FREELINE

Corporate Presentation

January 2023

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Our leadership in AAV gene therapy

Two clinical-stage programs	FLT190: Best-in-class potential for Fabry disease FLT201: First- and best-in-class potential for Gaucher disease
Near-term data readouts for both	FLT190: Data from first two cohorts of MARVEL-1 Phase 1/2 study in 1H2023 FLT201: Data from first cohort of GALILEO-1 Phase 1/2 study in 1H2023
Scientific leadership in AAV gene therapy	Rationally designed AAVS3 capsid delivers high protein expression at low doses Advanced multiple gene therapy candidates into development, combining cutting-edge protein engineering and rational vector and capsid design
Experienced management team	Expertise in gene therapy, clinical translation and development through product approval, launch and commercialization

Advancing programs with first- and/or best-in-class potential

FLT190

For treatment of Fabry disease

Data from first dose cohort show promising safety and durability; currently dosing in second cohort

FLT201

For treatment of Gaucher disease[1]

Most advanced AAV gene therapy program for Gaucher disease[1]; robust preclinical efficacy

Program	Research	IND enabling studies	Phase 1/2	Phase 3	Rights[2]
FLT190 Fabry disease					FREELINE
FLT201 Gaucher disease (Type 1)					FREELINE

[1] Gaucher disease Type 1
[2] Development and worldwide commercial rights; owned and in-licensed intellectual property rights.

The Opportunity

One-time therapy that stops disease progression, improves outcomes, and frees patients from burden of frequent and lengthy infusions

Debilitating, chronic and progressive disorders

Fabry disease

- Inherited deficiency in α-Gal A enzyme
- Progressive organ damage
- Reduced life expectancy despite existing treatments
- Renal failure and cardiac disease most common causes of premature death

Patient population[1]: ~16,000[2]

Gaucher disease

- Inherited deficiency in GCase enzyme
- Leads to enlarged spleen and liver, low platelets and red blood cells, and bone and lung dysfunction
- Existing treatments cannot penetrate all tissues, poorly addressing certain aspects of disease

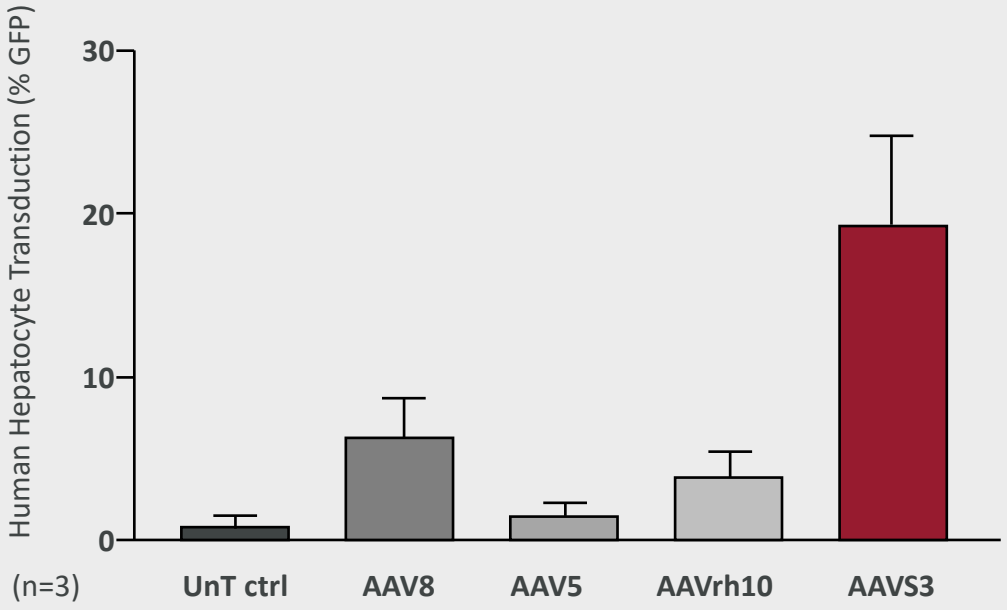
Patient population[1]: ~18,000[3]

[1] These figures represent the total theoretical genetic prevalence of the indications. The seroprevalence of antibodies against the AAV capsid renders approximately 30-50% of patients currently not eligible for AAV gene therapy.
[2] Ann Intern Med. 2003;138:338-346; Markets: EU4, UK, US, Japan.
[3] Hematology. 2017 Mar;22(2):65-73. doi: 10.1080/10245332.2016.1240391; Markets: EU4, UK, US, Israel.

Clinical-stage programs leverage highly potent AAVS3 capsid

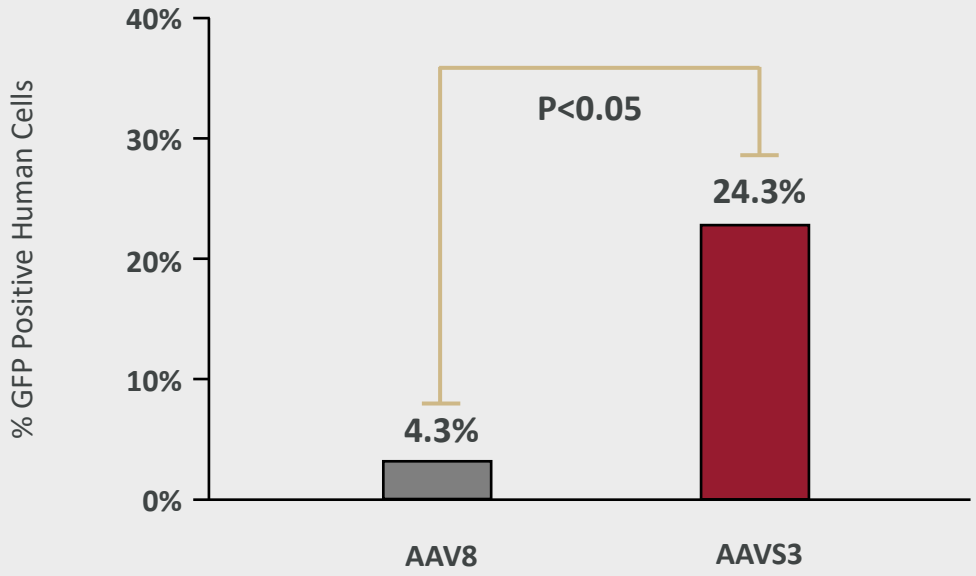
AAVS3 transduction significantly higher compared to other serotypes and untreated control

Transduction efficiency of AAVS3 in vitro compared with other vectors in primary human hepatocytes[1]



AAVS3 achieved nearly 6-fold higher transduction compared to AAV8

Transduction of human hepatocyte *in vivo* in AAV8 and AAVS3[2]



Data from 19 patients treated to date across our programs supports safety and potency of AAVS3 capsid

[1] Percentage of vectors containing GFP, green fluorescent protein, measured in primary human hepatocytes following transduction. AAVS3 pseudo typed vector used.
[2] Number of human hepatocytes expressing GFP following transduction. Measured in a human xenograft mouse model.

