A 3-month gene therapy single-dose IV administration pharmacology and safety study with ST-920 (isaralgagene civaparvovec) for Fabry disease in mice

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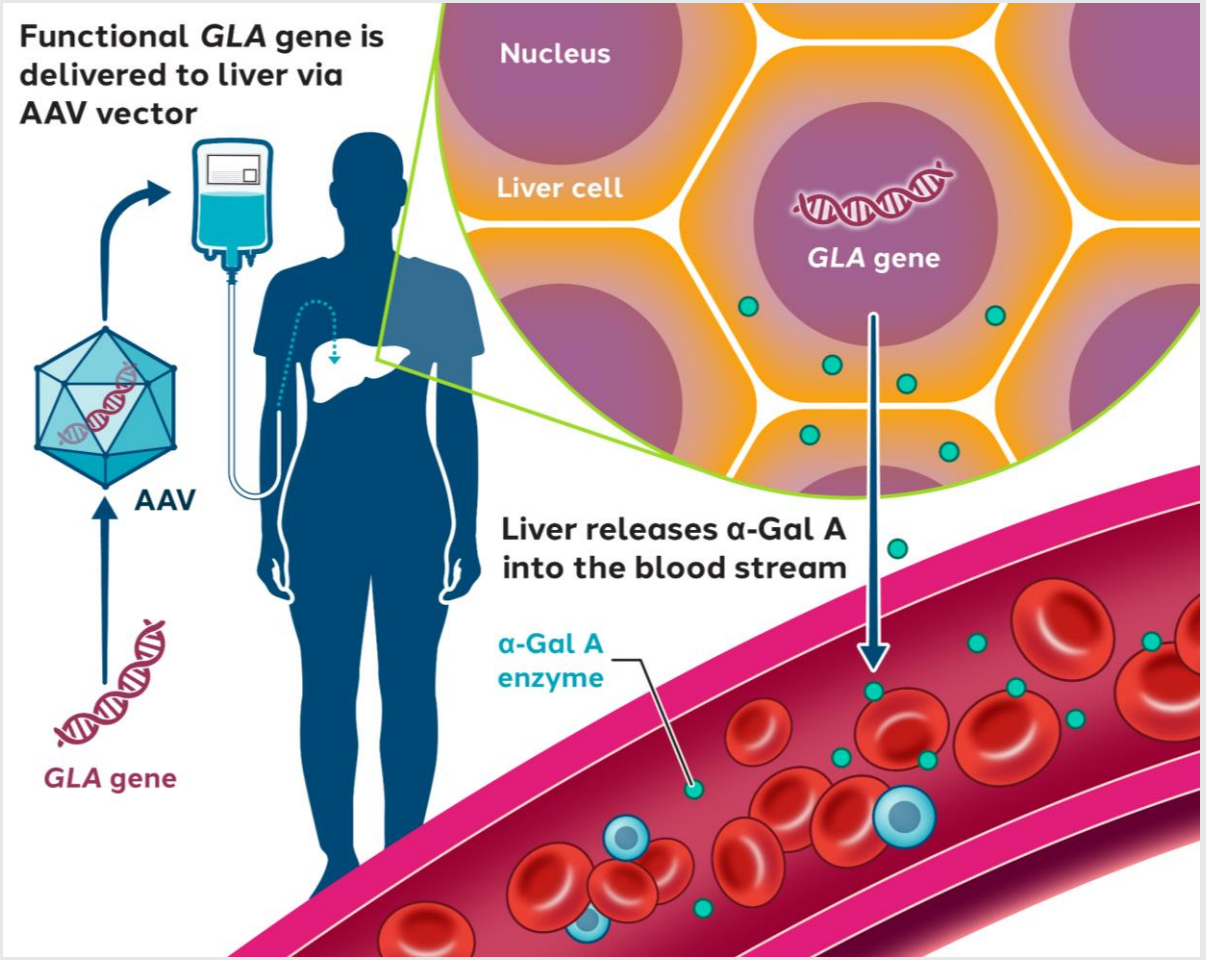
Abstract No. 224

ST-920 Employs a Recombinant AAV2/6 Vector with Human *GLA* cDNA for Continuous, Liver-specific α-Gal A Expression



