Non-clinical Evaluation of 4D-310 in Combination with Rituximab/Sirolimus

A Translational Study to Support Adoption of a Novel Prophylactic Immunomodulation Regimen in Clinical Trials in Adults with Fabry Disease

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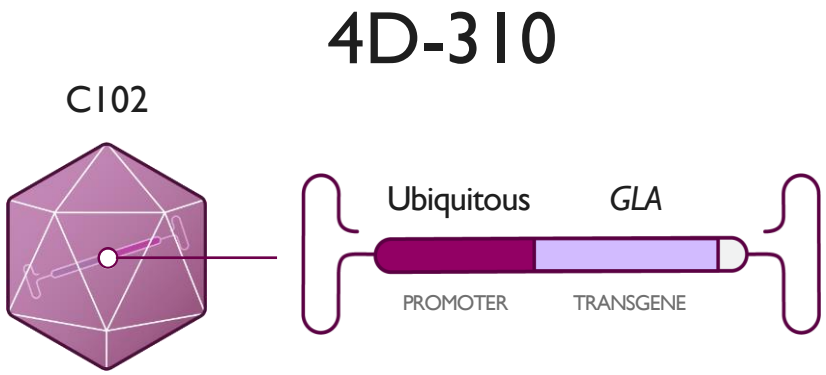
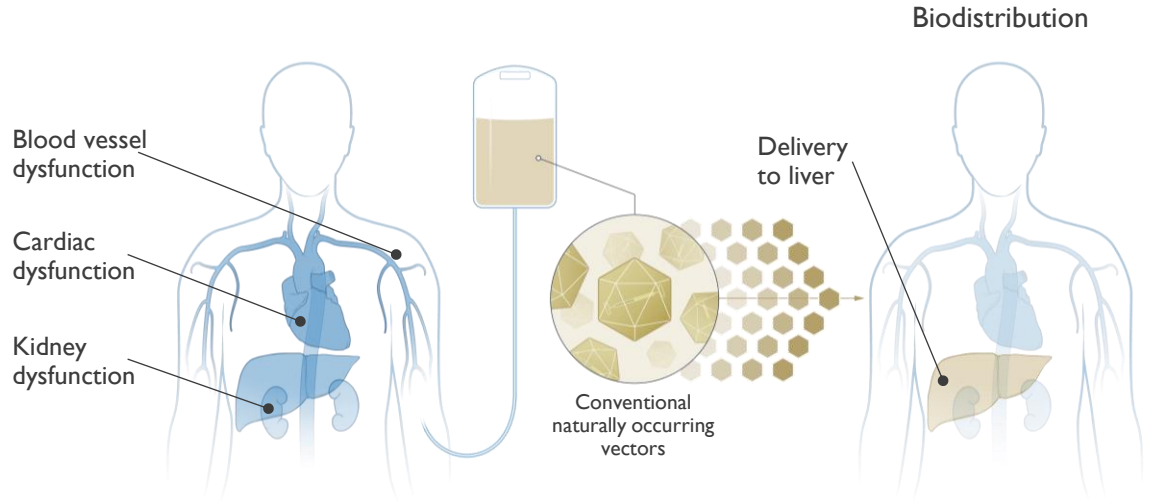
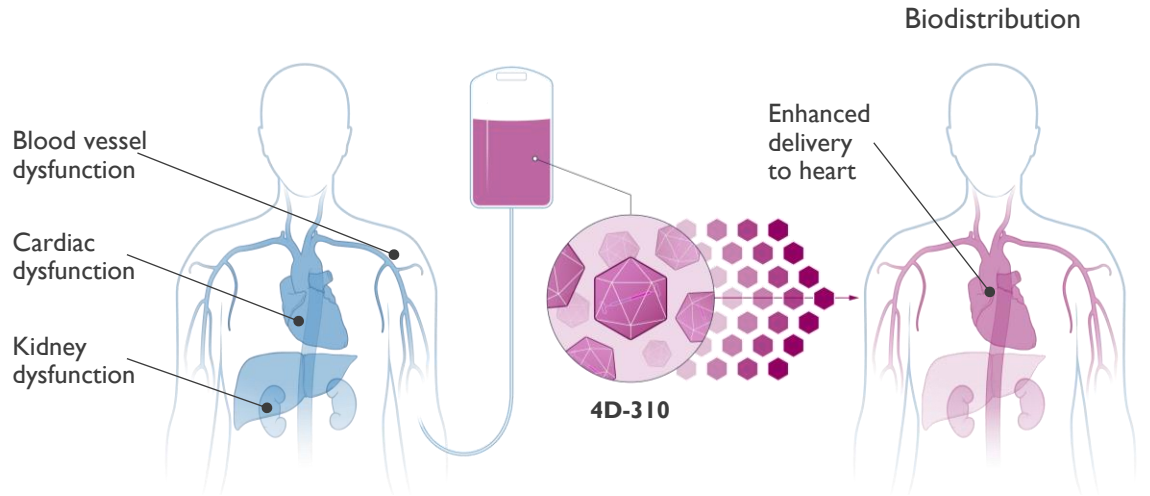
4D-310: Genetic Medicine for Fabry Disease Cardiomyopathy

