dose level (mg/kg/day)

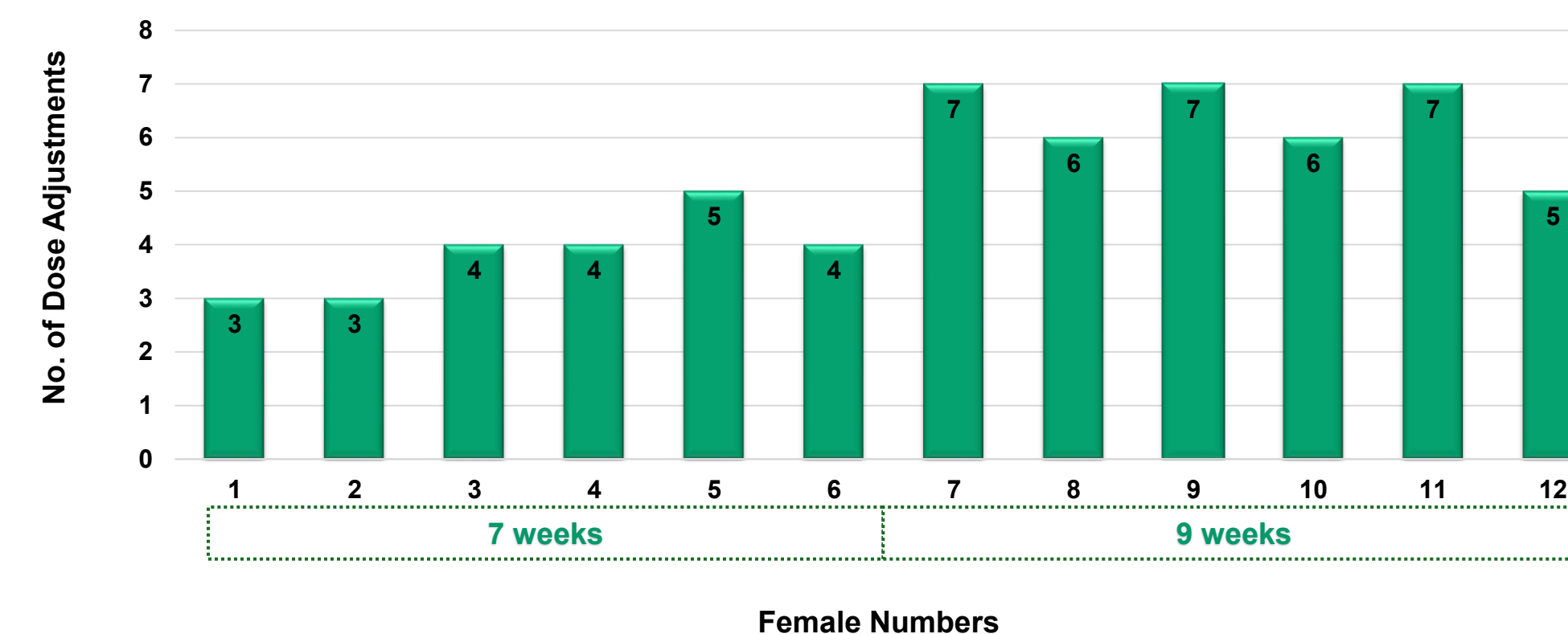


Figure 2. Female - Total number of dose adjustments over a period of 7 or 9 weeks

