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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

Melissa A. Calton, Joshua Holter, Caralee Schaefer, Kathryn Yoh, Ted Sullivan, Samantha Jensen, Edward Kim, Christian Vettermann, David Kirn, An Song

4D Molecular Therapeutics Inc., Emeryville, California

Background and Rationale

- 4D-310 is a clinical-stage genetic medicine that employs a cardiotropic AAV vector (C102) to deliver a codon-optimized human *GLA* transgene to cardiomyocytes for cell-autonomous α-galactosidase A (AGA) production via intravenous administration; activity is augmented by cross-correction from AGA secreted into blood.

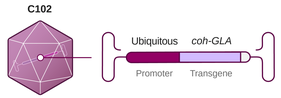


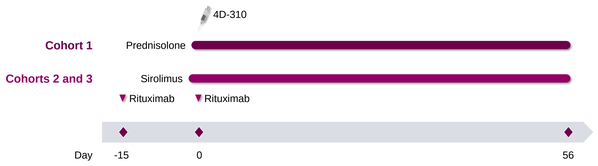
Figure 1. 4D-310 gene therapy product design.

- Thrombotic microangiopathy (TMA) and atypical hemolytic uremic syndrome (aHUS) caused by antibody-dependent activation of the classical complement pathway are known class effects of systemic AAV-based gene therapy.[1–4]
- Published reports have shown that prophylactic administration of rituximab/sirolimus attenuates humoral response to the AAV capsid following systemic AAV9-based gene therapy.[5]
- To support the adoption of rituximab/sirolimus as a prophylactic immunomodulatory regimen in the ongoing 4D-310 INGLAXA Phase 1/2 clinical trial (NCT04519749), we performed a 10-week nonhuman primate study to evaluate safety, anti-capsid antibody response, biodistribution, and transgene expression following administration of 4D-310 with either rituximab/sirolimus or prednisolone.

Methods

Study Design

Cohort	N	4D-310	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)†
3	3	3×10^{12} vg/kg	Oral sirolimus 0.5–8 mg/m^2/d (beginning on day –3)‡



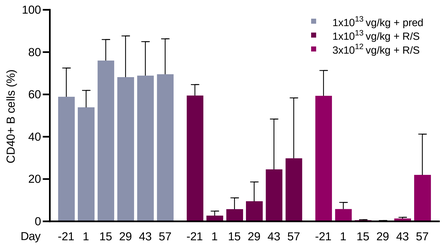
*Protocol-specified prophylactic immunomodulatory therapy in the initial dose cohort in the INGLAXA Phase 1 clinical trial evaluating 4D-310.
†Diphenhydramine 4 mg/m^2 administered via intramuscular injection prior to rituximab ‡Dose titrated to maintain a 24-hour trough concentration of 2–4 ng/mL.

Study Outcomes

Outcome	Marker(s)
Humoral immune response	Serum antibodies (Total and IgM), B cells
Complement activation	Serum/plasma complement factors (C3a, C4a, Bb, C3, C4, sC5b–9)
4D-310 biodistribution	Viral DNA (qPCR), *GLA* mRNA (ISH), AGA protein (IHC)
Transgene protein expression	Tissue AGA activity
Safety and tolerability	In-life examination, clinical pathology, anatomic pathology

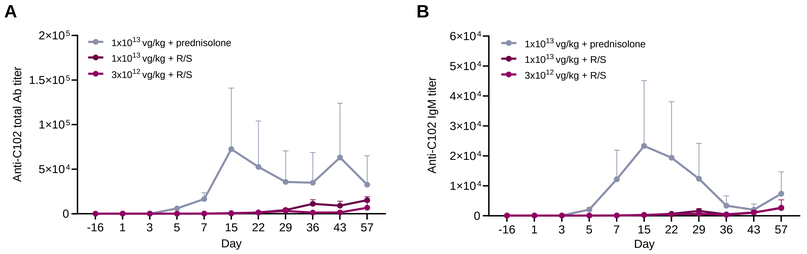
Results

B Cell Depletion



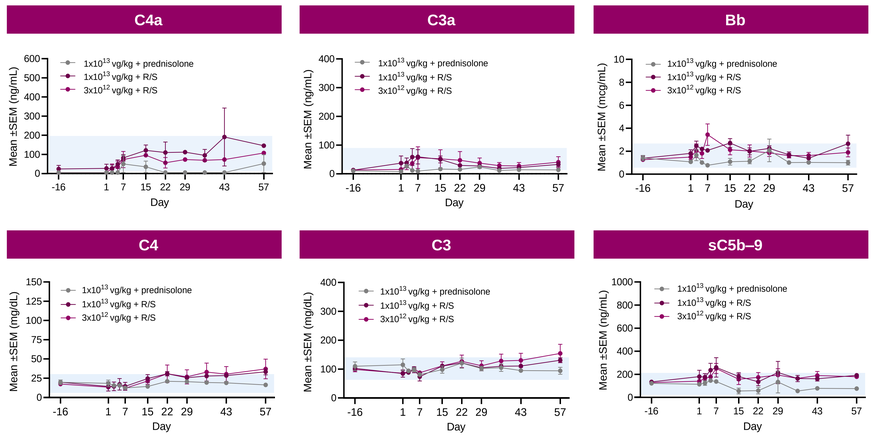
Rituximab/sirolimus results in efficient depletion of CD40+ B cells. Flow cytometry immunophenotyping and quantification of CD40+ B cells following administration of 4D-310 with either rituximab/sirolimus (n=3 animals per dose group) or prednisolone (n=2 animals). CD40+ cells were used to quantify B cells owing to the potential for rituximab to interfere with detection of CD20+ cells by flow cytometry. A >95% reduction in CD40+ B cells was observed on Day 1 compared to Day -21 in both rituximab/sirolimus groups. Data are shown as mean +SEM.