Low-dose IV Delivery to Cardiomyocytes for Cell-autonomous AGA Production



- **Vector**: C102 (evolved cardiotropic AAV)
- **Transgene**: *GLA* (encodes AGA enzyme)
- **Promoter**: Ubiquitous





*AAV, adeno-associated virus; AGA, α-galactosidase A.

Background and Rationale

- Initial dose cohort in the INGLAXA clinical trial evaluating 4D-310 in adults with Fabry disease received oral prednisolone for prophylactic immunosuppression
  - Evidence of antibody-dependent activation of the complement system leading to thrombotic microangiopathy—a reported class effect of systemic AAV-based gene therapy[1–4]
- Published reports have shown that rituximab/sirolimus attenuates humoral response to the AAV capsid following systemic administration of AAV9-based gene therapy [5]

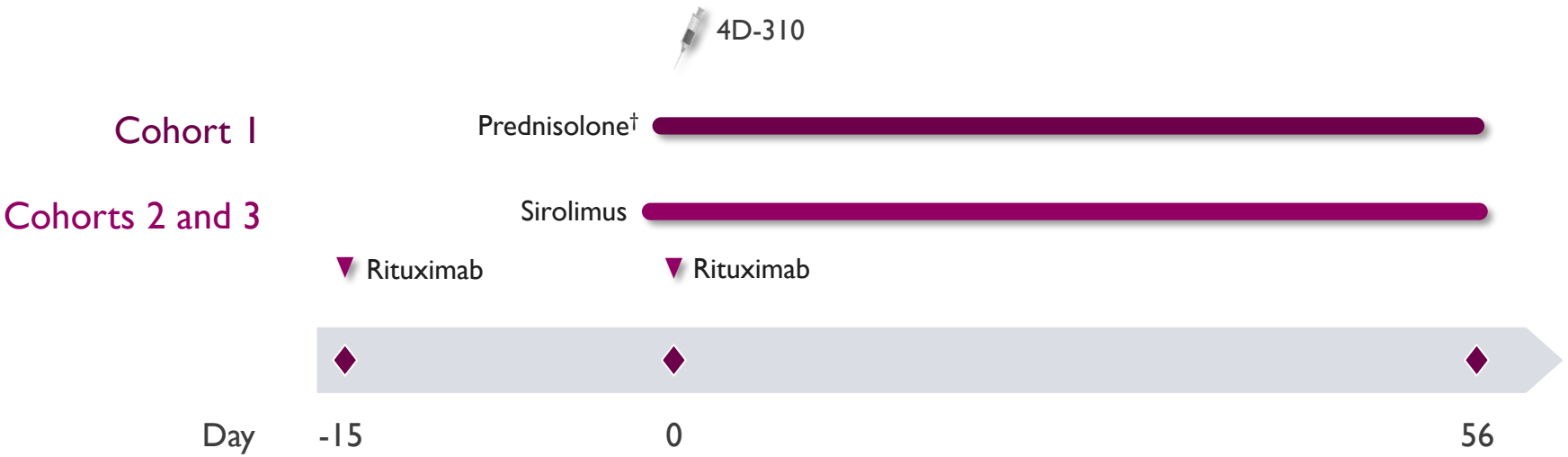
Study Objectives

- Demonstrate suppression of humoral response to C102 capsid by rituximab/sirolimus
- Evaluate the effect of rituximab/sirolimus on 4D-310 cell transduction and transgene expression
