FREELINE

Corporate Presentation

December 8, 2021

Legal disclaimer

FREELINE

This presentation contains statements that constitute “forward looking statements” as that term is defined in the United States Private Securities Litigation Reform Act of 1995, including statements that express the opinions, expectations, beliefs, plans, objectives, assumptions or projections of Freeline Therapeutics Holdings plc (the “Company”) regarding future events or future results, in contrast with statements that reflect historical facts. Examples include statements that the Phase 1/2 B-LIEVE dose-confirmation clinical trial of FLT180a is expected to be initiated in the first quarter of 2022, that the Company will be able to provide an interim data readout from such trial in the middle of 2022 or at all, that the Company will be able to present long-term durability data from its Phase 1/2 B-AMAZE clinical trial in 2021, that a dose of 7.7e11 vg/kg or the Company’s immune management regimen will be successful in preserving Factor IX (“FIX”) activity levels in the normal range, that the Company will be able to progress its Phase 1/2 MARVEL-1 clinical trial of FLT190 by dosing a third patient in the first quarter of 2022 and provide interim data readouts in the middle and end of 2022 or at all, that the Company will be able to complete trial site initiation in the Phase 1/2 dose-finding clinical trial for FLT201 in 2021 and provide an interim data readout from such trial in the middle of 2022 or at all, or that the Company will be able to improve operational efficiency to provide enhanced financial flexibility for future investments in its platform, as well as any other discussion of the Company’s strategies, financing plans, business plans and prospects, capital allocation objectives and manufacturing, research, pipeline and clinical trial plans, including anticipated development milestones for the Company’s product candidates. In some cases, you can identify such forward-looking statements by terminology such as “anticipate,” “intend,” “believe,” “estimate,” “plan,” “seek,” “potential,” “project” or “expect,” “may,” “will,” “would,” “could” or “should,” the negative of these terms or similar expressions. Forward-looking statements are based on management’s current beliefs and assumptions and on information currently available to the Company, and you should not place undue reliance on such statements. Forward-looking statements are subject to many risks and uncertainties, including the Company’s recurring losses from operations; the uncertainties inherent in research and development of the Company’s product candidates, including statements regarding the timing of initiation, completion and the outcome of clinical studies or trials and related preparatory work and regulatory review, regulatory submission dates, regulatory approval dates and/or launch dates, as well as risks associated with preclinical and clinical data, including the possibility of unfavorable new preclinical, clinical or safety data and further analyses of existing preclinical, clinical or safety data; the Company’s ability to design and implement successful clinical trials for its product candidates; the recent departures of a number of executive officers of the Company, and the Company’s ability to fill open positions, implement an orderly transition process and retain key talent; whether the Company’s cash resources will be sufficient to fund the Company’s foreseeable and unforeseeable operating expenses and capital expenditure requirements for the Company’s expected timeline; the potential for a pandemic, epidemic or outbreak of infectious diseases in the United States, United Kingdom or European Union, including the COVID-19 pandemic, to disrupt and delay the Company’s clinical trial pipeline; the Company’s failure to demonstrate the safety and efficacy of its product candidates; the fact that results obtained in earlier stage clinical testing may not be indicative of results in future clinical trials; the Company’s ability to enroll patients in clinical trials for its product candidates; the possibility that one or more of the Company’s product candidates may cause serious adverse, undesirable or unacceptable side effects or have other properties that could delay or prevent their regulatory approval or limit their commercial potential; the Company’s ability to obtain and maintain regulatory approval of its product candidates; the Company’s limited manufacturing experience which could result in delays in the development, regulatory approval or commercialization of its product candidates; and the Company’s ability to identify or discover additional product candidates, or failure to capitalize on programs or product candidates. Such risks and uncertainties may cause the statements to be inaccurate and readers are cautioned not to place undue reliance on such statements. Many of these risks are outside of the Company’s control and could cause its actual results to differ materially from those it thought would occur. The forward-looking statements included in this presentation are made only as of the date hereof. The Company does not undertake, and specifically declines, any obligation to update any such statements or to publicly announce the results of any revisions to any such statements to reflect future events or developments, except as required by law.

For further information, please refer to the Company’s reports and documents filed with the U.S. Securities and Exchange Commission. You may obtain these documents by visiting EDGAR on the SEC website at www.sec.gov.

Certain information contained in this presentation relates to, or is based on, studies, publications, surveys and other data obtained from third party sources and the Company's internal estimates and research. While the Company believes these third-party sources to be reliable as of the date of this presentation, they have not been independently verified, and makes no representation as to the adequacy, fairness, accuracy or completeness of, any information obtained from third party sources. In addition, all of the market data included in this presentation involve a number of assumptions and limitations, and there can be no guarantee as to the accuracy or reliability of such assumptions. Finally, although the Company believes its own internal research is reliable, such research has not been verified by any independent source.

