#338

Phase 1/2 Clinical Trial 4D-310 in Adults with Fabry Disease Cardiomyopathy: Interim Analysis of Cardiac and Safety Outcomes in Patients with 21–43 Months of Follow-up

Mark Thomas[a], Dau-Ming Niu[b], Nikola Stoyanov[a], Jerry Vockley[c], Damara Ortiz[c], Nadine Jacquez[d], Carol Chung[d], Jinsong Shen[d], Ted Sullivan[d], Alan Cohen[d], David Kirn[d]

[a]Royal Perth Hospital Perth, Australia; [b]Taipei Veterans General Hospital, Taipei, Taiwan; [c]University of Pittsburgh, Pittsburgh, Pennsylvania; [d]4D Molecular Therapeutics Inc., Emeryville, California

Introduction

- Fabry disease is an X-linked, monogenic, lysosomal storage disorder caused by loss-of-function mutations in the gene encoding the α-galactosidase A (AGA) enzyme.[1]
- Deficient AGA enzyme activity leads to accumulation of glycosphingolipid substrates in the heart, kidneys, and blood vessels, resulting in multiorgan dysfunction.[1]
- Cardiovascular disease is the most common cause of death, accounting for 75% of deaths in individuals with Fabry disease.[2,3]
- Current therapies do not adequately address the cardiovascular manifestations of Fabry disease,[2–4] highlighting a significant unmet medical need.
- 4D-310 is an investigational genetic medicine that employs the targeted AAV vector C102 to deliver a human *GLA* transgene directly to cardiomyocytes for cell-autonomous AGA production following intravenous administration; activity is augmented by cross-correction from AGA secreted into blood.

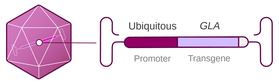


Figure 1. 4D-310 gene therapy product design.

- Here we report interim results from a first-in-human Phase 1/2 clinical trial evaluating 4D-310 in adults with Fabry disease cardiomyopathy with a duration of follow up ranging from 21 to 43 months.

Methods

Study Design

- INGLAXA-1 is an open-label, Phase 1/2 clinical trial (NCT04519749) evaluating the safety and clinical activity of a single IV dose of 4D-310 in adults with classic or late onset Fabry disease.
  - Eligibility criteria include age ≥18 years with a pathogenic *GLA* mutation and Fabry-related clinical manifestations; participants may be on or off enzyme replacement therapy.
  - The primary study end point is the incidence and severity of adverse events. Additional end points include changes from baseline in serum AGA activity, serum lysoGb3 concentration, and measures of cardiac function and biochemical composition.
- INGLAXA-2 is a multinational, open-label, Phase 1/2 clinical trial (NCT05629559) evaluating 4D-310 in adults with Fabry-associated cardiovascular involvement.
  - INGLAXA-2 includes cardiac biopsies; tissue samples are evaluated for *GLA* RNA by *in situ* hybridization and RT-qPCR, and for AGA protein by immunohistochemistry.
- Analysis population
  - Interim analysis of clinical activity and safety outcomes included all study participants in dose cohort 1 (1×10^{13} vg/kg) with evaluable data as of 5 December 2024.

Cardiac Outcomes

Outcome	Assessment Method
Transgene delivery and expression, Gb3 accumulation *Exploratory endpoint (INGLAXA-2)*	Cardiac biopsy*
Cardiac contractility (global longitudinal strain) *FDA-recommended supportive endpoint*	Echocardiogram†
Exercise capacity (peak VO_2) *FDA-recommended primary endpoint*	Cardiopulmonary exercise test†
Cardiac quality of life (physical limitations, symptoms) *FDA-recommended primary endpoint*	KCCQ summary scores

*Transgene delivery assessed by qPCR; RNA expression analyzed by RT-qPCR and *in situ* hybridization; AGA protein evaluated by immunohistochemistry; Gb3 accumulation in cardiomyocytes evaluated by electron microscopy.
†Assessed by independent central reading center. KCCQ, Kansas City Cardiomyopathy Questionnaire.