- Confirm the safety of 4D-310 administered in combination with rituximab and sirolimus

1. Schiffmann et al. WORLD Symposium 2023. 2. Hamilton et al. *Front Immunol* 2021;12:675897. 3. Mingozzi et al. *Blood* 2013;122:23–36. 4. Loo et al. *Bioanalysis* 2022;14:737–93. 5. Salabarria et al. *J Clin Invest* 2024;134:e173510.

Study Design
Nonhuman Primate Study

Cohort	N	4D-310 (Day 0)	Immunomodulatory Regimen
1	2	1×10^{13} vg/kg	Prednisolone 3 mg/kg/d (beginning on day –1)*
2	3	1×10^{13} vg/kg	IV rituximab 750 mg/m^2 (days –15 and 0)† Oral sirolimus 0.5–8 mg/m^2/d (beginning on day –3)‡
3	3	3×10^{12} vg/kg	



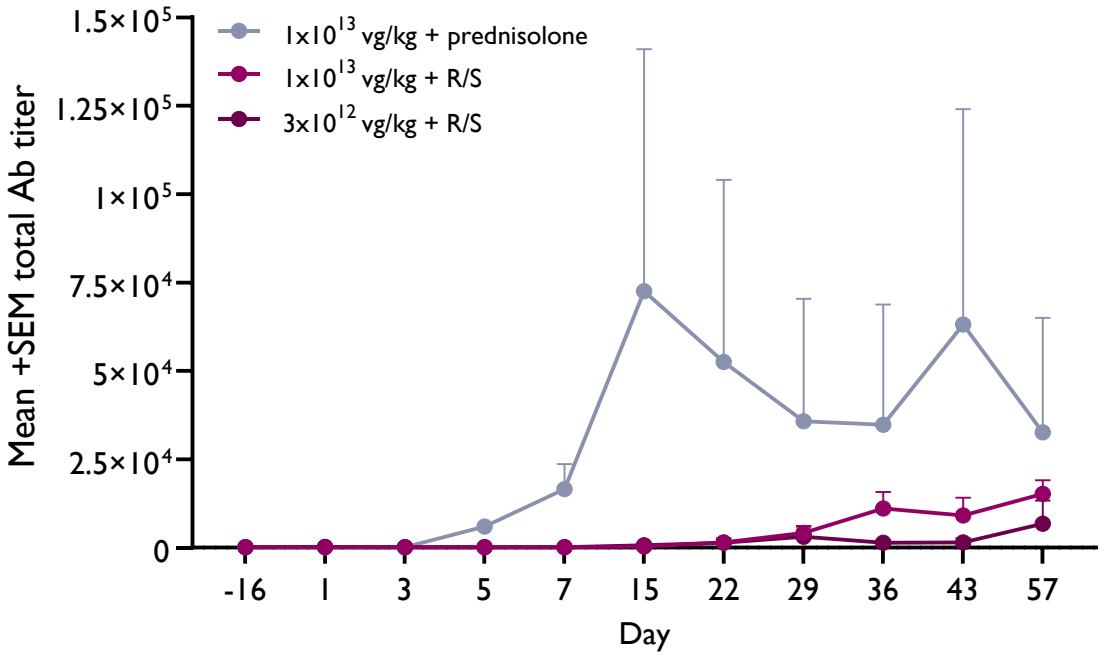
Study Outcomes
- Immune response
- Transgene expression
- Safety and tolerability

*Protocol-specified prophylactic therapy in the initial dose cohort in INGLAXA Phase 1 clinical trial. †IM diphenhydramine 4 mg/m^2 administered prior to rituximab. ‡Dose titrated to maintain a 24-hour trough concentration of 2–4 ng/mL.

