Phase 1/2 Clinical Trial Evaluating 4D-310 in Adults with Fabry Disease Cardiomyopathy: Interim Analysis of Cardiac and Safety Outcomes in Patients with 12–33 Months of Follow-up

Mark Thomas, MBBS
Royal Perth Hospital
Perth, Australia

Fabry Disease Cardiomyopathy

Leading Cause of Death and Significant Unmet Medical Need

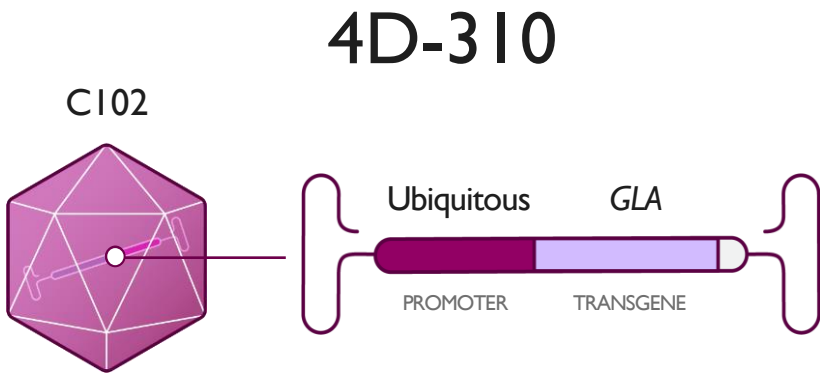
- Cardiac dysfunction is the most common clinical manifestation in Fabry disease[1]
  - Cardiovascular disease is the most common cause of death (75%)[1]
  - 10-fold increased risk of sudden cardiac death compared to the general population[1]
- Current therapies do not adequately address Fabry-related cardiac manifestations[2–5]
  - Enzyme replacement therapy results in transient increases in serum AGA, but does not improve cardiac function[6]
  - Emerging evidence suggests a nominal effect on exercise capacity with migalastat in patients with amenable *GLA* variants[7]; however, ~65% of Fabry patients carry a variant that is not amenable to treatment with migalastat[8]
  - No therapy has been shown to clear accumulated Gb3 from cardiomyocytes in patients with Fabry disease
- Effective therapy for the cardiac manifestations of Fabry disease therefore represents a significant unmet medical need

AGA, a-galactosidase A; Gb3, globotriaosylceramide.

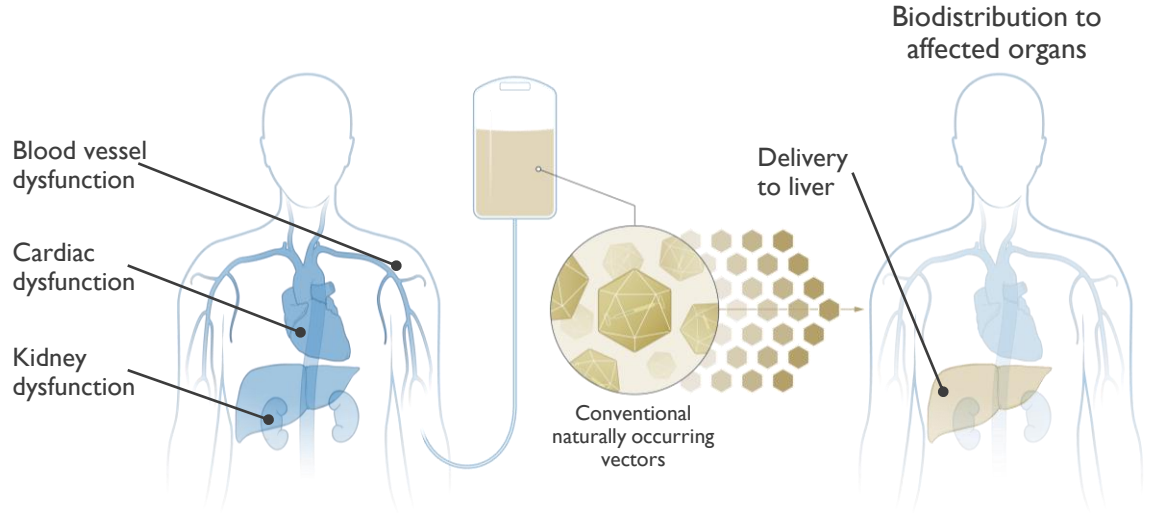
1. Baig S et al. *Europace* 2018;20:153–61. 2. Waldek S et al. *Genet Med* 2009;11:790–796. 3. Banikazemi M et al. *Ann Intern Med* 2007;14:77–86. 4. Tsukimura T et al. *Mol Genet Metab Rep* 2020;25:100650. 5. Azevedo O et al. *Int J Mol Sci* 2021;22:4434. 6. Lobo T et al. *Intern Med J* 2008;38:407–14. 7. Camporeale A et al. *J Med Genet* 2023;60:850–8. 8. Hughes et al. *J Med Genet.* 2017;54:288–96.