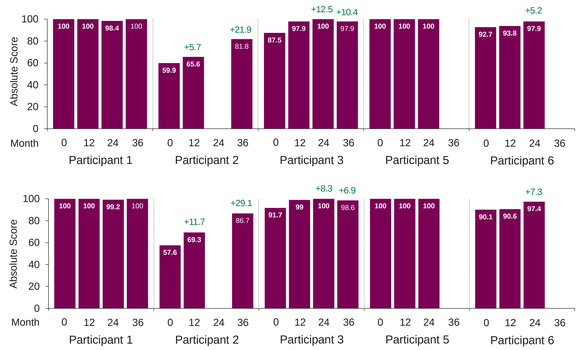
Results

Demographics and Baseline Characteristics

	INGLAXA 1				INGLAXA 2	
Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6
Age, years	51	32	26	19	57	69
Race/ethnicity	Hisp/Latino	White	White	NR	Asian	White
Disease classification	Classic	Classic	Classic	Late onset	Late onset	Late onset
Serum AGA, nmol/hr/mL*	0.42	0.00	0.30	0.06	1.62	0.18
Serum lyso-Gb3, ng/mL†	6.28	101.0	8.78	45.0	3.79	3.2
ERT experience	Yes	Yes	Yes	No	Yes	Yes
ERT status at enrollment	On	Off	On	Naïve¶	On	Off¶
Anti-AGA antibody titer	1:947	1:99,900	1:13,900	Negative	Negative	Negative
Peak VO_2, % predicted	na	33.0	66.1	30.3	76.0	120.2
Global longitudinal strain, %	–17.10	–22.17	–18.83	–23.27	–21.95	–20.63
eGFR, mL/min/1.73 m^2‡	107	130	125	142	64.9	61
Body mass index, kg/m^2	31.5	21.7	33.6	34.1	26.7	26.9
Blood pressure, mmHg	133/82	111/70	148/87	121/58	140/89	141/82
LVMI, g/m^2§	86.7	81.8	67.8	73.1	58.4	105.9

*Reference range, 4.44–27.42 nmol/hr/mL. †Reference range, ≤1.0 ng/mL. ‡Reference range, >60 mL/min/1.73m^2. ¶On migalastat at enrollment. §Reference range, 49–85 g/m^2.
AGA, α-galactosidase A; eGFR, estimated glomerular filtration rate; ERT, enzyme replacement therapy; Gb3, globotriaosylceramide; LVMI, left ventricular mass index.

Kansas City Cardiomyopathy Questionnaire



Scores range from 0 to 100 (higher score=less severe); minimal clinically important difference (overall summary score), 5 points [5]. *High antibody titer; entered study off ERT.

Cardiopulmonary Exercise Test: Peak VO_2

			Change from Baseline	
Patient	Measurement	Baseline	Month 12	Month 24
1	mL/kg/min (% predicted)	na	+2.0† (+6.3)	+7.8† (+24.6)
2‡	mL/kg/min (% predicted)	14.0 (33.0)	+7.0 (+17.0)	na
3	mL/kg/min (% predicted)	23.0 (66.1)	–2.2 (–7.8)	–4.1 (–15.6)
5	mL/kg/min (% predicted)	24.8 (76.0)	+1.8 (+8.3)	–0.1 (+3.3)
ERT¶	VO_2 max (mL/kg/min)	24.1	–1.8	–2.3

Minimal clinically important difference, 1.5 mL/kg/min [6].
*Not calculable (missing baseline data).
†Calculated as change from Month 6 values (21.4 mL/kg/min, 72% predicted).
‡High antibody titer, entered study off ERT.
¶Mean value, historical control (N=14); median duration of ERT, 48 months [7].

- Improved or stable Peak VO_2 was observed in 3 of 4 evaluable participants during follow up for up to 2 years

Global Longitudinal Strain

			Change from Baseline (%)		
Patient	Baseline		Month 12	Month 24	Month 36
1	–17.10	Borderline	–2.5	–2.9	–2.8
2‡	–22.17	Normal	–1.1	na	–0.9
3	–18.83	Low normal	–3.3	–2.8	–3.4
5	–21.95‡	Normal	–1.2‡	+0.4‡	
6	–20.63	Normal	–0.3	–2.4	
ERT¶	–13.2	na	+1.1	na	na

GLS was measured in 3 apical views (4-, 3- and 2-chamber); the average value is shown.
*GLS range (borderline), –16.0 to –18.0% [8]. Minimal detectable difference, 1.5% [9].
†High antibody titer, entered study off ERT.
‡GLS average of 4- and 2-chamber views (3-chamber view not available).
¶Mean value, historical control (N=18); median duration of ERT, 4.2 years (range, 1.4–12.2) [10].