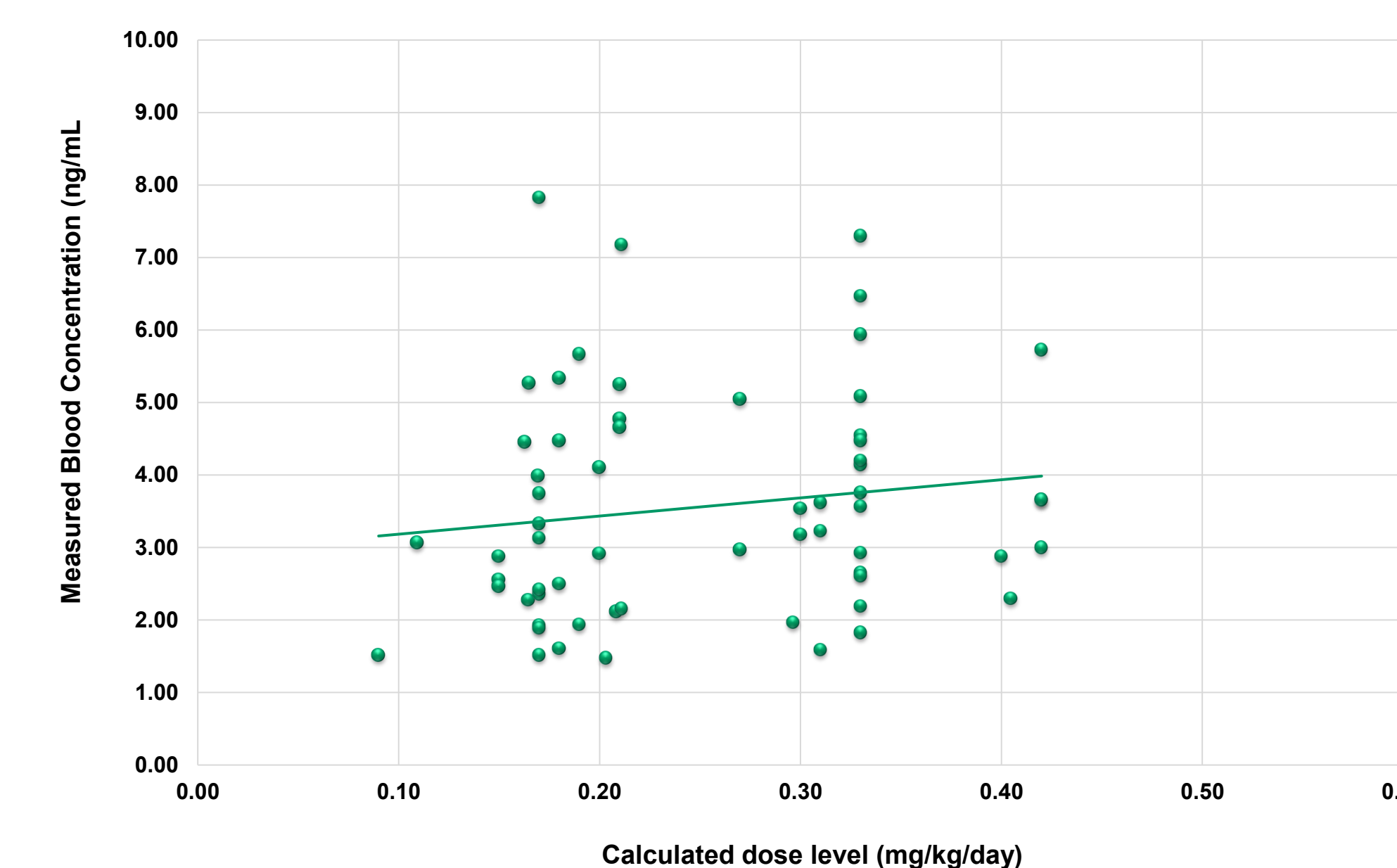


Figure 3. Male - Blood sirolimus concentrations (ng/mL) vs. calculated dose level (mg/kg/day)



Figure 4. Male - Total number of dose adjustments over a period of 4 or 9 weeks

CONCLUSIONS

In nonhuman primate gene therapy studies, sirolimus dosing regimens varied widely in schedule and duration, frequently requiring dose adjustments and repeated blood sampling to maintain target trough concentrations. Although higher mg/kg/day doses were generally associated with increased sirolimus blood levels, substantial inter-animal variability limited consistent attainment of target exposures. The proposed dosing range of 0.3 to 0.5 mg/kg/day provides a practical and evidence-based framework to more reliably achieve therapeutic sirolimus concentrations, reduce the need for frequent dose modification and confirmatory sampling, and enable more consistent cross-study comparisons. Collectively, adoption of this refined dosing approach is expected to improve study efficiency while supporting animal welfare through reduced handling and procedural burden.

REFERENCES

Rapamune (sirolimus) Prescribing Information. U.S. Food and Drug Administration.

Sirolimus Dosage Guide. Drugs.com (FDA-aligned summary, updated Feb 2026).