Experienced team to drive progress and execution

Michael Parini, CEO and Director

20 years as senior executive in leading biopharmaceutical companies

VERTEX · Pfizer

Pam Foulds, MD, Chief Medical Officer

20+ years of medical and clinical leadership

Auregen BioTherapeutics · Aegerion Pharmaceuticals · Biogen · genzyme

Henning Stennicke, PhD, Chief Scientific Officer

25+ years of scientific leadership experience

novo nordisk® · Sanford Burnham Prebys · University of Copenhagen

Paul Schneider, Chief Financial Officer

20+ years of global financial, commercial and operational experience

EXO THERAPEUTICS · CASEBIA THERAPEUTICS · Aegerion Pharmaceuticals · Shire

Jay Bircher, Chief Technical Operations Officer

30 years of quality and technical operations experience

Abeona THERAPEUTICS · BD · gsk · AMGEN

Nicole Jones, Chief People Officer

25+ years of global human resources experience

ALEXION · MERCK · Fidelity INVESTMENTS

Cash runway into 2024 beyond key clinical milestones

FLT190 in Fabry disease	Report initial safety and efficacy data from second cohort and updated data from first cohort of MARVEL-1 Phase 1/2 trial	**1H 2023**
FLT201 in Gaucher disease	Report initial safety and efficacy data from first cohort of GALILEO-1 Phase 1/2 trial	**1H 2023**

Cash runway includes anticipated proceeds from sale of Freeline's German subsidiary, Freeline Therapeutics GmbH, which is expected to close in the first quarter of 2023, subject to customary closing conditions, including receipt of regulatory approvals.

Fabry Disease

"Travelling to and from the hospital, the time spent for infusions, and trying to keep my spirit up and adjusting to treatment is a significant challenge."

- *Fabry disease patient perspective*

No curative therapy exists; high unmet need despite ERT

Fabry Disease Characteristics:

- Rare X-linked lysosomal storage disorder (LSD) resulting from deficient activity, or absence, of α-Galactosidase A (α-Gal A)
- Deficiency of α-Gal A results in accumulation of harmful substrates, including Gb3 and lyso-Gb3
- Characterized by progressive multi-systemic damage, including kidney, heart and vasculature

Limitations of ERT

Patients and Healthcare Systems

Lifelong, intravenous infusions every 2 weeks places significant burden on patients and healthcare systems

Incomplete Response

Despite treatment with ERT, patients experience debilitating symptoms and disease progression, resulting in shortened lifespan

Average life expectancy[2]	General population	Fabry population
US Males	74.7 years	58.2 years

Burden on patients[1]

ERT treatment burden

"Travelling to and from the hospital, the time spent for infusions, and trying to keep my spirit up and adjusting to treatment is a significant challenge."

Need for greater efficacy

"My son who is 35 was having bi-weekly infusions with not much positive results."

"The pain doesn't just affect me but so does the fatigue. Sometimes the pain literally wipes me out."

[1] Market research - interviews, survey and analysis
[2] Waldek S, Patel MR, Banikazemi M, Lemay R, Lee P. Life expectancy and cause of death in males and females with Fabry disease: findings from the Fabry Registry. Genet Med. 2009 Nov;11(11):790-6
ERT = Enzyme Replacement Therapy

FLT190: Potential best-in-class AAV gene therapy for Fabry disease

Competitor landscape

- Short half-life ERT therapies do not deliver sustained enzyme levels and patients continue to experience debilitating symptoms and disease progression

Patient population[2]

~16,000[3]

Gene therapy opportunity[1]

Patients
Attracted to freedom from bi-weekly infusions provided by gene therapy

Physicians
Will prescribe gene therapy with long-lasting efficacy results that are comparable to or better than ERT

Payers
Recognize potential for cost savings compared to chronic lifelong ERT

Target product profile

- Durable increased α-Gal A activity levels above the normal range
- Greater penetration of tissues, including heart and kidney
- Sustained reduction in lyso-Gb3
- Eliminates the need for ERT
- Improves outcomes
- Long-term tolerability and safety

[1] Market research - interviews, survey and analysis
[2] These figures represent the total theoretical genetic prevalence of the indications. The seroprevalence of antibodies against the AAV capsid renders approximately 30-50% of patients currently not eligible for AAV gene therapy.
[3] Ann Intern Med. 2003;138:338-346; Markets: EU4, UK, US, Japan.

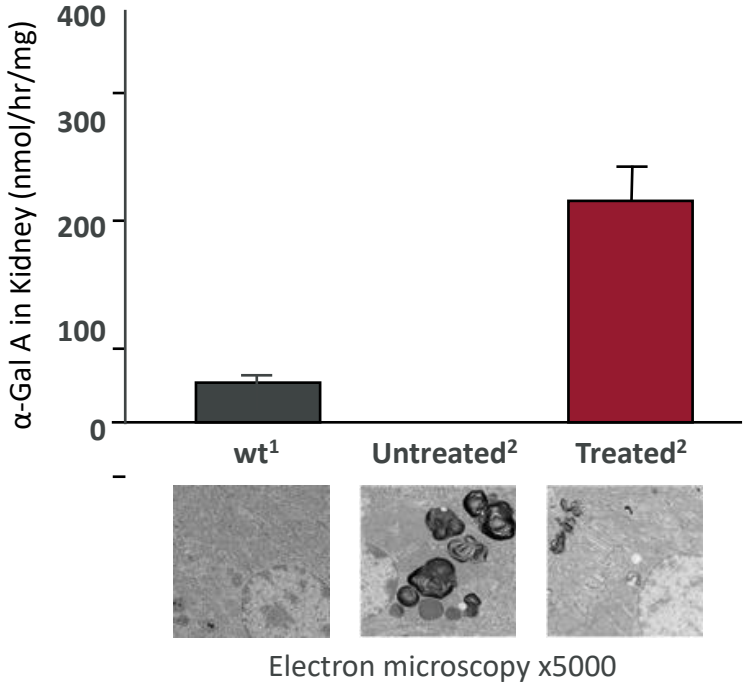
Increased α-Gal A in key tissues in Fabry mouse model