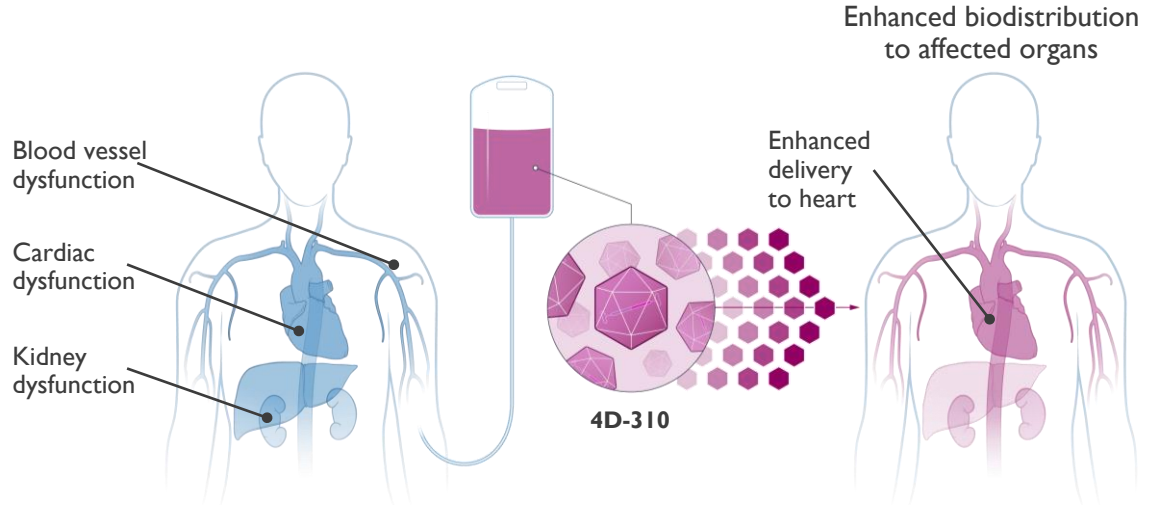
4D-310: Direct Cardiac Mechanism of Action

Low-dose IV Delivery to Cardiomyocytes



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





*AAV, adeno-associated virus

4D-310 Study Design

Open-label Phase 1/2 Trial in Adults with Classic or Late-onset Fabry Disease

Geography	U.S. (INGLAXA 1); Taiwan and Australia (INGLAXA 2)
Patient Population	Adult males/females; classic or late-onset Fabry disease; cardiac involvement* (on/off ERT)
C102 NAb Screening	Exclusion: high titer NAb to C102 (titer >1:1,000)
AGA Ab Screening	Exclusion: high titer Ab to AGA (titer ≥1:25,000)
4D-310 Dose	$1x10^{13}$ vg/kg (IV)
Immune Regimen	Corticosteroid prophylactic immunosuppression†
Primary Endpoint	Incidence and severity of adverse events
Secondary Endpoints	Cardiac imaging, function, quality of life
Histological Endpoints	Transgene delivery, RNA and AGA protein expression (INGLAXA 2)

*Eligibility for INGLAXA 2 required evidence of left ventricular hypertrophy on echo or cardiac MRI within 12 months prior to screening.
†Conversion to rituximab/sirolimus prophylactic immunomodulation regimen pending.
Ab, antibody; AGA, a-galactosidase A; ERT, enzyme replacement therapy; NAb, neutralizing antibody.