Advantages

- Convenient one-time administration
- Potential to eliminate ERT infusions
- Durable efficacy
- Low immunogenicity
- No prophylaxis with steroids or other immunomodulating agents required

Design of Pharmacology & Toxicology Studies Supporting Clinical Use of New Therapy

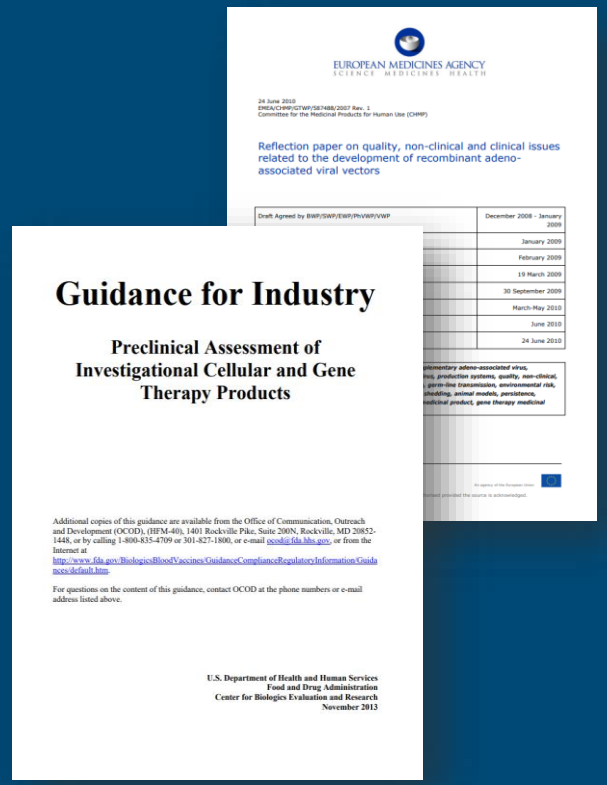
The design and conduct of preclinical pharmacological and toxicological studies:

- Inform regulatory decisions that help define safe administration of an investigational CGT product in humans

Elements and design of preclinical testing based on:

- Specific product characteristics
- Putative mechanism of action (MOA)
- Target disease indication
- Method of product delivery

Primary Objectives of Pharmacology/ Toxicology Studies Supporting Cell and Gene Therapy Development



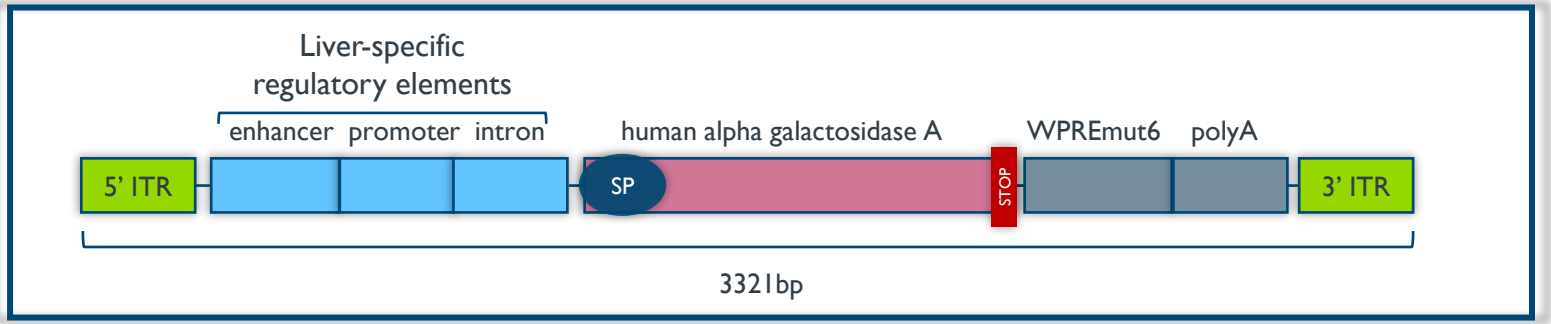
Objectives of Pharmacology & Toxicology Studies