Strong exposure to α-Gal A in key organs

Exposure levels in GLA knockout mice exceeds wildtype in both kidney and heart at 2e12 vg/kg

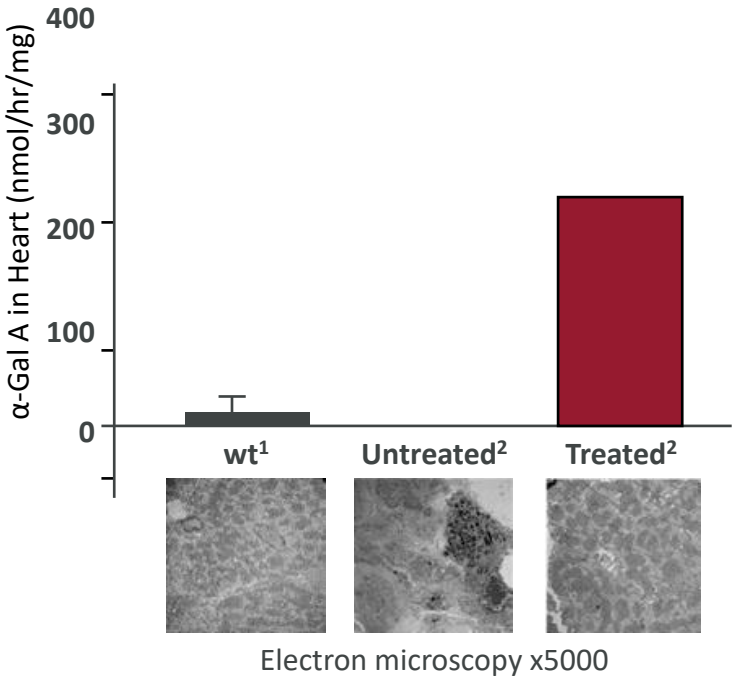
Electron microscopy demonstrates elimination of substrate from both kidney and heart

Kidney
α-Gal A activity levels



Electron microscopy x5000

Heart
α-Gal A activity levels



Electron microscopy x5000

[1] Untreated wild-type (non-GLA KO) mice. [2] GLA KO mice.
FLT190 vector genome pseudo-typed with AAV8 in GLA knockout ("GLA KO") mice; Dose: 2e12 vg/kg. Error bars: mean ± SD.
Time point: 16-week disease development prior to treatment; analysis 14 weeks post-treatment. Gb3/Lyso-Gb3 data (n=4, 2 males and 2 females).
WORLD*Symposium* 2019: Jey Jeyakumar et al. Liver-directed gene therapy corrects Fabry disease in mice.
Fabry mouse model: Ohshima T, Murray GJ, Swaim WD, Longenecker G, Quirk JM, Cardarelli CO, Sugimoto Y, Pastan I, Gottesman MM, Brady RO, Kulkarni AB. (1997). alpha-Galactosidase A deficient mice: a model of Fabry disease. PNAS: 18;94(6):2540-4.

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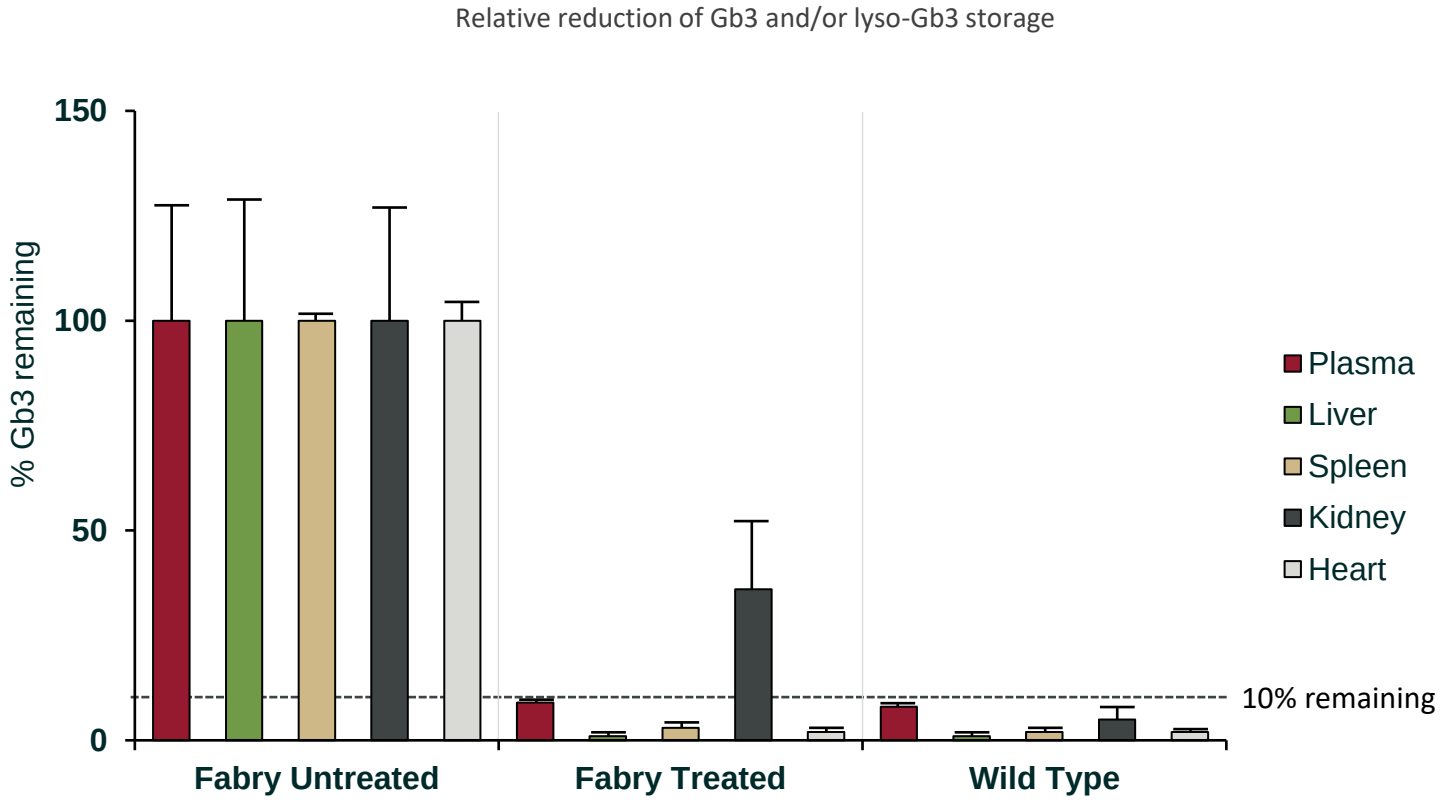
Cleared pathological substrates in Fabry mouse model

Efficient substrate elimination

Reduction in Gb3 observed by mass spectrometry in all key tissues as well as urine

Reduction of lyso-Gb3 to below 10% of GLA knockout observed in plasma, heart and kidney



The relative reduction of Gb3 and/or Lyso-Gb3 storage in Fabry mice administered $2x10^{12}$ vg/kg of FLT190 vector genome pseudotyped with AAV8. The percentage remaining storage was calculated based on untreated Fabry mice. Data are mean ± SD, n=4 animals per time point, analysed 14 weeks after treatment at 7.5 months of age. n=4 for untreated age-matched control group, n=2 for wild type mice.