Clinical-stage biotechnology company developing transformative AAV-mediated gene therapies for patients suffering from inherited systemic debilitating diseases

- Proprietary capsid enables high protein expression at low doses in humans
- Freeline positioned to target diseases perceived as beyond the reach of first-generation AAV gene therapy
  - GD: FLT201, novel protein-engineered treatment for Gaucher disease Type 1, shows promising pre-clinical data; on track to initiate clinical trial by year-end
  - FIX: FLT180a for treatment of hemophilia B demonstrated Factor IX activity levels in normal range enabling potential for functional cure[1]
  - Fab: FLT190 for treatment of Fabry disease in the clinic; on track for interim data readout in H1 2022
- Proprietary protein engineering, analytics and CMC platform that deliver novel, high-quality gene therapy product candidates at commercial scale
- Improving operational efficiency to provide enhanced financial flexibility for future investments in our platform

1. Certain adult hemophilia B patients.

Potent AAVS3 capsid and platform drives protein expression

FREELINE

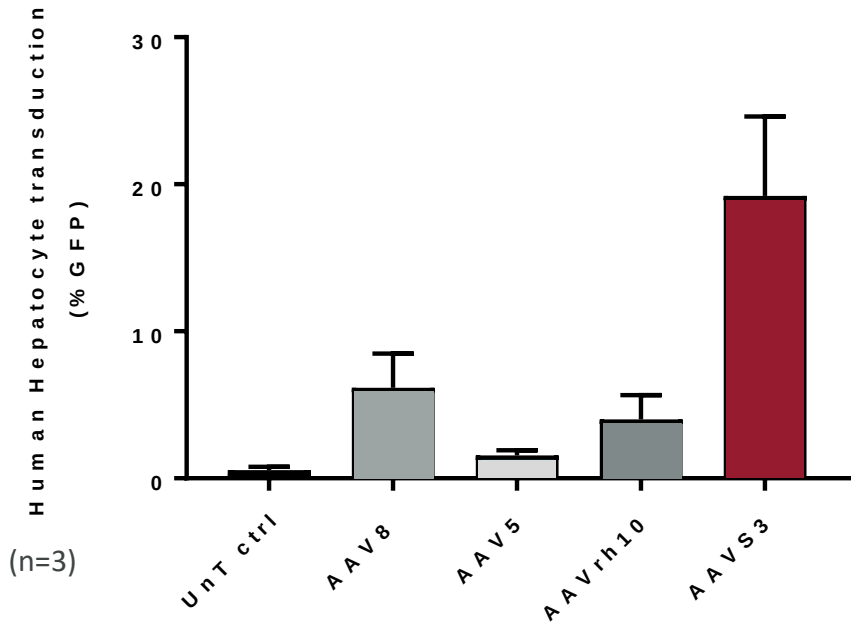
Our rationally designed AAVS3 capsid enables:

- Potent liver transduction
- High protein expression at lower dose levels
- Improved safety margin

AAVS3 capsid is engineered to efficiently transduce human hepatocytes.

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

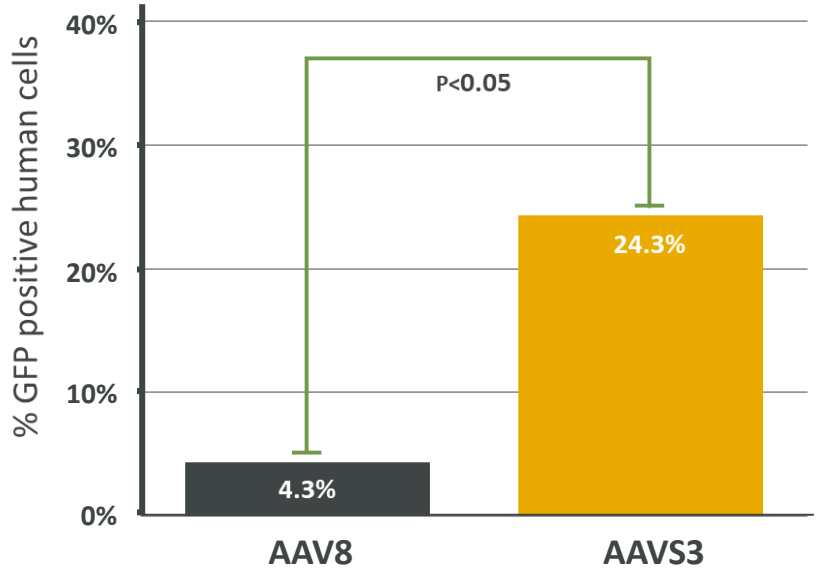
AAVS3 transduction significantly higher compared to other serotypes and untreated control



(n=3)

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

AAVS3 achieved 6-fold higher transduction compared to AAV8



1. Percentage of vectors containing GFP, green fluorescent protein, measured in primary human hepatocytes following transduction. AAVS3 pseudotyped vector used.