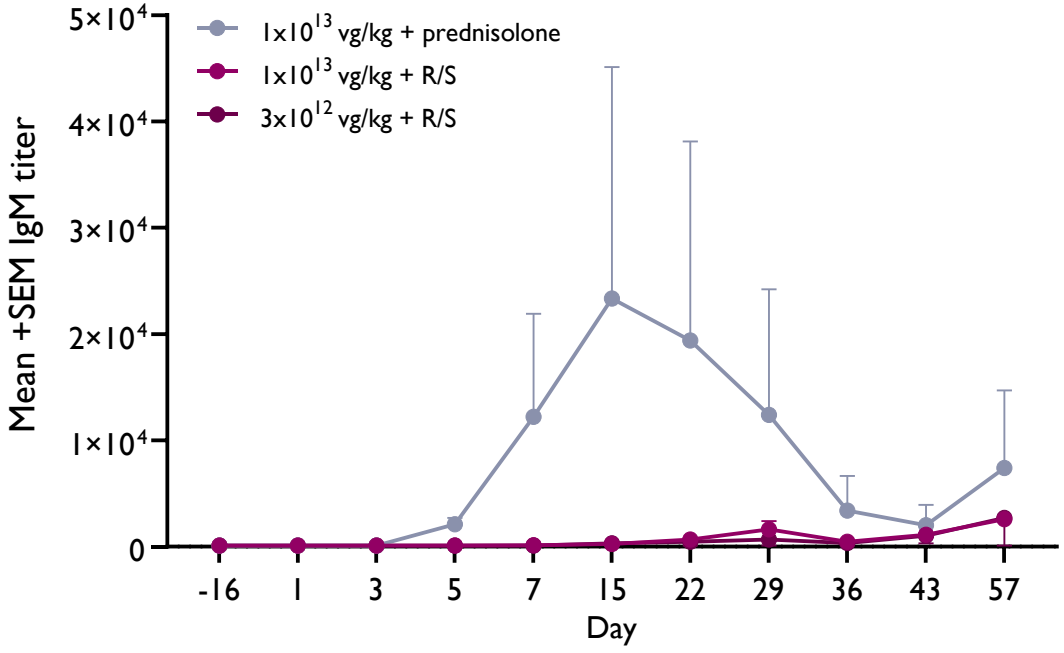
Immune Response

Rituximab/Sirolimus Attenuated Total and IgM Antibody Responses to C102 Capsid

Anti-C102 Total Ab*



Anti-C102 IgM



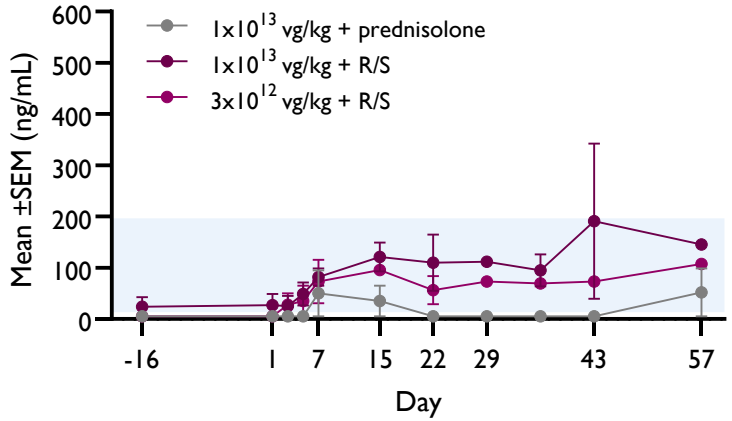
- Marked attenuation of total and IgM antibody response to the C102 capsid, with a delayed onset of response and decreased peak antibody titers compared to prednisolone

*IgG, IgM, and IgA antibodies. Ab, antibody; SEM, standard error of measurement.