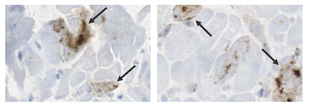
- Left ventricular function improved or remained stable in all evaluable participants

Cardiac Biopsy (Transgene Expression)

***GLA* mRNA (RT-qPCR)**

Week 6			Week 26		
Copies/ mg NA	Copies/ cell	Copies/ CM†	Copies/ mg NA	Copies/ cell	Copies/ CM†
2.2x10^5	4.3	16.2	1.3x10^5	2.6	9.8

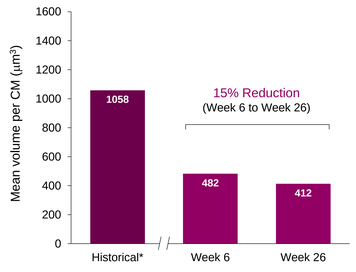
AGA Protein (IHC)

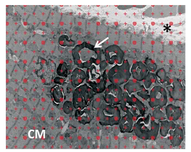


- Single participant with repeated cardiac biopsy (wks 6 and 26)*
- Paired analysis demonstrated widespread transduction and durable transgene expression
  - 4D-310 vector DNA (qPCR)
  - *GLA* RNA (ISH, RT-qPCR)
  - AGA protein (IHC)
- 4D-310 transgene expression observed predominantly in cardiomyocytes
- No evidence of inflammation

*Male (57 years) with late-onset Fabry disease (IVS4 + 919G>A). †Calculated based on an estimated 30% ratio of cardiomyocytes to all heart cells.
CM, cardiomyocyte; IHC, immunohistochemistry; ISH, *in situ* hybridization; NA, nucleic acid.

Gb3 Inclusion Body Volume (Cardiomyocytes)





Electron Microscopy Image Analysis
Point grid superimposed on cardiomyocytes for estimation of Gb3 inclusion body volume by image analysis algorithm. White arrow, Gb3 inclusion; asterisk, interstitium.

*Sample collected ~7 years prior to enrollment and analyzed independently by the investigator [11]. CM, cardiomyocyte; Gb3, globotriaosylceramide.

Safety

- No clinically significant cardiac or liver toxicities during follow up for 3 years
- Previous cases of atypical hemolytic uremic syndrome (n=3) fully resolved
  - Improved/stable cardiac outcomes observed on subsequent assessments in all 3
- No new 4D-310–related adverse events > Grade 1 since the last interim update

Conclusions

- Evaluation of interim results from the INGLAXA Phase 1/2 clinical trial demonstrated evidence of clinically meaningful improvements in left ventricular function, exercise capacity, and quality of life in adults with Fabry disease for up to 3 years after a single IV dose of 4D-310 (1E13 vg/kg).
- 4D-310 was generally well tolerated; there were no clinically significant cardiac or liver toxicities no new 4D-310–related Adverse events > Grade 1 since the last interim update in February 2024.
- Study enrollment is expected to resume in early 2025, with the addition of a new dose cohort and a modified immunomodulatory regimen.

References

1. Desnick R. et al. Alpha-galactosidase A deficiency: Fabry disease. In: The Metabolic and Molecular Bases of Inherited Disease, 8th ed, Scriver CR et al (Eds), McGraw Hill, New York 2001. **2.** Baig S, et al. Ventricular arrhythmia and sudden cardiac death in Fabry disease: a systematic review of risk factors in clinical practice. *Europace* 2018;20:153–61. **3.** Waldek S, et al. Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry. *Genet Med* 2009;11:790–796. **4.** Banikazemi M, et al. Disease Clinical Trial Study Group. Agalsidase-beta therapy for advanced Fabry disease: a randomized trial. *Ann Intern Med* 2007;14:77–86. **5.** Spertus JA, et al. Interpreting the Kansas City Cardiomyopathy Questionnaire in clinical trials and clinical care: JACC State-of-the-Art Review. *J Am Coll Cardiol* 2020;76:2379–90. **6.** Wilkinson TJ, et al. The "Minimum Clinically Important Difference" in frequently reported objective physical function tests after a 12-week renal rehabilitation exercise intervention in nondialysis chronic kidney disease. *Am J Phys Med Rehabil* 2019;98:431–7. **7.** Lobo T, et al. Cardiovascular testing in Fabry disease: exercise capacity reduction, chronotropic incompetence and improved anaerobic threshold after enzyme replacement. *Intern Med J* 2008;38:407–14. **8.** Yang H et al. Research to practice: Assessment of left ventricular global longitudinal strain for surveillance of cancer chemotherapeutic-related cardiac dysfunction. *JACC Cardiovasc Imaging* 2018;11:1196–1201. **9.** Lambert J, et al. Variability in echocardiography and MRI for detection of cancer therapy cardiotoxicity. *Heart* 2020;106:817–23. **10.** Nordin S, et al. Myocardial storage, inflammation, and cardiac phenotype in Fabry disease after one year of enzyme replacement therapy. *Circ Cardiovasc Imaging* 2019:e009430. **11.** Chang et al. Globotriaosylceramide accumulation in adult male patients with Fabry disease and IVS4 + 919G>A GLA mutation is progressive with age and correlates with left ventricular hypertrophy and reduced left ventricular ejection fraction. MedRxiv [Preprint]. 2023 Dec 11:2023.12.09.23298489.