- Establishment of biological plausibility
- Identification of biologically active dose levels
- Establishment of feasibility and reasonable safety of the investigational product's proposed clinical route of administration
- Selection of potential starting dose level, dose escalation schedule and dosing regimen for clinical studies
- Support of patient eligibility criteria
- Identification of physiologic parameters that can guide clinical monitoring
- Identification of potential public health risks (general public, caregivers, family members, close contacts and intimate contacts)

FDA Guidance Document: Preclinical Assessment of Investigational Cellular and Gene Therapy Products 2013

ST-920 Vector Construct

ST-920 - isaralgagene civaparvovec



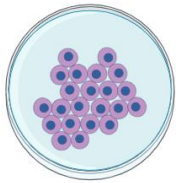
- AAV2 ITR & AAV6 capsid
- Liver-specific promoter elements
  - Enhancer and hepatic control region from human apolipoprotein E (ApoE) gene and human α-1-antitrypsin (hAAT) promoter
  - A modified chimeric intron (HBB-IgG) added to increase transgene expression
- Human codon optimized *GLA* cDNA transgene, including the native *GLA* signal peptide. Same amino acid sequence as endogenous α-Gal A and agalsidase beta
- WPREmut6 element to enhance transcription and protein expression
- Poly A sequence derived from bovine growth hormone polyadenylation signal

ST-920 Preclinical Program: Pharmacology

- Characterize pharmacology profile of new therapy
- Demonstrate that investigation therapy has the pharmacological activity needed to advance in development

In vitro studies

- Transduction of mouse, nonhuman primate and human primary hepatocytes, hepatocyte cell lines, and/or iPSC hepatocytes



  - Production and demonstration of enzymatic activity of α-Gal A
  - Characterization of α-Gal A glycosylation pattern, mannose-6-phosphate receptor uptake

In vivo studies

- Studies in transgenic mouse models of Fabry disease (GLAKO and GLAKO/Gb3)

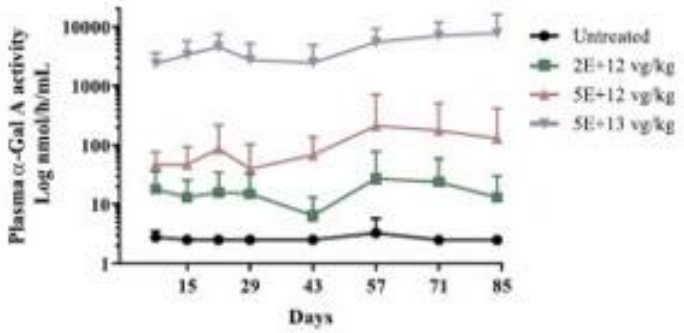
  - Plasma and tissue levels of α-Gal A and reduction of tissue lyso-Gb3
  - Relationship between dose, plasma and tissue α-Gal A and substrate reduction
  - Safety assessment

ST-920 Gene Therapy is Produced in Liver and Pharmacologically Effective in Affected Tissues in Fabry Mouse Models

3-Month GLAKO Mouse Study Results

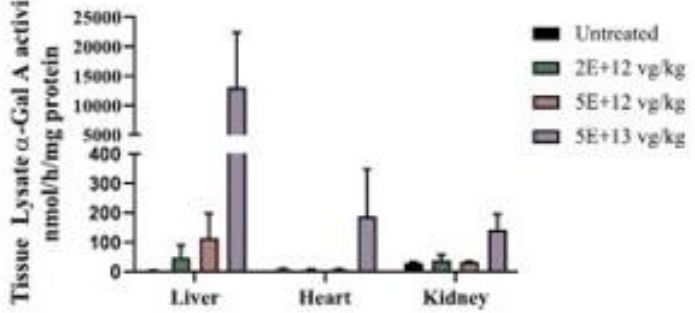
D

Plasma α-Gal A activity



E

Liver α-Gal A activity



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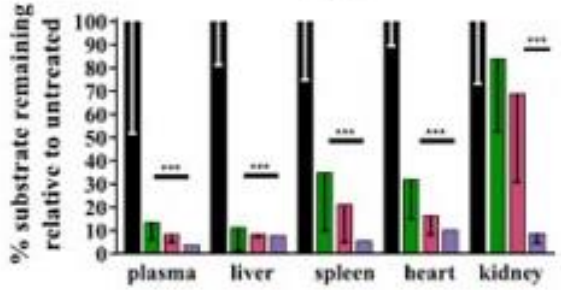
Tissue Gb3