Cardiac Assessments

Biopsy, Imaging, Function, Quality of Life

Study Assessment	Method	Time Points
Transgene delivery and expression, Gb3 accumulation *Exploratory endpoint* (INGLAXA 2)	Cardiac Biopsy*	Weeks 6 and 26
Cardiac contractility (global longitudinal strain) *FDA-recommended supportive endpoint*	Echocardiogram†	Months 6, 9, 12, 18, 24
Exercise capacity (peak VO_2) *FDA-recommended primary endpoint*	CPET†	Months 6, 9, 12, 18, 24
Cardiac quality of life (physical limitations, symptoms) *FDA-recommended primary endpoint*	KCCQ	Months 6, 9, 12, 18, 24

*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.
CPET, cardiopulmonary exercise test; KCCQ, Kansas City Cardiomyopathy Questionnaire; MRI, magnetic resonance imaging.

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	Hispanic/Latino	White	White	NR	Asian	White
Disease classification	Classic	Classic	Classic	Late onset	Late onset	Late onset
GLA variant	c.1023A>C	c.708G>T	c.974G>A	c.671A>G	IVS4+919 G>A	c.644 A>G
Serum AGA activity, 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/1.73m^{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
Left ventricular mass index, 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; NR, not reported.

Cardiac Biopsy

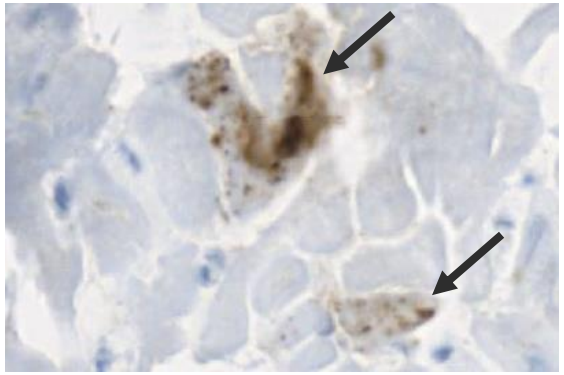
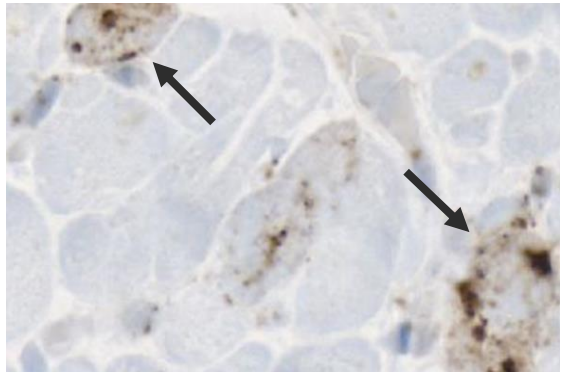
Robust and Durable Transgene Expression in Cardiomyocytes

- Single participant with repeated cardiac biopsy (Weeks 6 and 26)*
- Paired analysis of biopsies 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 inflammation

mRNA (RT-qPCR)

Week 6			Week 26		
Copies/ µg NA	Copies/ cell	Copies/ CM†	Copies/ µg NA	Copies/ cell	Copies/ CM†
2.2×10^5	4.3	16.2	1.3×10^5	2.6	9.8

AGA Protein (IHC)