Humoral Immune Response



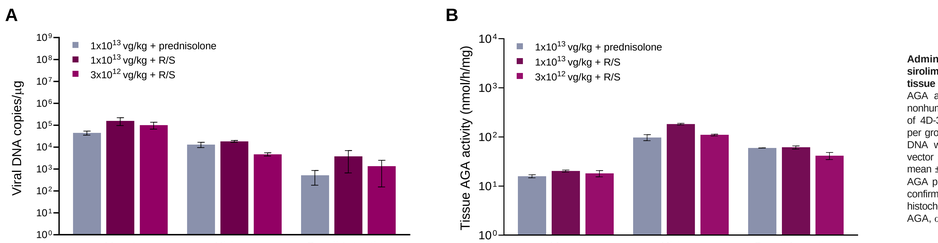
Rituximab/sirolimus attenuated treatment-emergent total and IgM antibody responses to the C102 capsid compared to prednisolone. Longitudinal assessment of (A) anti-C102 total antibody titer (IgG, IgA, and IgM) and (B) anti-C102 IgM antibody titer following administration of 4D-310 1×10^{13} or 3×10^{12} vg/kg with rituximab/sirolimus (n=3 per group) or 4D-310 1×10^{12} vg/kg with prednisolone (n=2) to nonhuman primates. Data are shown as mean +SEM. R/S, rituximab/sirolimus.

Complement Activity



Complement activation biomarkers generally remained within the normal range following administration of 4D-310 with rituximab/sirolimus. Absolute concentration of complement markers following administration of 4D-310 1×10^{13} or 3×10^{12} vg/kg with rituximab/sirolimus (n=3 per group) or 4D-310 1×10^{13} vg/kg with prednisolone (n=2) to nonhuman primates. Blue shaded areas indicate the normal range. Mild and transient elevations in activated complement markers were observed two animals. Complement activation preceded the development of anti-C102 antibodies in both animals and no human AGA-specific antibodies were detected in either animal, indicating that the observed increase in complement activity was unrelated to humoral immune response to 4D-310.

4D-310 Cell Transduction and Transgene Expression



Administration of 4D-310 with rituximab/sirolimus did not affect cell transduction or tissue AGA activity. (A) Viral DNA copies and (B) AGA activity in tissue samples collected from nonhuman primates at Day 57 post administration of 4D-310 with rituximab/sirolimus (n=3 animals per group) or prednisolone (n=2 animals). Tissue DNA was extracted and analyzed for 4D-310 vector genomes by qPCR. Data are shown as mean ±SEM. Transgene-derived *GLA* mRNA and AGA protein expression in tissue samples were confirmed by *in situ* hybridization and immunohistochemistry, respectively (data not shown). AGA, α-galactosidase A.

Safety

- Administration of 4D-310 with rituximab/sirolimus to nonhuman primates was safe and well tolerated
  - No morbidity or mortality
  - No adverse clinical or histopathological findings
  - No changes in body or organ weights
- Findings from analysis of clinical pathology markers commonly associated with TMA/aHUS (platelet counts, schistocytes, reticulocytes, LDH, and BUN) were unremarkable
  - No evidence of schistocytes
- Early AAV-induced transient elevation in serum ALT concentration was ameliorated by rituximab/sirolimus

Conclusions

- Administration of 4D-310 with rituximab/sirolimus to nonhuman primates was safe and well tolerated
- Rituximab and sirolimus markedly attenuated humoral responses to the C102 capsid
  - Delayed onset of both total and IgM antibody responses and reduced peak antibody titers compared to prednisolone
- Complement markers generally remained within the normal range
- No adverse effect on 4D-310 biodistribution or tissue AGA activity
- These findings support the use of rituximab/sirolimus as a prophylactic immunomodulatory regimen in clinical trials evaluating 4D-310

References

1. Schiffmann R et al. Cardiac Effects of 4D-310 in Adults with Fabry Disease in a Phase 1/2 Clinical Trial: Functional, Quality of Life, and Imaging Endpoints in Patients with 12 Months of Follow-up [Poster #323]. WORLDSymposium 2023. **2.** Hamilton BA, Wright JF. Challenges Posed by Immune Responses to AAV Vectors: Addressing Root Causes. *Front Immunol* 2021;12:675897. **3.** Mingozzi F, High KA. Immune Responses to AAV Vectors: Overcoming Barriers to Successful Gene Therapy. *Blood* 2013;122:23–36. **4.** Loo et al. *Bioanalysis* 2022;14:737–93. **5.** Salabarria SM et al. Thrombotic Microangiopathy Following Systemic AAV Administration is Dependent on Anti-capsid Antibodies. *J Clin Invest* 2024;134:e173510.