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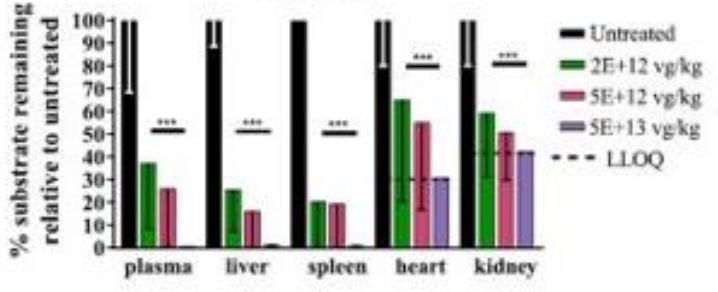
Tissue Lyso Gb3



Yasuda (2020) Molecular Therapy: Methods and Clinical Development 18: 607-619

ST-920 Program Toxicology

- Continue to characterize new therapy
- Demonstrate safety at anticipated dose range to test in patients
- Determine safety margin, clinical starting dose and dose-escalation plan

Toxicology Studies

- Non-GLP 3-month study with ST-920PC* in GLAKO mice
- GLP 1-month studies with ST-920 and ST-920PC in C57BL/6 mouse
- **GLP 3-month study ST-920 in C57BL/6 mouse**
- GLP 2-month study ST-920PC in cynomolgus monkey

Endpoints

- Clinical observations, body weight, body weight gain
- AAV6 toxicokinetics
- Plasma and liver α-Gal A activity