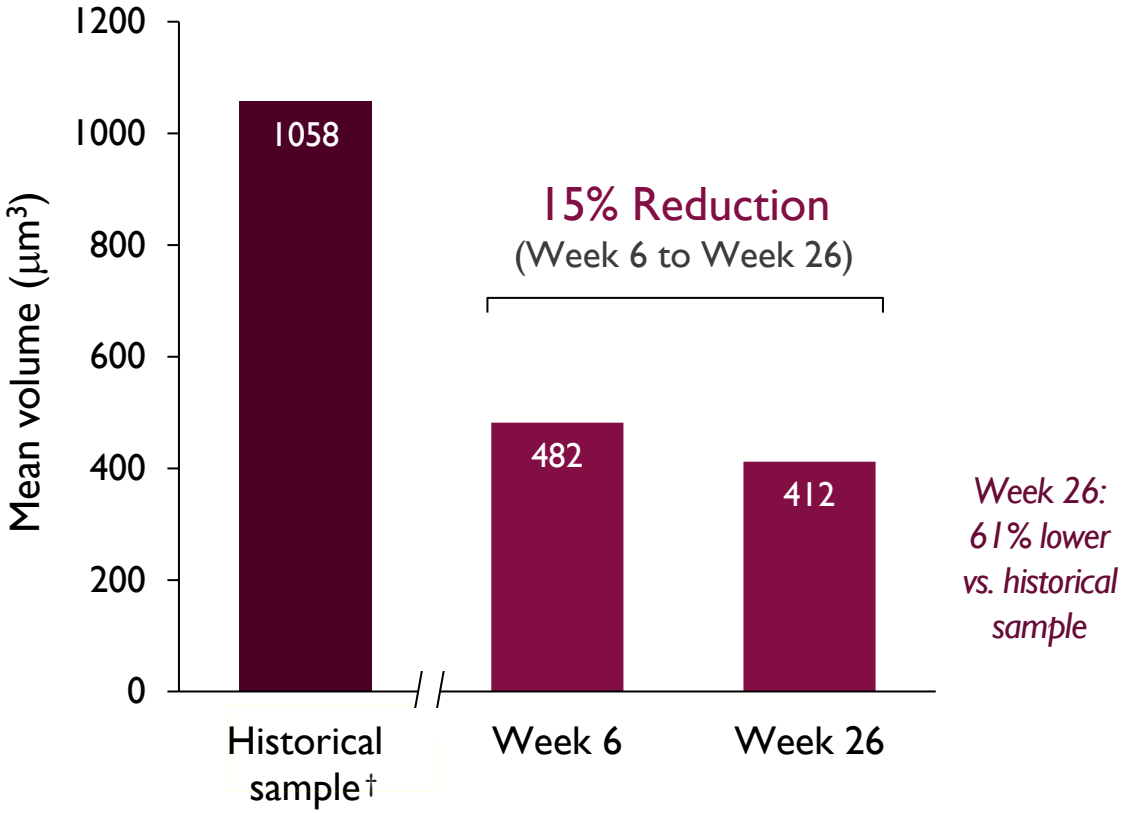
Week 6	Week 26
	

*Male (57 yrs) 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; qPCR, quantitative polymerase chain reaction; RT-qPCR, reverse transcription-qPCR; NA, nucleic acid.