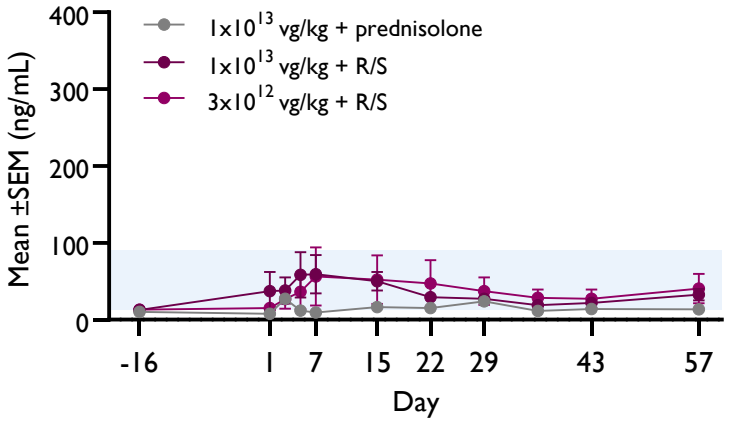
Complement Activity

Complement Markers Generally Remained within Normal Range

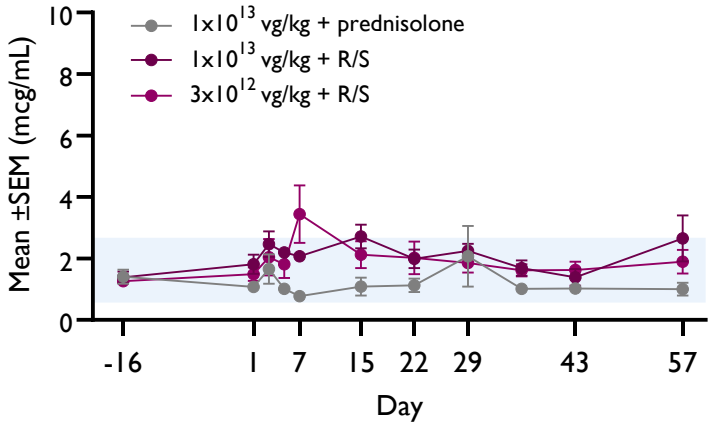
C4a



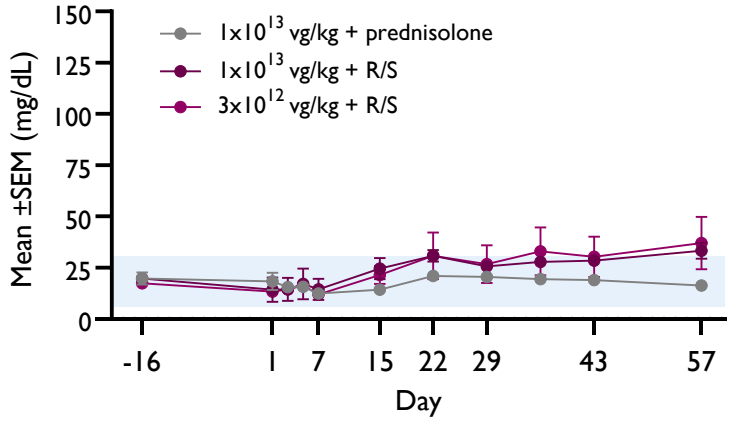