2. Number of human hepatocytes expressing GFP following transduction. Measured in a human xenograft mouse model.

Potent AAVS3 capsid and platform drives protein expression

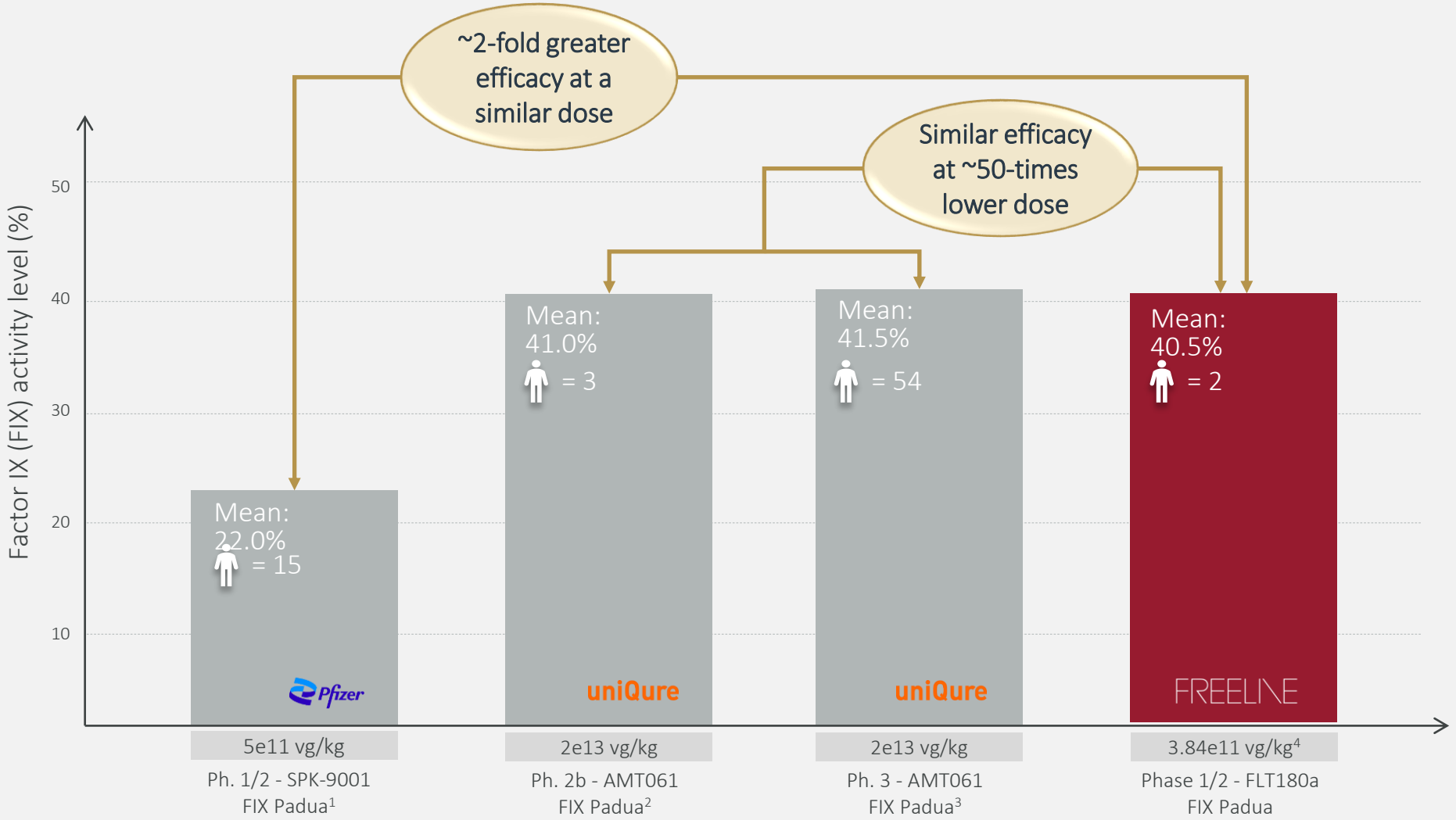
FREELINE

Our rationally designed AAVS3 capsid enables:

- Potent liver transduction
- High protein expression at lower dose levels
- Improved safety margin

Mean 52-week Factor IX Activity Level

Clinical data from Phase 1/2 dose-finding trial of FLT180a demonstrates potency of AAVS3 capsid relative to competitors.



The FLT180a dose listed, 3.84e11, is equivalent to 4.5e11 under the previous equivalent dosing nomenclature.

1. Pfizer R&D Day Sep 2020 – up to four-year follow-up data in 15 patients from Phase 1/2 trial.
2. Miesback et al; Blood 2018 131:1022-1031.
3. uniQure R&D day June 22, 2021 – 12-month follow-up data in 54 patients from the HOPE-B Phase 3 trial.
4. As of the data cut-off date of July 16, 2021 measured using one-stage assay, central laboratory measurement. Two patients dosed at this dose level, with mean value calculated based on following Week 52 FIX activity levels: Patient 1, 45%, Patient 2, 36%.