- Liver α-Gal A mRNA
- AAV6 vector biodistribution and shedding
- Hematology, clinical chemistry, urinalysis
- Macroscopic and microscopic pathology

*ST-920PC is parent construct of ST-920 which lacks the MPREmut6 element

GLP 3-Month Mouse Toxicology Study

Study Objectives:

Characterize ST-920 pharmacology, toxicokinetics, biodistribution and safety

ST-920 3-Month Study Group Designations

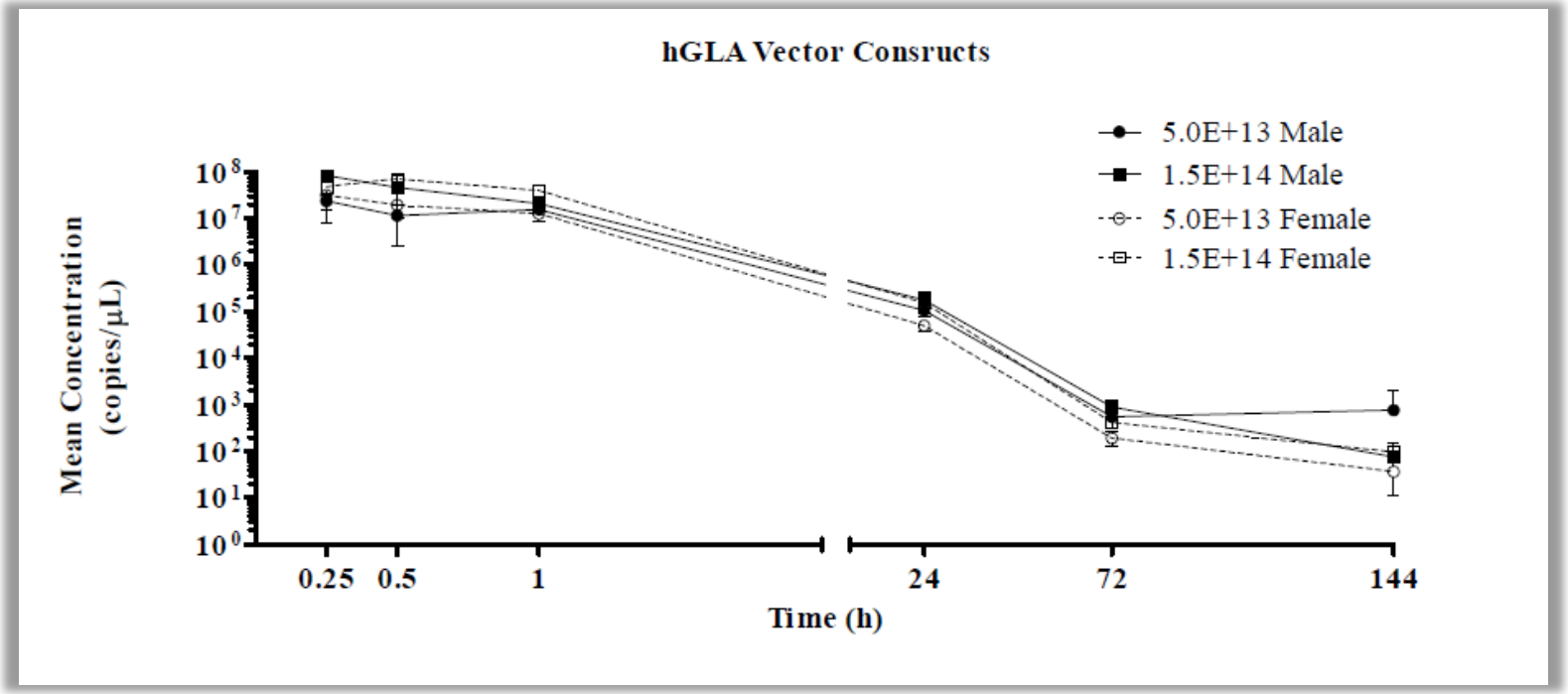
Group No.	Test Material	AAV Dose[a] (vg/mouse)	AAV Dose[a] (vg/kg)	No. of Animals			
				Main Study Cohort		TK/BD Study Cohort	
				Males	Females	Males	Females
1	Vehicle Control	0	0	10	10	5	5
2	ST-920 (Mid dose)	1.25E+12	5.0E+13	10	10	12	12
3	ST-920 (High dose)	3.75E+12	1.5E+14	10	10	12	12