C3a



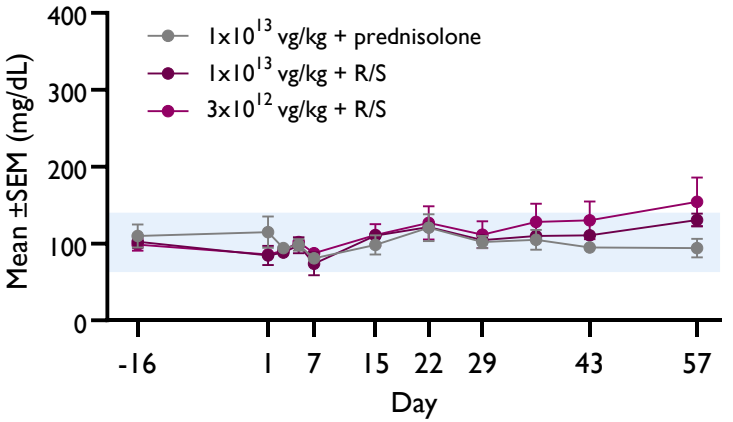
Bb



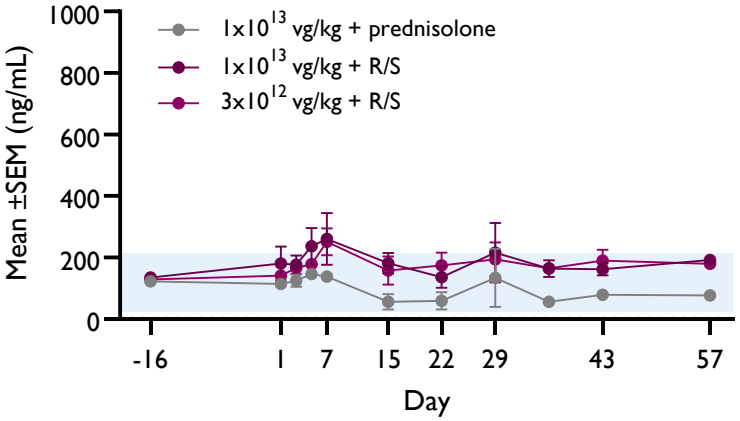
C4



C3



sC5b–9

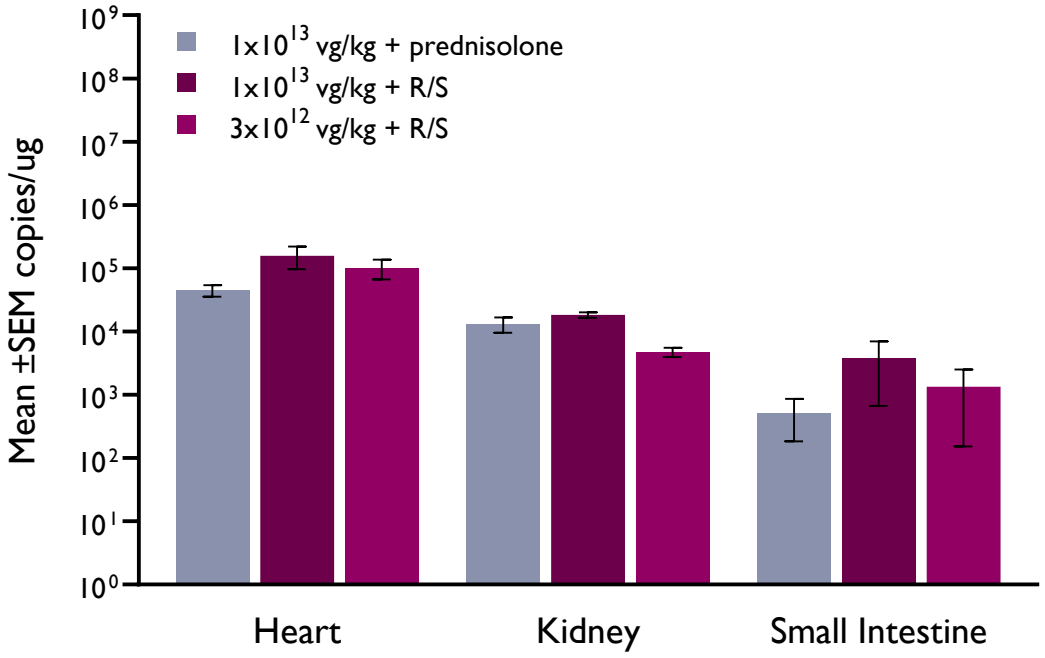


Shaded area indicates normal range for nonhuman primates.