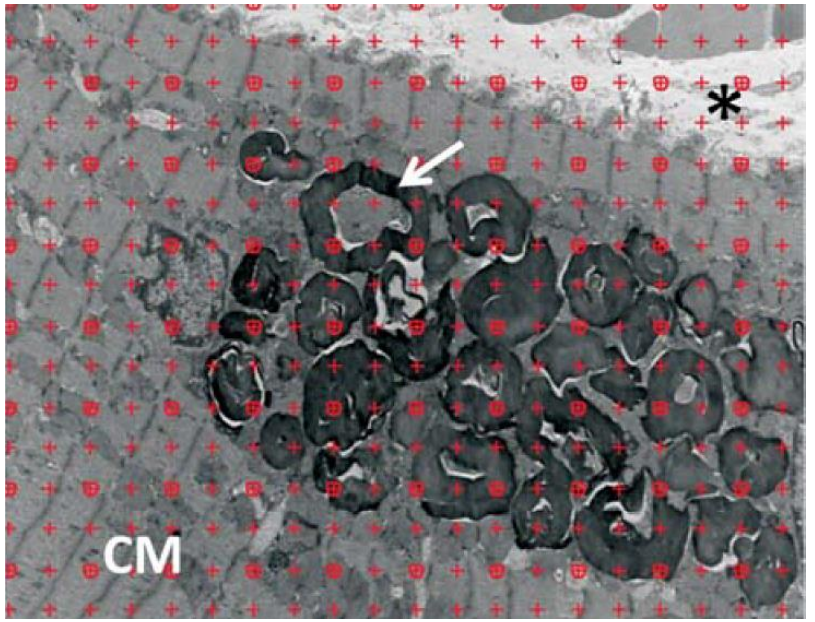
Cardiac Biopsy

Reduction in Substrate Accumulation in Cardiomyocytes*

Mean Gb3 Inclusion Body Volume per Cardiomyocyte



Ultra-high resolution electron microscopy and image analysis used to identify cardiomyocytes and quantify the volume of Gb3 inclusions[1]



Point grid superimposed on cardiomyocytes for estimation of Gb3 inclusion volume. White arrow, Gb3 inclusion; asterisk, interstitium.

*Male (57 years) with late-onset FD (IVS4+919G>A). †Sample collected ~7 years prior to enrollment and analyzed independently by investigator [1]. 1. Chang et al. 2023.12.09.23298489; doi: https://doi.org/10.1101/2023.12.09.23298489.

Global Longitudinal Strain

Ventricular Function Improved or Remained Stable in All Evaluable Participants

Patient	Baseline		Change from Baseline (%)*		
			Month 6	Month 12	Month 24
1	–17.10	Borderline	–1.1	**–2.5**	**–2.9**
2†	–22.17	Normal	na	–1.1	na
3	–18.83	Low normal	–0.5	**–3.3**	**–2.8**
5	–21.95‡	Normal	na¶	–1.2‡	
6	–20.63	Normal	–0.4	–0.3	
ERT§	–13.2			+1.1	–

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% [1]. Minimal detectable difference, 1.5% [2].
†Mean value, historical control (N=18); median duration of ERT, 4.2 years (range, 1.4–12.2) [3].
‡GLS average of 4- and 2-chamber views (3-chamber view not available).
¶Not evaluable.
§High antibody titer, entered study off ERT

1. Yang H et al. *JACC Cardiovasc Imaging* 2018;11:1196–1201. 2. Lambert J et al. *Heart* 2020;106:817–23. 3. Nordin S et al. *Circ Cardiovasc Imaging* 2019:e009430.

Cardiopulmonary Exercise Test

Peak VO_2 Improved in 3 of 4 Evaluable Participants

Patient	Measurement	Baseline	Change from Baseline		
			Month 6	Month 12	Month 24
1	mL/kg/min (% predicted)	na	na*	**+2.0**† (+6.3)†	**+7.8**† (+24.6)†
2‡	mL/kg/min (% predicted)	14.0 (33.0)	na	**+7.0** (+17.0)	na
3	mL/kg/min (% predicted)	23.0 (66.1)	+0.4 (–0.3)	–2.2 (–7.8)	–4.1 (–15.6)
5	mL/kg/min (% predicted)	24.8 (76.0)	**+2.6** (+9.4)	**+1.8** (+8.3)	
ERT¶	VO_2 max (mL/kg/min)	24.1		–1.8	–2.3