Product Pipeline: On track to have third program in the clinic by end of 2021

Program	Research[1]	IND enabling studies[2]	Phase 1/2	Phase 3	Patient No. US, UK & EU4[3]	Development & WW Commercial rights[4]
Hemophilia B FLT180a (RMAT designation)					~ 9,000	FREELINE
Fabry FLT190 (Orphan designations)					~ 9,000	FREELINE
Gaucher (Type 1) FLT201 (Orphan designations)					~ 6,000	FREELINE
Hemophilia A FLT210					~ 38,000	FREELINE

1. In the research stage, we conduct *in vitro* and *in vivo* preclinical studies to evaluate different product candidates to select those with the best tolerability and potency profiles.

2. In the IND enabling studies stage, we conduct preclinical *in vivo* studies in disease-specific mouse models and good laboratory practice, or GLP, toxicity studies in non-human primates and generate the CMC information and analytical data required for an investigational new drug, or IND, submission to the FDA for a clinical trial authorization, or CTA, submission to the EMA.

3. These figures represent the total approximate diagnosed population for each indication. The seroprevalence of antibodies against the AAV capsid renders approximately 30-50% of patients currently not eligible for gene therapy.

4. Owned and in-licensed intellectual property rights.

Hemophilia epidemiology: World Federation of Hemophilia 2018.

Fabry Disease epidemiology: Metchler et al 2012; Spada et al 2016; Fabry Register; Fabry Outcome Survey; Waldek et al 2009; Deegan et al 2006.

Gaucher Disease epidemiology: Nalysnyk et al 2016; Weinreb et al 2008 & 2013; Charrow et al 2000; National Gaucher Foundation; Orphanet; NIH Technology Assessment Panel on Gaucher; Poorthuis 1999; Stirnemann et al 2012; Puopetova 2010; Mehta et al 2006.

FREELINE

The Freeline mission:
To be life changers

Hemophilia B

FLT180a (Verbrinacogene setparvovec): Potential to provide a functional cure by normalizing FIX activity levels

FREELINE

Key learnings from the Phase 1/2 B-AMAZE dose-finding clinical trial

- Demonstrated efficacy with FIX activity in the normal range achieved with relatively low vector doses. A dose of 7.7e11 vg/kg is expected to result in consistent FIX levels in the normal range and will be taken forward in the dose-confirmation study (B-LIEVE)
- Stable and durable response up to 3.5 years post-treatment to date
- No spontaneous bleeds that required treatment with supplemental FIX[1]
- Favorable safety profile
- Proactive immune management approach expected to preserve FIX activity levels in the normal range
- Learnings from immune management regimen applied to other programs

1. One patient in the 6.4e11 vg/kg dose cohort (7.5e11 vg/kg under the previous equivalent dosing nomenclature) lost expression due to transaminitis and resumed FIX prophylaxis.

FREELINE

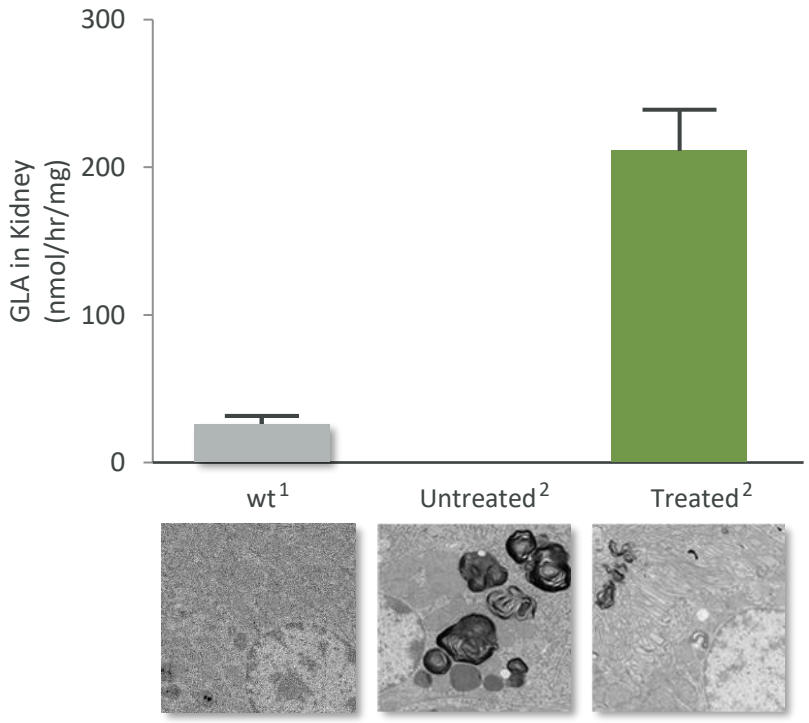
The Freeline mission:
To be life changers

Fabry disease

FLT190 demonstrates increased GLA expression and reduction in pathologic substrate in key tissues in Fabry mouse model