4D-310 Biodistribution

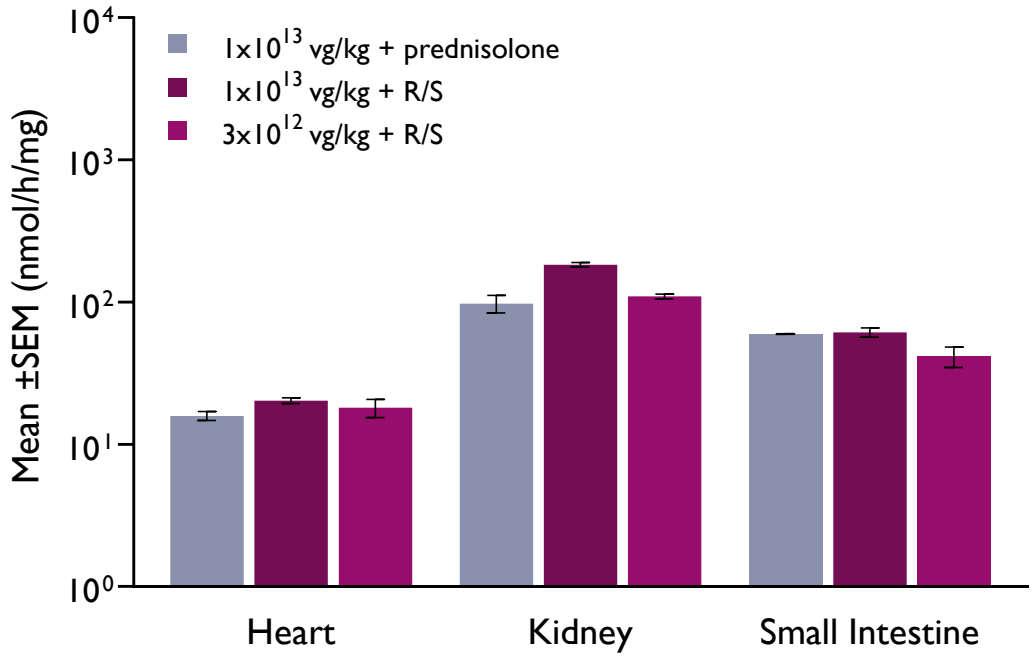
Rituximab/Sirolimus Did Not Affect Cell Transduction or Tissue AGA Activity

4D-310 Vector DNA*



- No meaningful differences in cell transduction observed across treatment groups

Tissue AGA Activity



- Generally similar levels of tissue AGA activity in the R/S and prednisolone groups

*Assess by qPCR. AGA, α-galactosidase A; R/S, rituximab/sirolimus; SEM, standard error of measurement.

Safety and Tolerability

4D-310 + Rituximab/Sirolimus

- Combined administration of 4D-310 and rituximab/sirolimus was well tolerated
- Findings consistent with prior studies
  - No morbidity or mortality
  - No adverse clinical or histopathological findings
  - No changes in body or organ weights
- Clinical pathology
  - No evidence of schistocytes (hallmark of TMA)
  - Early AAV-induced ALT elevation ameliorated with R/S compared to prednisolone

ALT, alanine aminotransferase. TMA, thrombotic microangiopathy.

Summary

- Administration of 4D-310 and rituximab/sirolimus to NHPs was well tolerated and attenuated humoral responses to the C102 capsid compared to prednisolone
  - Reductions in both total and IgM antibody response compared to prednisolone
  - Reduced risk of antibody-mediated complement activation
- No adverse effect on 4D-310 biodistribution or tissue AGA activity
- These findings support use of rituximab/sirolimus as a prophylactic immunomodulatory regimen in clinical trials evaluating 4D-310