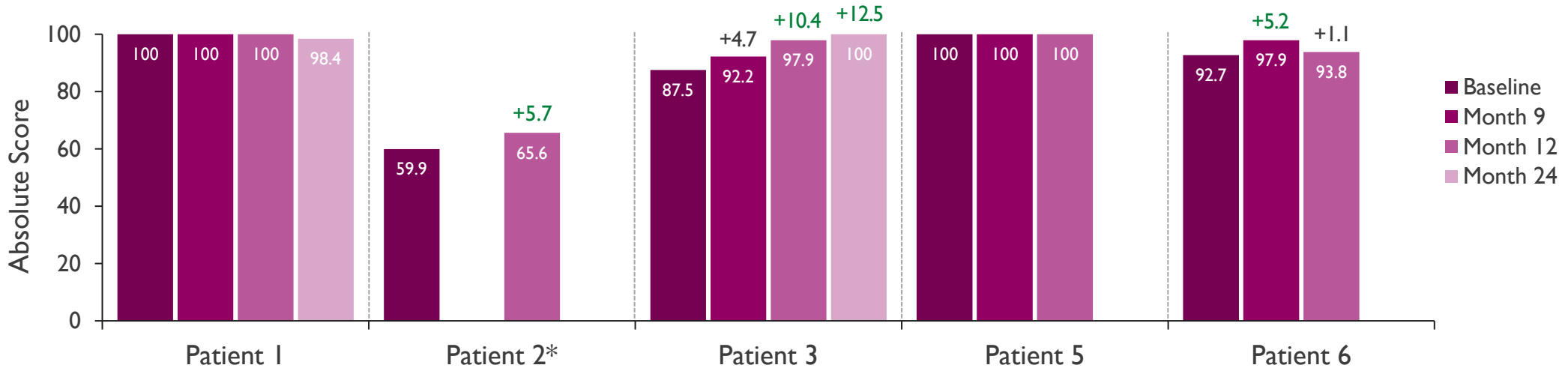
Minimal clinically important difference, 1.5 mL/kg/min [1].
*Not calculable (missing baseline data).
†Calculated as change from Month 6 values (21.4 mL/kg/min, 72% predicted).
‡High antibody titer, entered study off ERT.
¶Mean value, historical control (N=14); median duration of ERT, 48 months [2].

1. Wilkinson. *Am J Phys Med Rehabil* 2019;98:431. 2. Lobo T et al. *Intern Med J* 2008;38:407–14.

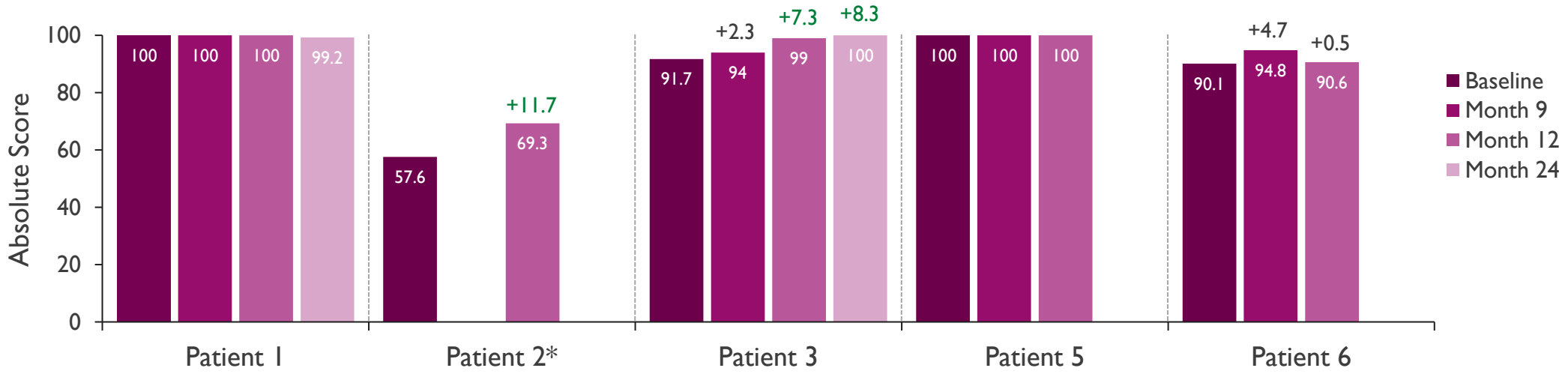
Kansas City Cardiomyopathy Questionnaire