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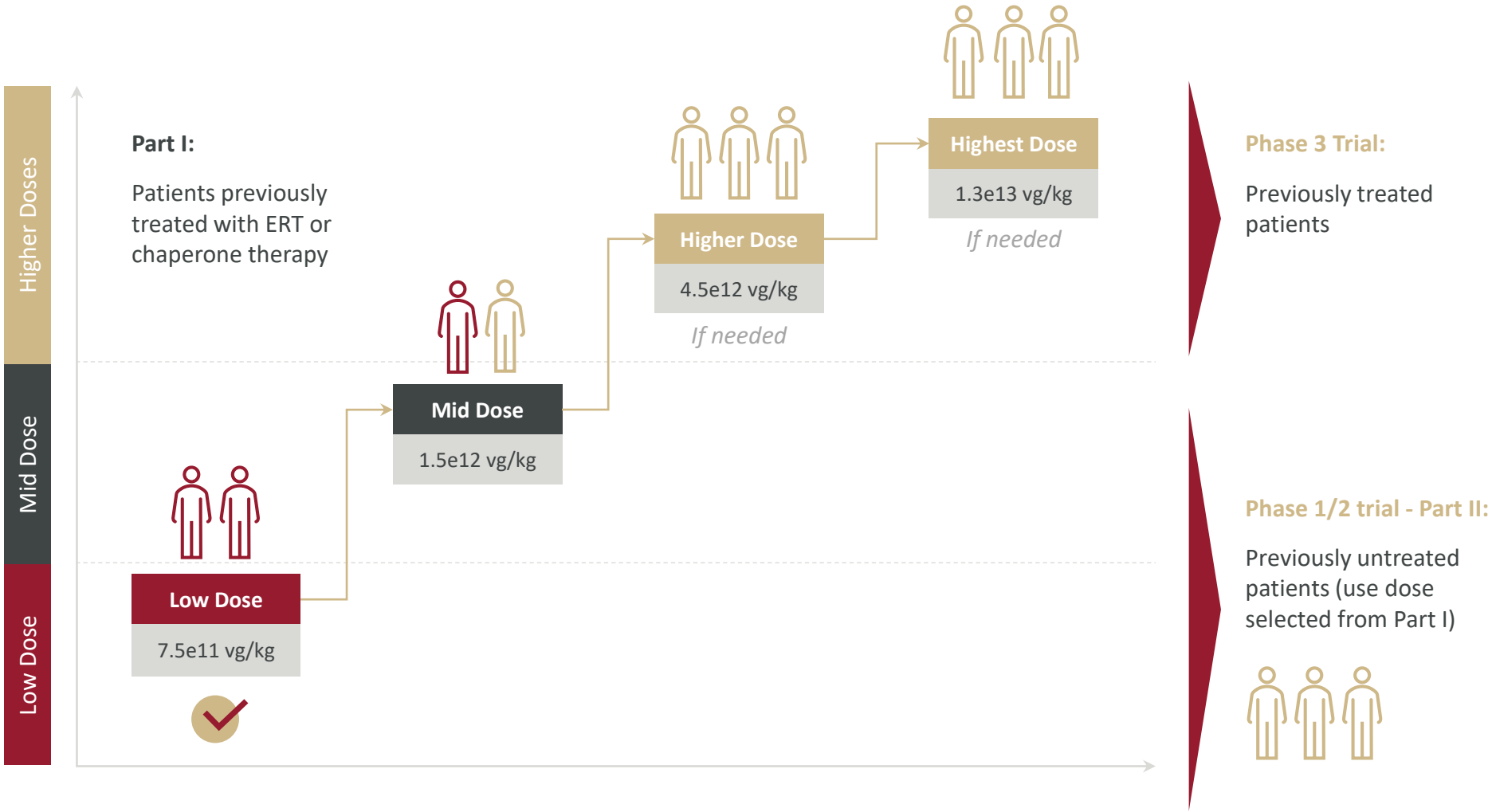
MARVEL-1 Phase 1/2 dose-finding trial design