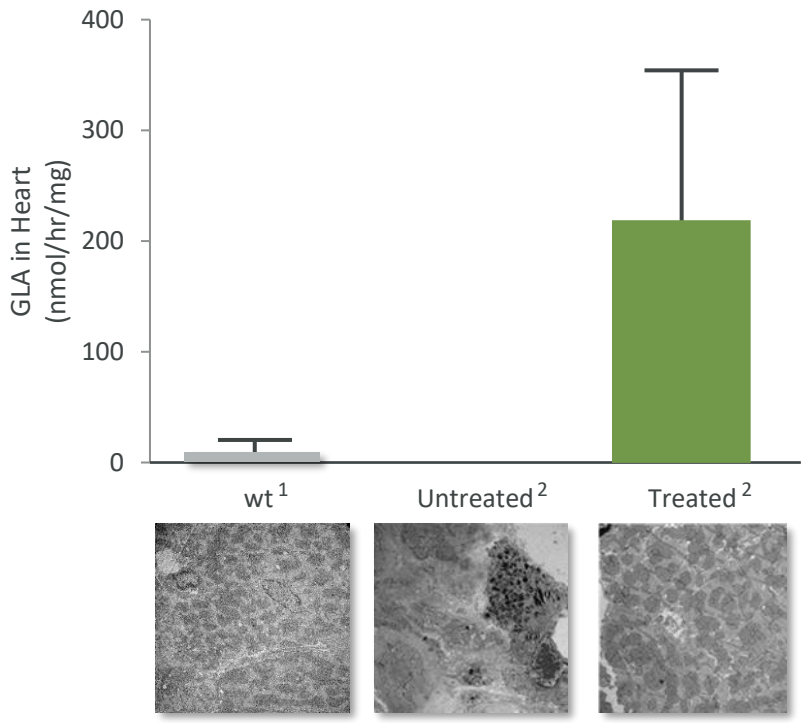
FREELINE

Kidney GLA activity levels



Electron microscopy x5000

Heart GLA activity levels



Electron microscopy x5000

WORLD symposia 2019: Jey Jeyakumar et al. Liver-directed gene therapy corrects Fabry disease in 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).

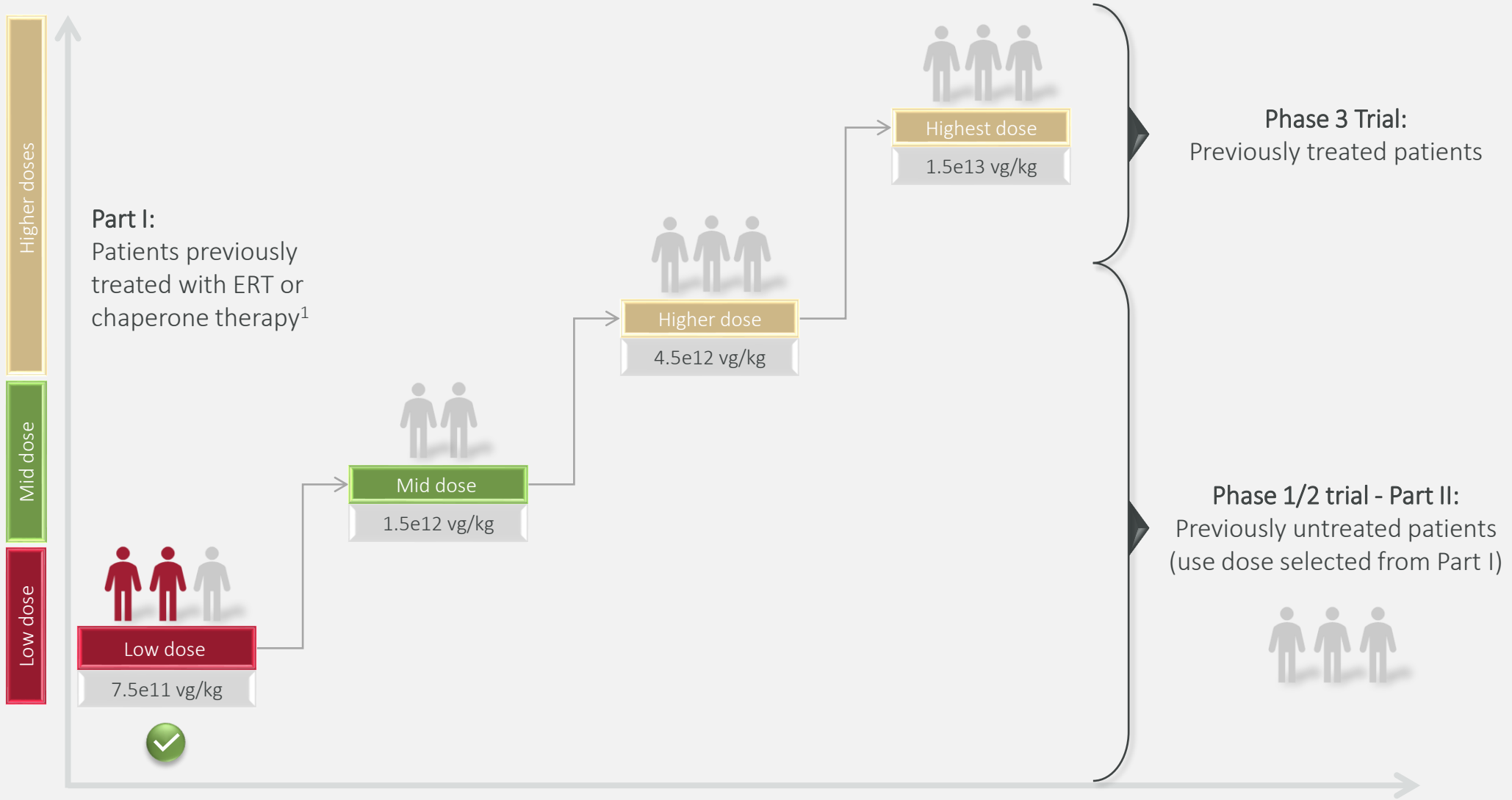
1.Untreated wild-type (non-GLA KO) mice.