Stable or Improved in All Evaluable Patients

Clinical Summary Score



Overall Summary Score



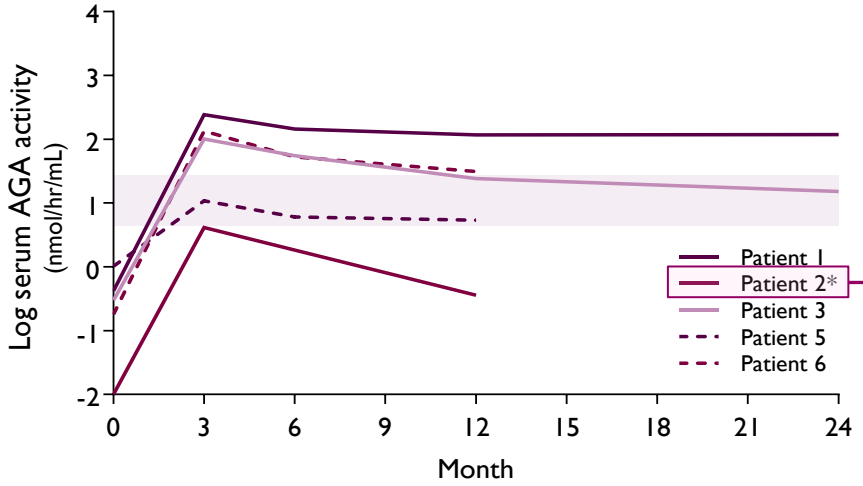
Scores range from 0 to 100 (higher score=less severe); minimal clinically important difference (overall summary score), 5 points [1]. *High antibody titer; entered study off ERT.

1. Spertus JA et al. *JACC* 2020;76:2379–90.

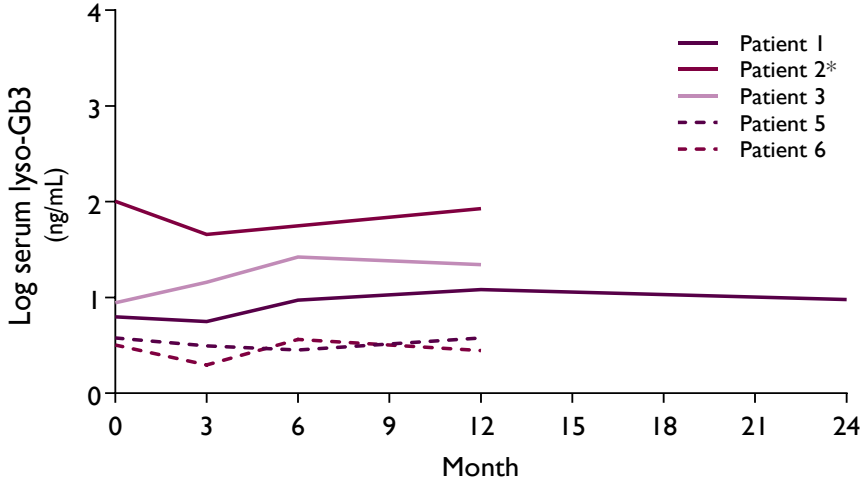
Serum Biomarkers

Considerable Inter- and Intrasubject Variability, No Correlation with Cardiac Outcomes

Serum AGA Activity



Serum Lyso-Gb3



Cardiac Outcomes (Patient 2)