Dosing in second cohort ongoing

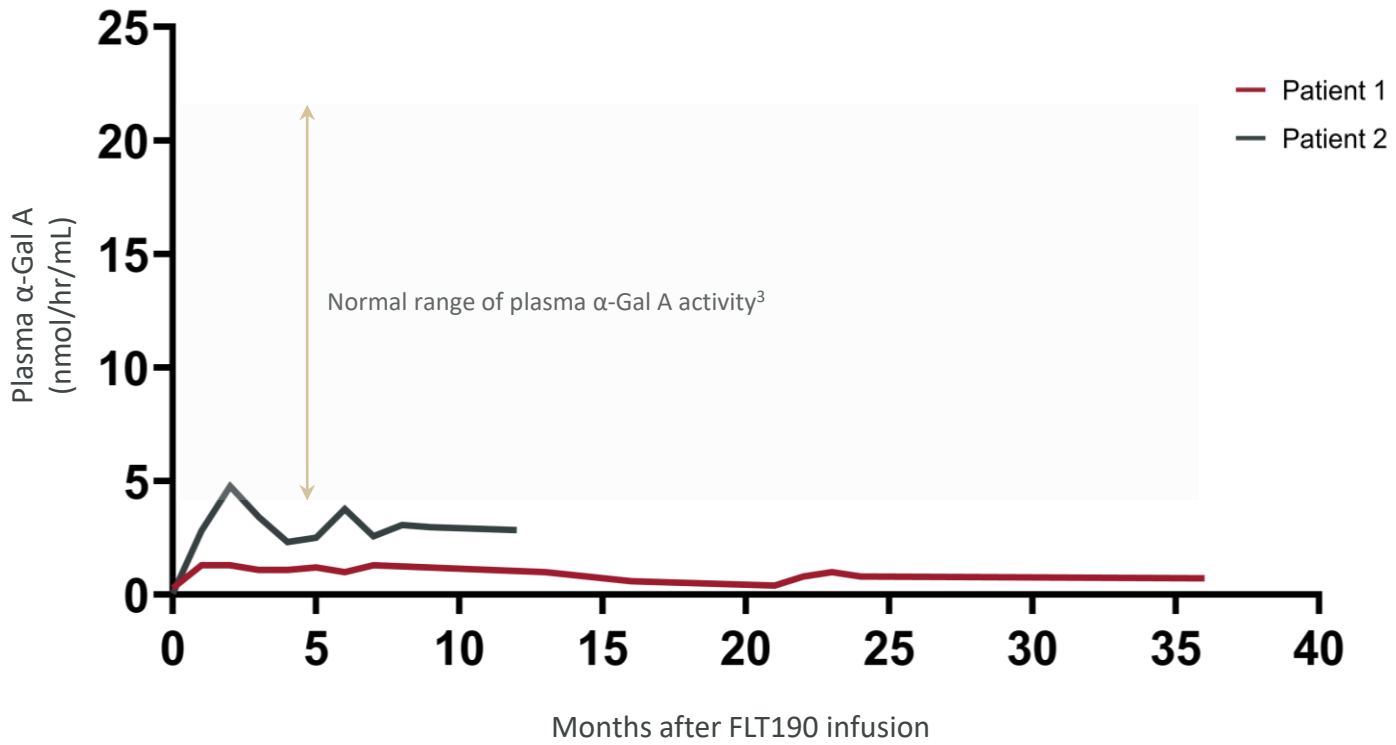
Adaptive trial:
To establish a dose of FLT190 that delivers sustained increases in α-Gal A activity to levels that reduce substrate accumulation



✓ Marks cohort has completed dosing

Data from first cohort show FLT190 is well-tolerated with durable α-Gal A activity up to three years post-dosing[1]

Plasma α-Gal A[2] Activity Over Time Following Treatment with 7.5e11 vg/kg of FLT190



FLT190 continues to be well-tolerated

- Early transient myocarditis resolved on its own in both patients
- No findings of long-term cardiac sequelae
- No further events of myocarditis or elevations of troponin

Potentially dose-dependent response

- ~40% increase in total dose with ~400% increase in activity[4]

Patient 2 remains off ERT

[1] Data from Patient 1 and Patient 2 in first dose cohort of MARVEL-1 trial as of August 2022
[2] α-Gal A: Plasma α-galactosidase A, the missing enzyme in Fabry disease.
[3] Current assay normal range: 4.0-21.9 nmol/hr/mL.
[4] The total vector genome (vg) dose Patient 2 received was approximately 40% higher than Patient 1 due to differences in their weights

Gaucher Disease (Type 1)

"… The main reason for considering a gene therapy would be to be free of the infusions. It would be brilliant, absolutely brilliant…"

- *Gaucher disease patient perspective*

No curative therapy exists for Gaucher disease

Disease Characteristics:

- Rare genetic LSD resulting from deficiency of glucocerebrosidase (GCase)
- Leads to accumulation of glucocerebroside (GL-1)
- Type 1 characterized by enlarged liver and spleen, anemia, thrombocytopenia, and bone and lung dysfunction, with absence of primary CNS disease

Limitations of ERT

Patients and Healthcare Systems

Lifelong intravenous infusions every two weeks places significant burden on patients and healthcare systems

Incomplete Response

Patients continue to experience cellular dysfunction and disease progression, particularly in the lung and bone

Burden on patients[1]

ERT Treatment Burden

“It would be a lot easier if I didn’t have to do the infusions every two weeks…it takes two or three hours.”

“The main reason for considering a gene therapy would be to be free of the infusions. It would be brilliant, absolutely brilliant.”

Need for greater efficacy

“I have a fear of pain. For many, many years, I was very cautious in all kinds of activities from bending down to walking, to how I would sit on a plane so I wouldn’t get jostled.”

1 Market research - interviews, survey and analysis

FLT201: Potential first- and best-in-class AAV gene therapy for Gaucher disease (Type 1)

Competitor landscape

- Short-half life therapies do not deliver sustained enzyme levels or penetrate hard-to-reach tissues
- Limited competition in the Gaucher gene therapy market
- *Ex vivo* lentiviral gene therapy is more burdensome and invasive than AAV. Manufacturing scale-up is also highly complex

Patient population[2]

~18,000[3]

Gene therapy opportunity[1]

Patients
Attracted to freedom from bi-weekly infusions provided by gene therapy

Physicians
Will prescribe durable gene therapy with efficacy comparable to or better than ERT

Payers
Are attracted to the potential cost savings associated with displacing chronic ERT

Target product profile

- Durable increases in GCase levels above the normal range
- Greater penetration of tissues, including bone and lung
- Sustained reduction in lyso-Gb1
- Eliminates the need for ERT/SRT
- Improves outcomes
- Long-term tolerability and safety

[1] Market research - interviews, survey and analysis
[2] These figures represent the total theoretical genetic prevalence of the indications. The seroprevalence of antibodies against the AAV capsid renders approximately 30-50% of patients currently not eligible for AAV gene therapy.
[3] Hematology. 2017 Mar;22(2):65-73. doi: 10.1080/10245332.2016.1240391; Markets: EU4, UK, US, Israel.
SRT = Substrate Reduction Therapy

Our scientists engineered GCase variant that is more stable and achieved higher expression than wildtype

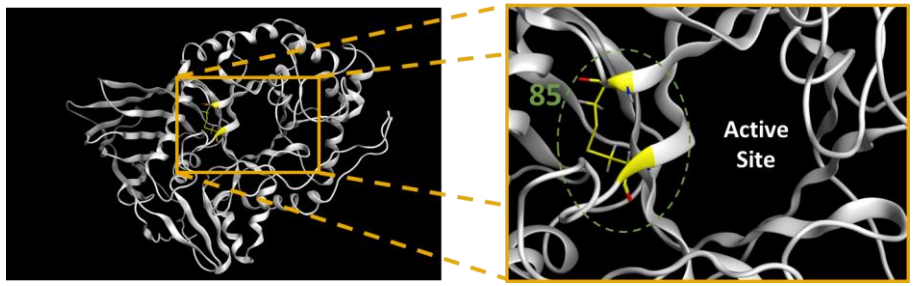
Key features of GCase variant

- > 20-fold increase in half-life in lysosomal pH compared with wild type (wt)
- Specific activity unchanged compared to wt GCase
- Compared to wt, 6-10 fold increase in half-life, enables increased *in vivo* steady state plasma levels for GCase 85