2. GLA KO mice.

Phase 1/2 dose-finding trial assessing the safety and efficacy of FLT190 in adult Fabry patients

FREELINE

Adaptive study design

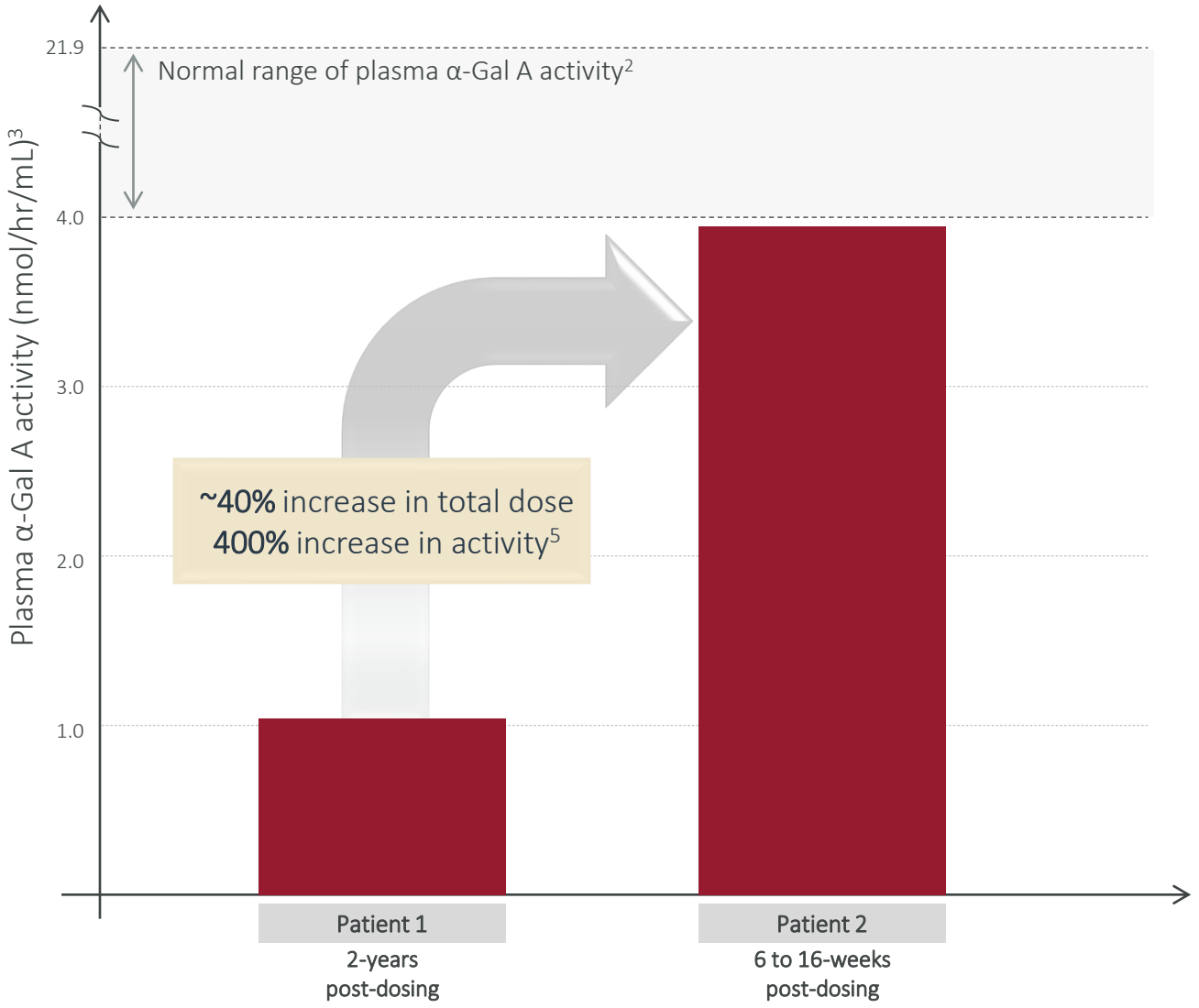


1. ERT= Enzyme Replacement Therapy.

Marks cohort with patients dosed

11

Interim clinical data from first and second patient dosed at the lowest dose cohort with FLT190[1]



Patient 2

- Sustained increase in activity to an average of 3.9 nmol/hr/mL; remains off ERT.
- No rise in ALT/AST levels; received optimized immune management regimen.
- Treatment well-tolerated with no SAEs.
- Transient increase in troponin-T has returned to baseline.