Back-up

Acknowledgements

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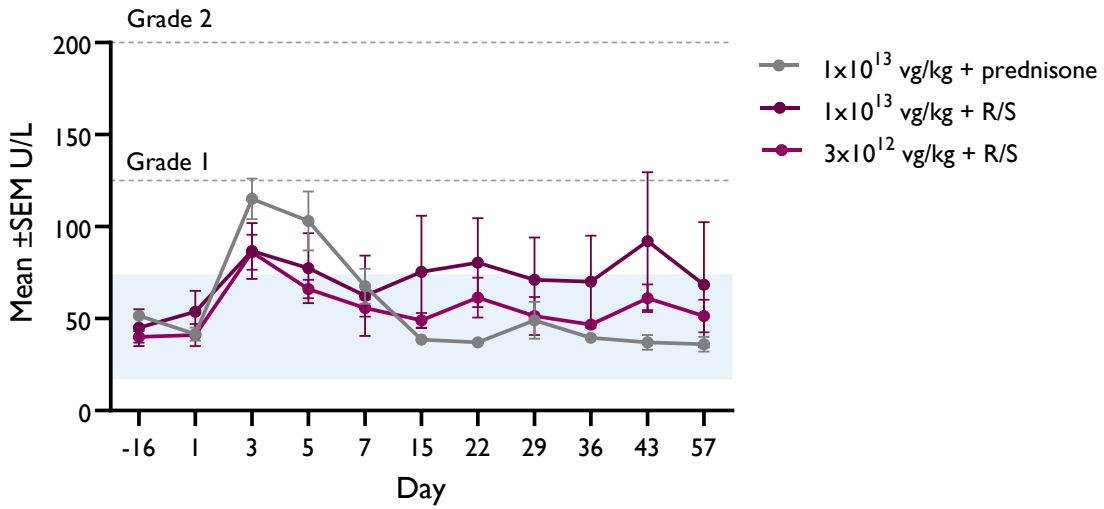
An Song
Research and Translational Development

**Former employee, 4D Molecular Therapeutics, Inc.*

Safety and Tolerability

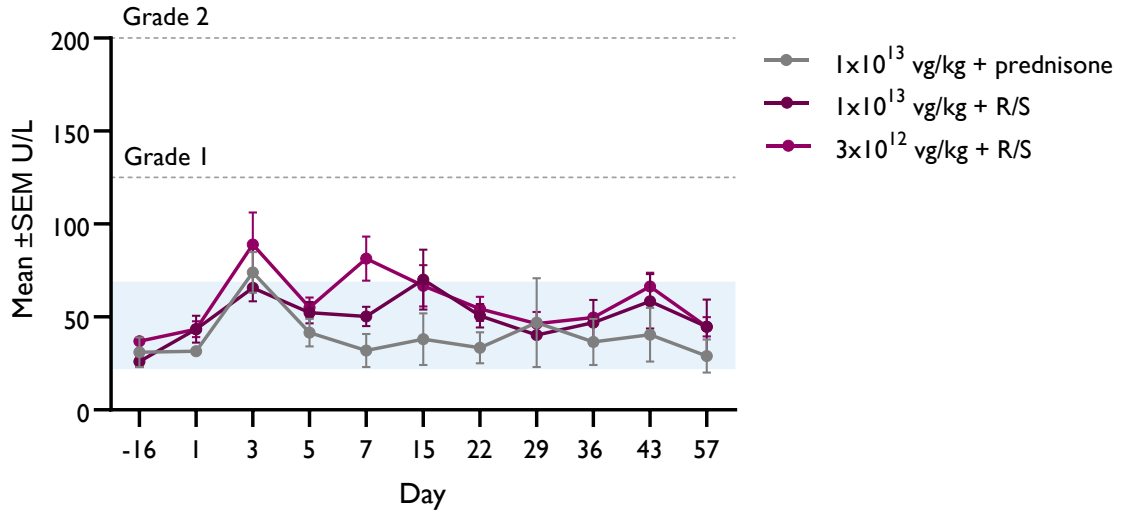
Serum Liver Enzymes

ALT



- Early mild and transient elevation in serum ALT is a known class effect of AAVs
- R/S was associated with lower peak serum ALT on day 3 compared to prednisone

AST



- Early mild and transient increases in mean serum AST observed following 4D-310 administration
- Mean serum AST concentrations remained within the normal range after the first week in all groups

ALT, alanine aminotransferase; AST, aspartate aminotransferase; R/S, rituximab + sirolimus.