GCase variant 85 structure

Two internal amino acid substitutions

- Does not impinge on the active site
- Minimizes 3D structural change



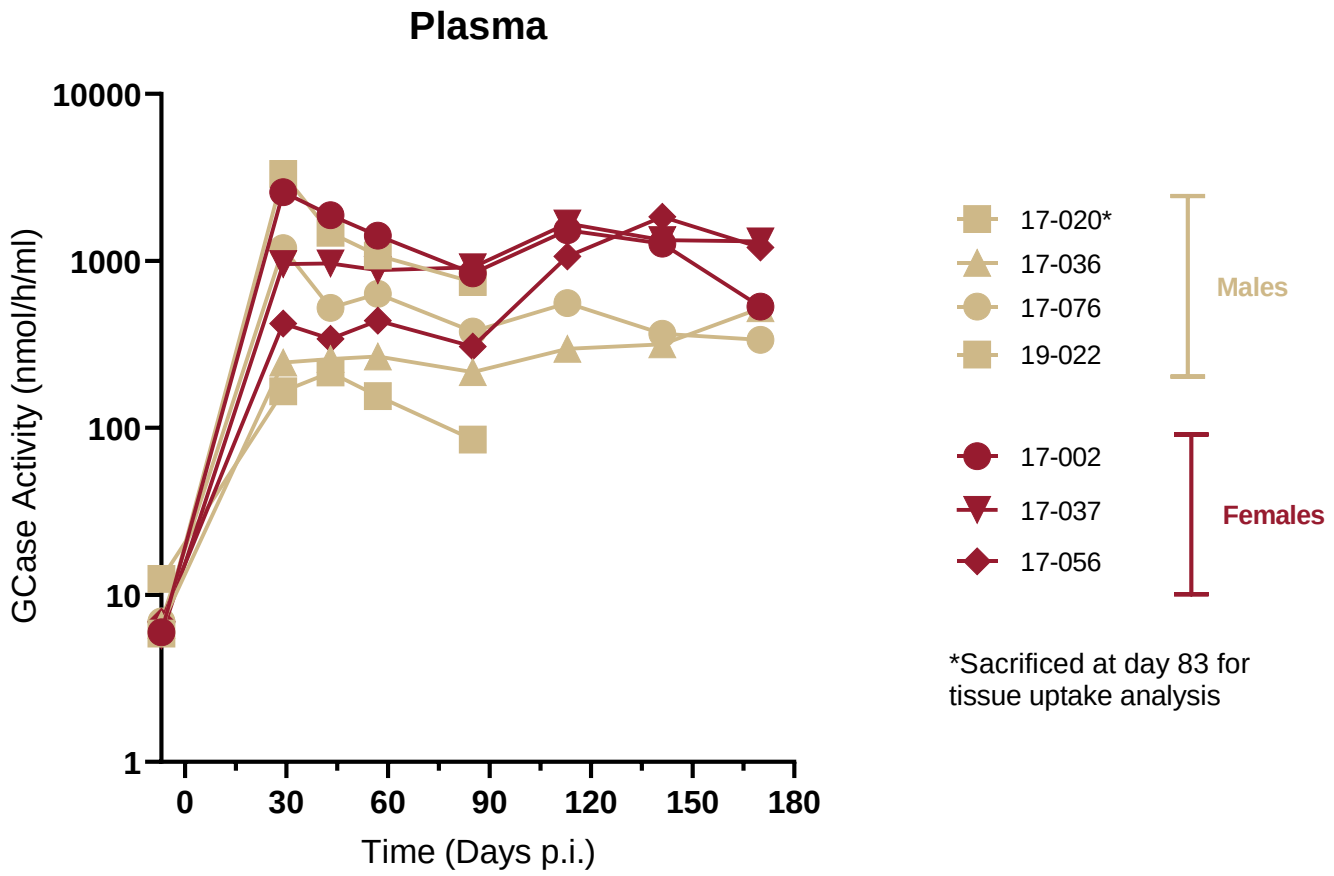
Biophysical properties of variant 85 and wildtype GCase					
	Lysosomal pH	Physiological pH	Mouse serum	Mouse plasma	Human serum
	Half-life (minutes)				
WT GCase	388	70	7.4	77	24
Variant 85	>8639	141	74	508	143
Improvement	**>21X**	**2X**	**10X**	**6.6X**	**6X**

WORLD*Symposium* 2021: Fabrizio Comper et al. Generation of β-Glucocerebrosidase Variants with Increased Half-life in Human Plasma for Liver Directed AAV Gene Therapy Aimed at the Treatment of Type 1 Gaucher Disease

Demonstrated high GCase expression in non-human primates

Achieved steady increases in GCase plasma levels

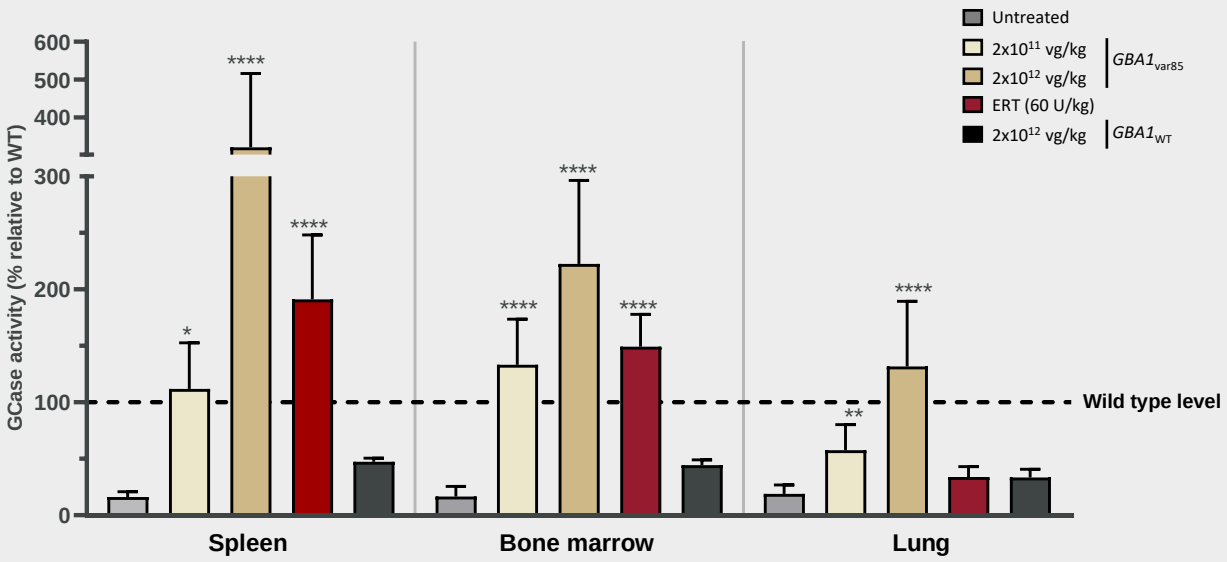
- A single injection of FLT201 was well tolerated
- Resulted in a rapid increase of GCase in plasma that was sustained for at least six months (trial ongoing)