- Male and female C57BL/6 mice
- Single, one-time ST-920 administered IV via tail vein (200 µL)
- Vehicle control and ST-920
  - 5.0E+13 vg/kg
  - 1.5E+14 vg/kg

ST-920 Toxicokinetics

- Blood collections 0.25, 0.5, 1, 24, 72 and 144 hours post dose
- Dose related increase in AUC(0-t) and Cmax; similar between males and females
- AUC(0-t) exposure increased in a less than dose-proportional manner in males and a dose-proportional manner in females
- Half-life : ~7 hours

ST-920 Toxicokinetic Profile in Mice

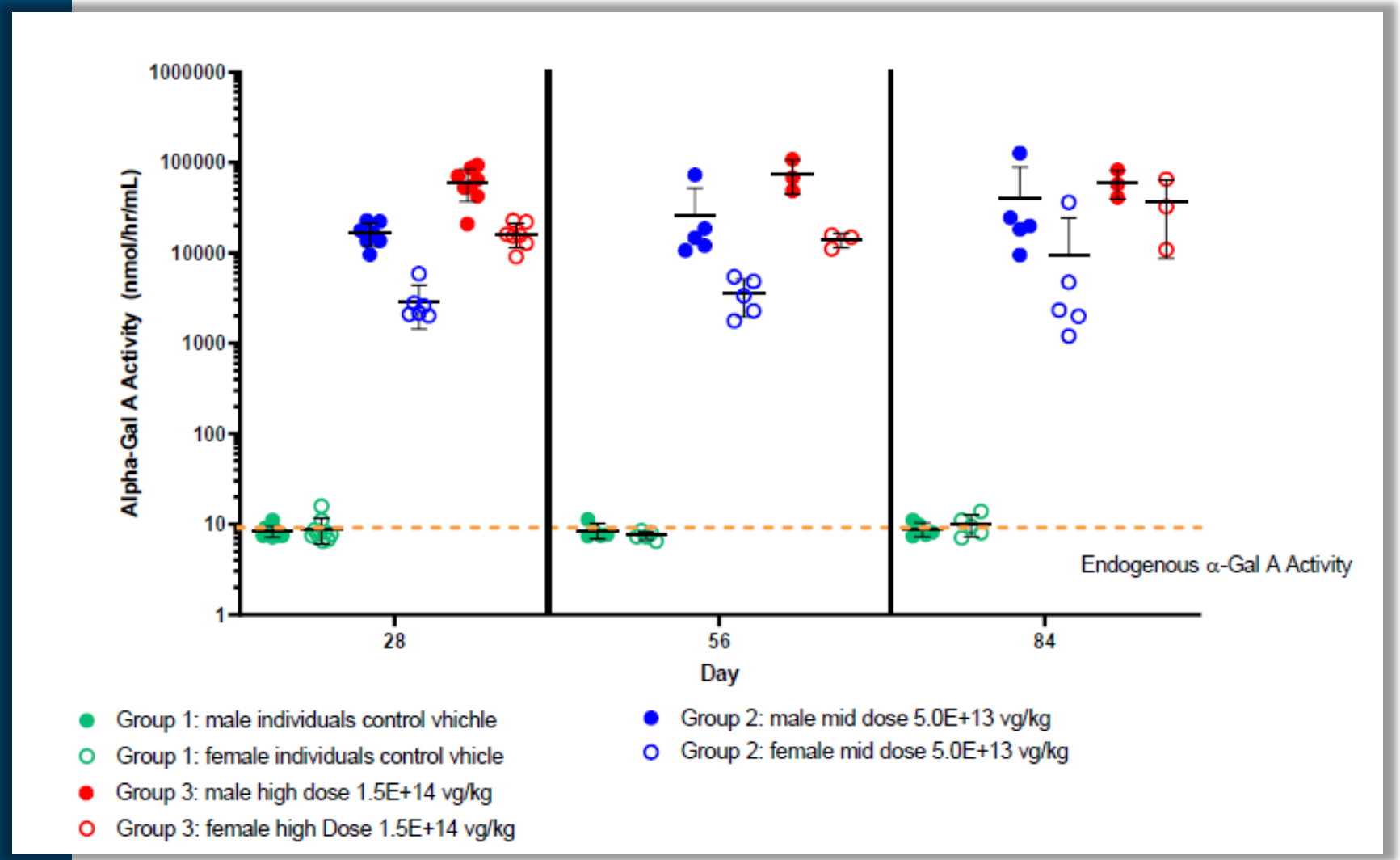


Dose (vg/kg)	Gender	AUC(0-t) (h*copies/µL)	AUC(0-t)/D	C0 (copies/ µL)	Cmax (copies/ µL)	Tmax (h)	Tlast (h)	CL (µL/h/kg)	Vd (µL/kg)	$T_{1/2}$ (h)
5.0E+13	Male	203,000,000	0.00000406	47,300,000	23,300,000	0.25	144	NR	NR	NR
1.5E+14	Male	307,000,000	0.00000205	151,000,000	83,100,000	0.25	144	488,000	5,030,000	7.14
5.0E+13	Female	170,000,000	0.00000341	51,500,000	31,300,000	0.25	144	293,000	3,000,000	7.10
1.5E+14	Female	510,000,000	0.00000340	47,500,000	70,100,000	0.50	144	294,000	3,110,000	7.34

Supraphysiological Plasma α-Gal A Activity in Male and Female Mice

- Males : **4,100** x higher than normal wild-type plasma enzyme activity
- Females : **2,200** x higher than normal wild-type plasma enzyme activity