Patient 1

- Remains at elevated activity two years post-dose and generally steady at an average of three times baseline.[4]
- Experienced no enduring clinical sequelae of the transient mild myocarditis episode previously reported in 2019.

1. As of the data cut-off date of October 6, 2021.
2. Assay normal range: 4.0-21.9 nmol/hr/mL.
3. α-Gal A: Plasma α-galactosidase A, the missing enzyme in Fabry disease.
4. Patient 1 had a subtherapeutic response with plasma α-Gal A at 0.8-1.3 nmol/hr/mL.
5. The total vector genome (vg) dose Patient 2 received was approximately 40% higher than Patient 1 due to differences in their weights.

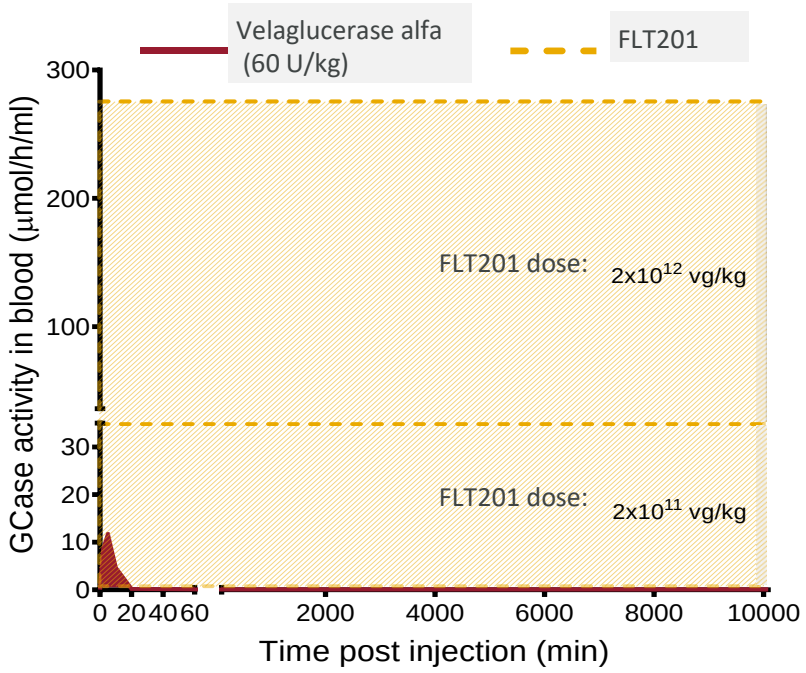
FREELINE

Gaucher disease

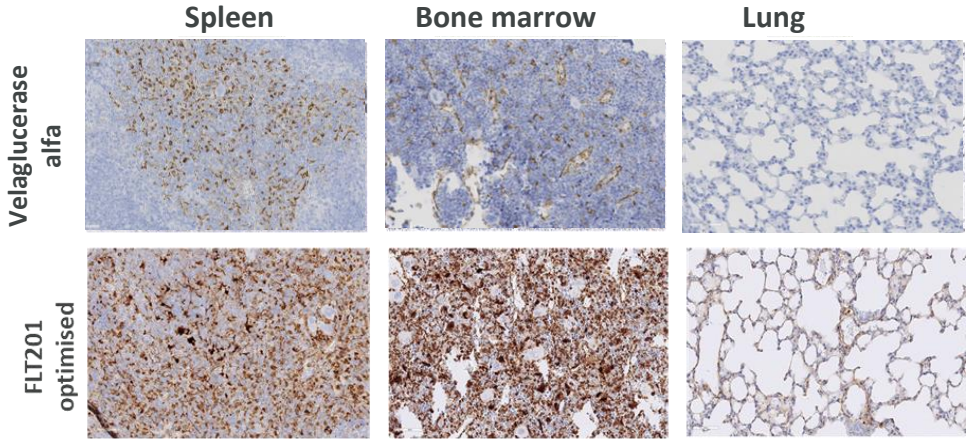
Low doses of FLT201 in Gaucher mice result in higher expression and increased uptake in tissues affected by Gaucher Type 1 disease

- Novel GCase variant produced by FLT201 is more stable in plasma than wild-type protein
- This leads to a greater than 20-fold increase in potency vs. wild-type protein and better substrate clearance in key tissues in Gaucher mice

Increased protein stability compared with velaglucerase alpha



Enhanced and sustained GCase uptake observed in key tissues as compared with velaglucerase alpha