Dose: FLT201 $2x10^{12}$ vg/kg (long-term observational trial)

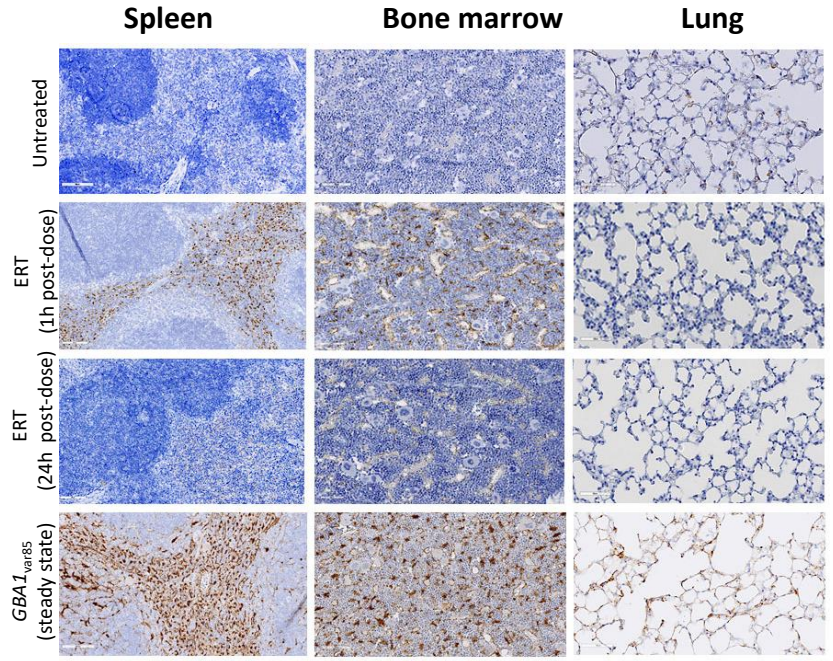
FLT201 led to higher GCase expression at low doses and increased uptake in key tissues in wildtype mice

Dose-dependent increase in GCase levels in key tissues compared to ERT or wildtype



Data represented as mean ± SD. n= 9 to 16 per treatment group. * P≤0.05, ** P≤0.001, P≤0.001, **** P ≤ 0.0001, one-way ANOVA.

Enhanced and sustained GCase uptake observed in key tissues compared to ERT



Anti-GCase – DAB, Haematoxylin counterstain, AAV8

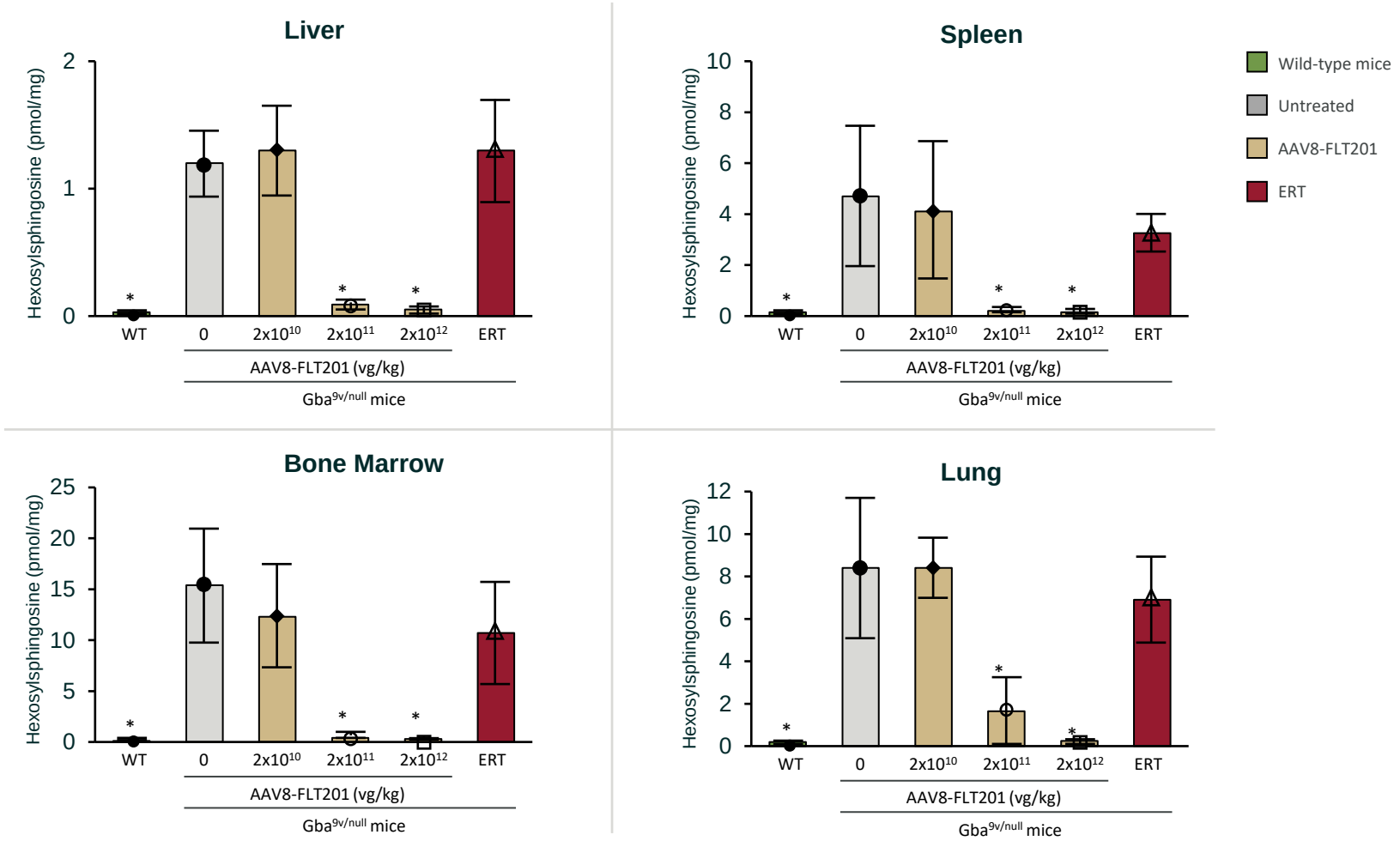
American Society of Gene & Cell Therapy 2021 Annual Meeting: Romuald Corbau et al. FLT201, a Novel Investigational AAV-Mediated Gene Therapy Candidate for Gaucher Disease Type 1
WORLD*Symposium* 2021: Romuald Corbau et al. FLT201: An AAV-Mediated Gene Therapy for Type 1 Gaucher Disease Designed to Target Difficult to Reach Tissues