Outcome	Baseline	Month 12	Change
Peak VO_2 (mL/kg/min)	14.0	21.0	+7.0
Peak VO_2 (% predicted)	33.0	50.0	+17.0
GLS (%)	–22.17	–23.27	–1.1
KCCQ			
Clinical Summary score	59.9	65.6	+5.7
Overall Summary score	57.6	69.3	+11.7

- Consistent with 4D-310 design characteristics, no correlation observed between serum AGA activity and cardiac outcomes

*High antibody titer (1:99,900) at baseline, entered study off ERT. Serum AGA normal range, 4.44–27.42 nmol/hr/mL (depicted as shaded area on graph). Lyso-Gb3 normal range, ≤1.0 ng/mL AGA, α-galactosidase A; Lyso-Gb3, globotriaosylsphingosine.

Interim Safety and Tolerability

INGLAXA 1 and 2 Clinical Trials

- Total of 6 participants received IV 4D-310 ($1x10^{13}$ vg/kg)
  - Duration of follow-up: 12–33 months
- No clinically significant cardiac or liver toxicities
- Previously reported cases of atypical hemolytic uremic syndrome (n=3) fully resolved
  - Evidence of improved or stable cardiac outcomes on subsequent assessments in all 3 participants
- No new 4D-310–related adverse events > Grade 1 since the last interim update in February 2023

Clinical Trial Status
INGLAXA 1 and 2

- Enrolled participants continue to complete scheduled study assessments
- Alignment with US FDA on plan to lift the clinical hold on the US study
  - Protocol amended to minimize aHUS risk associated with IV AAV
    - Pre-screen for complement activation
    - Addition of rituximab/sirolimus prophylactic immunosuppression regimen
  - Nonhuman primate safety study evaluating IV 4D-310 combined with rituximab/sirolimus underway
    - Results anticipated in mid-2024

aHUS, atypical hemolytic uremic syndrome; AAV, adeno-associated virus.

Summary

- Single IV dose of 4D-310 ($1x10^{13}$ vg/kg) demonstrated evidence of clinical activity on multiple distinct cardiac outcomes
  - Left ventricular function (ECHO)
  - Exercise capacity (CPET)
  - Quality of life (KCCQ)
- 4D-310 was generally well tolerated
  - No clinically significant cardiac or liver toxicities
  - Previously reported cases of aHUS (n=3) fully resolved, no new 4D-310–related AEs > Grade 1
- Cardiac biopsy (Weeks 6 and 26)*
  - Robust and durable 4D-310–mediated transgene expression in cardiomyocytes
  - All samples positive for transgene RNA (ISH) and AGA protein (IHC)
  - 15% reduction in Gb3 inclusions in cardiomyocytes between Weeks 6 and 26 (61% lower vs historical sample)

*INGLAXA 2 (n=1). AE, adverse event; aHUS, atypical hemolytic uremic syndrome; CPET, cardiopulmonary exercise test; IHC, immunohistochemistry; ISH, *in situ* hybridization; KCCQ, Kansas City Cardiomyopathy Questionnaire.

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- Co-authors:

Dau-Ming Niu, MD, PhD
Taipei Veterans General Hospital, Taipei, Taiwan

Nikola Stoyanov, MBBS, FRACP
Royal Perth Hospital, Australia

Jerry Vockley, MD, PhD
University of Pittsburgh, Pittsburgh, PA

Damara Ortiz, MD
University of Pittsburgh, Pittsburgh, PA

Nadine Jacquez
4D Molecular Therapeutics, Inc., Emeryville, CA

Carol Chung, PhD
4D Molecular Therapeutics, Inc., Emeryville, CA

Ted Sullivan
4D Molecular Therapeutics, Inc., Emeryville, CA

Jinsong Shen, MD, PhD
4D Molecular Therapeutics, Inc., Emeryville, CA

Alan Cohen, MD
4D Molecular Therapeutics, Inc., Emeryville, CA

David Kirn, MD
4D Molecular Therapeutics, Inc., Emeryville, CA