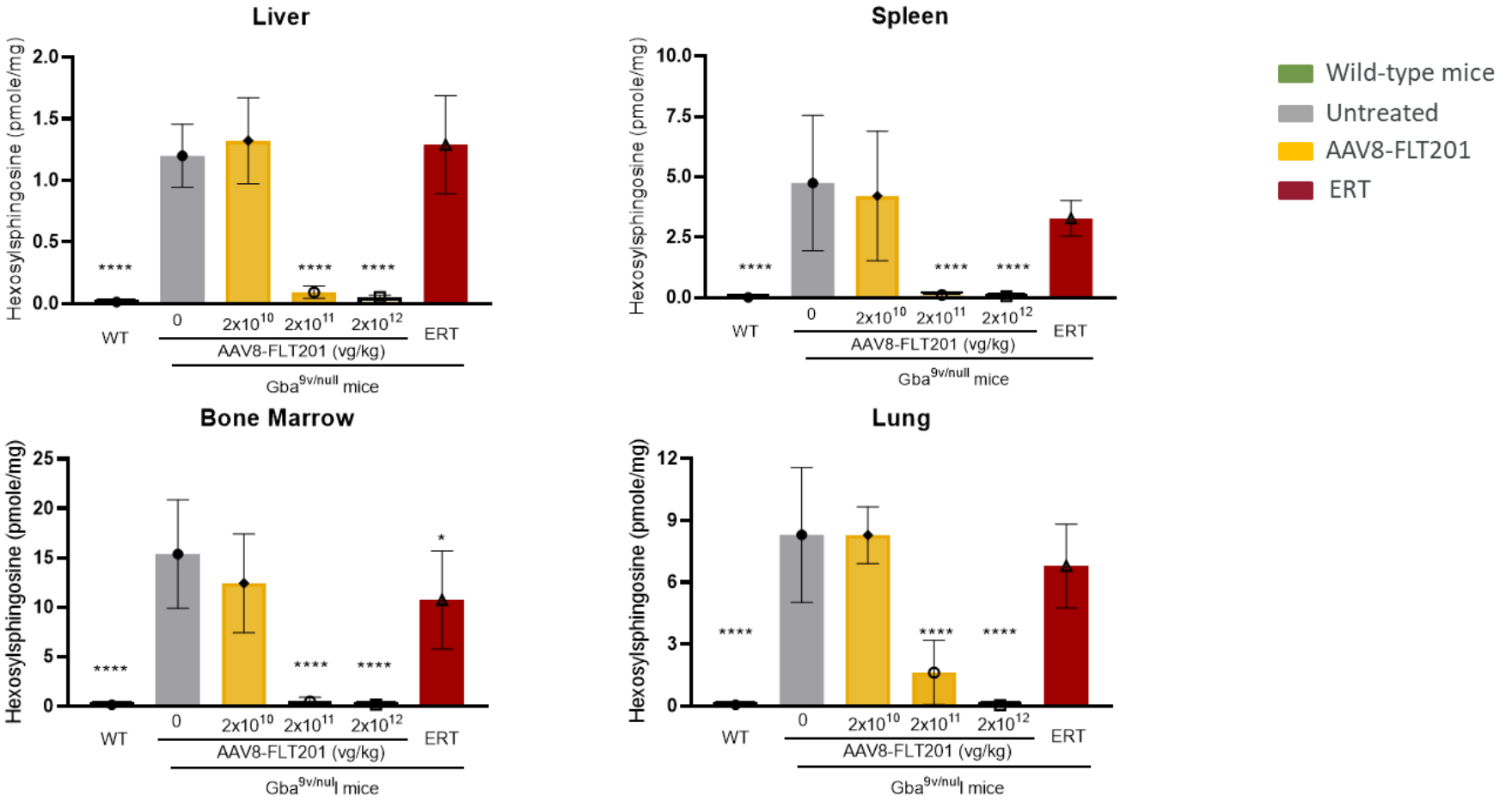


Anti-GCase – DAB, Haematoxylin counterstain, AAV8

Velaglucerase alfa is an Enzyme Replacement Therapy (ERT) for Gaucher disease.

FLT201 achieves GCase tissue penetration, enzymatic activity and substrate clearance in hard-to-reach tissues

- Restoration of GCase activity after FLT201 injection was observed in Gba-deficient mice in difficult-to-reach tissues as shown by decreased levels of lyso-GB1 substrate[1]
- Dose-dependent reductions of lyso-GB1 were observed in all tissues analyzed including bone marrow and lung



Data from study conducted in collaboration with Professor Ying Sun (Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA) and presented at the 17th Annual WORLDSymposium.

Velaglucerase alpha - an ERT used for Gaucher disease.

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.

1. Lack of GCase enzyme in humans leads to the accumulation of lyso-GB1 and Gaucher disease.

*p ≤ 0.0001.

FREELINE

The Freeline mission:
To be life changers

Milestones

Data rich 2022 – on track to demonstrate product value

FREELINE

2021-2022 Objectives

- **Gaucher disease Type 1** – Phase 1/2 dose-finding trial site initiation by year-end 2021; data expected in the middle of 2022
- **Hemophilia B** – Present long-term durability data from Phase 1/2 B-AMAZE dose-finding clinical trial by year end 2021
- **Hemophilia B** – Initiate Phase 1/2 B-LIEVE dose-confirmation trial in first quarter 2022; data expected in the middle of 2022
- **Fabry disease** – Progress Phase 1/2 MARVEL-1 dose-finding clinical trial with third patient dosed in first quarter 2022; data expected in the middle and end of 2022


FREELINE
Thank you