ERT=Velaglucerase alfa

Cleared pathological substrate in key tissues in Gaucher mice

Efficient substrate elimination

Dose-dependent reductions of lyso-Gb1 observed in all tissues analyzed, including bone marrow and lung

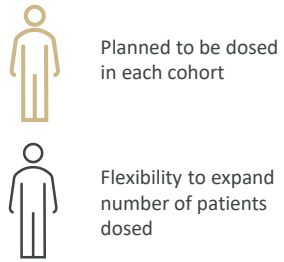


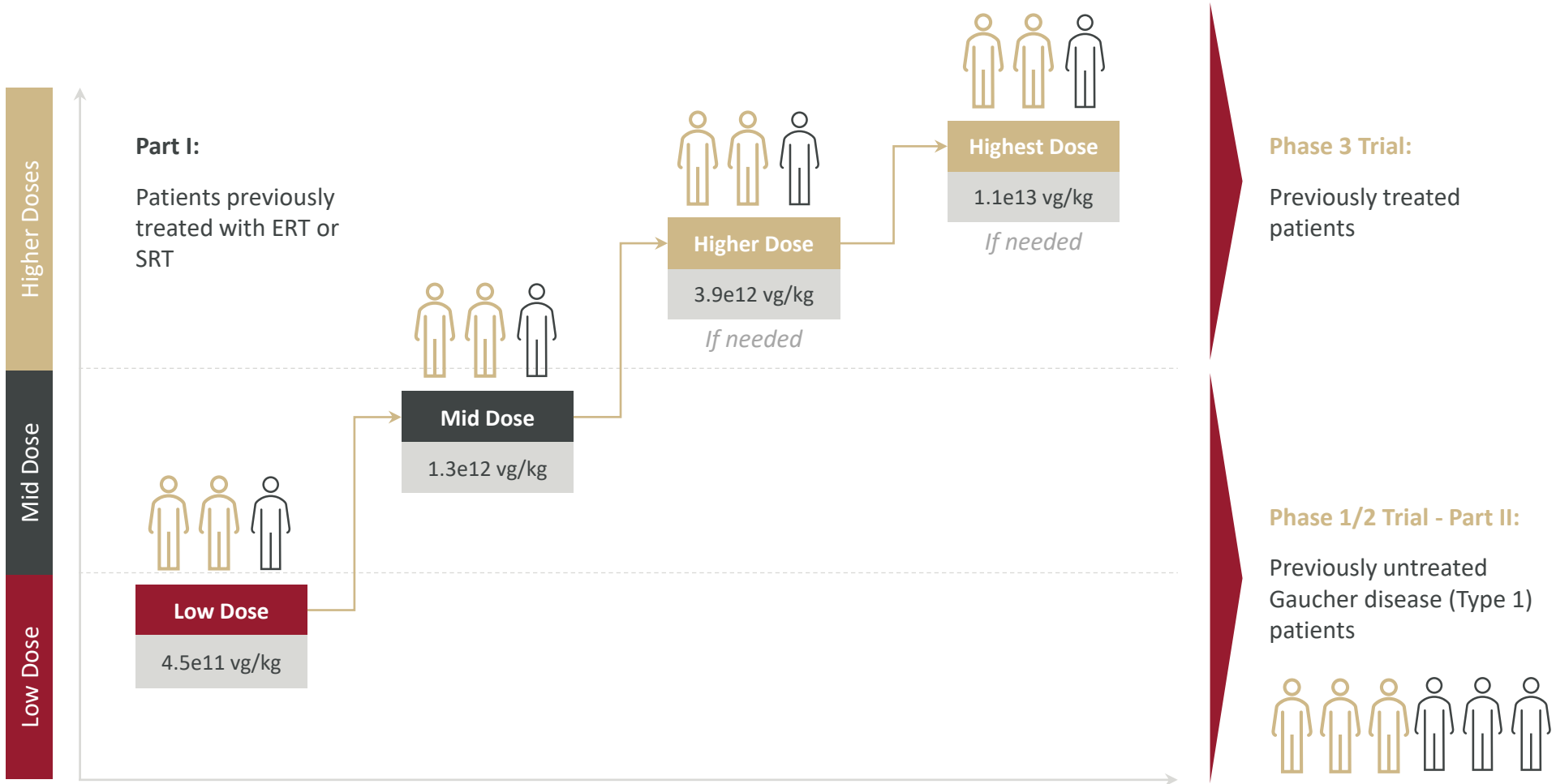
WORLD*Symposium* 2021: Romuald Corbau et al. FLT201: An AAV-Mediated Gene Therapy for Type 1 Gaucher Disease Designed to Target Difficult to Reach Tissues
AAV8-FLT201 = AAV8 pseudo-typed FLT201 genome. ERT = Velaglucerase alfa 60 U/kg biweekly (equivalent of the standard of care in humans). WT = wild-type mice. Evaluated 12 weeks post-injection. *$p \leq 0.0001$.

GALILEO-1 Phase 1/2 dose-finding trial design

Multiple patients in screening

Adaptive trial:
To establish a dose of FLT201 that delivers sustained increases in GCase activity to levels that reduce substrate accumulation and improve clinical parameters





Trial protocol allows for testing up to four doses in Part I of the clinical trial. Depending on dose response in the initial cohorts, we may not ultimately need to escalate to the later doses.
The Data Monitoring Committee (DMC) may recommend the next dose level at the next planned dose level, at same, higher or lower dose level based on emerging safety/tolerability, PK, PD and efficacy data.

A transformational year ahead for Freeline

2023

Two clinical-stage programs with first- and/or best-in-class potential

Data readouts for both programs in 1H 2023, with cash runway into 2024

Strengthening our leadership in AAV gene therapy

Experienced team to drive progress through focused and disciplined execution

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Thank you