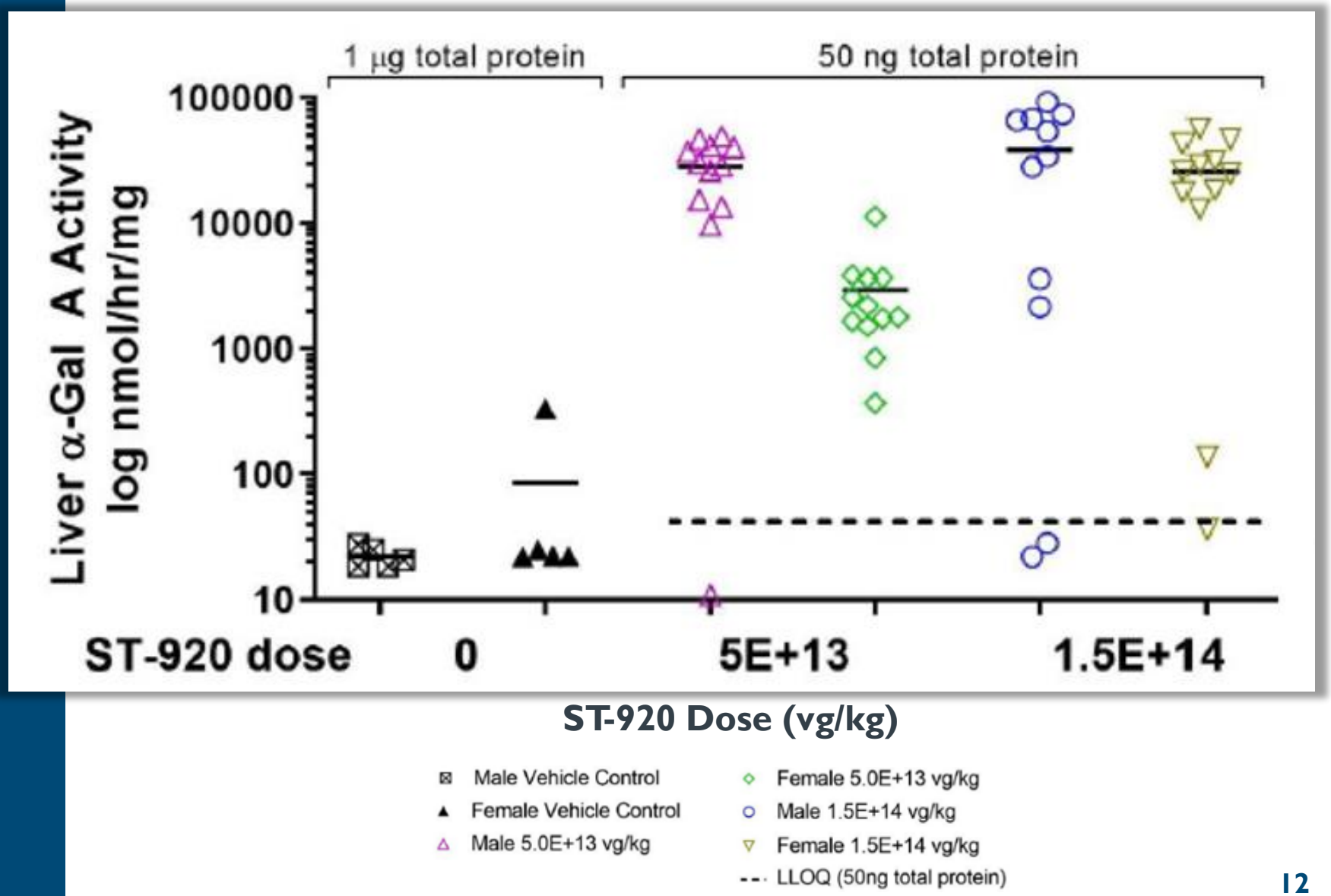
Plasma α-Gal A Activity in Mice



Supraphysiological Liver α-Gal A Activity in Male and Female Mice

- Males : **1,800** x higher than normal wild-type liver α-Gal A enzyme activity
- Females : **1,300** x higher than normal wild-type α-Gal A enzyme activity

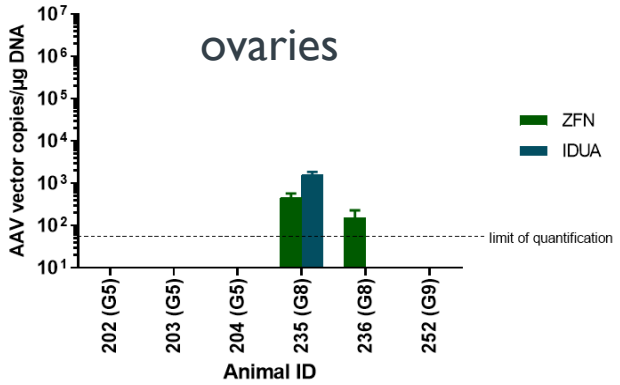
Liver α-Gal A Activity in Mice



AAV6 Vector Biodistribution Shows Highest Levels in Liver, Much Lower Levels in Other Tissues, and No Detectable Levels in Semen

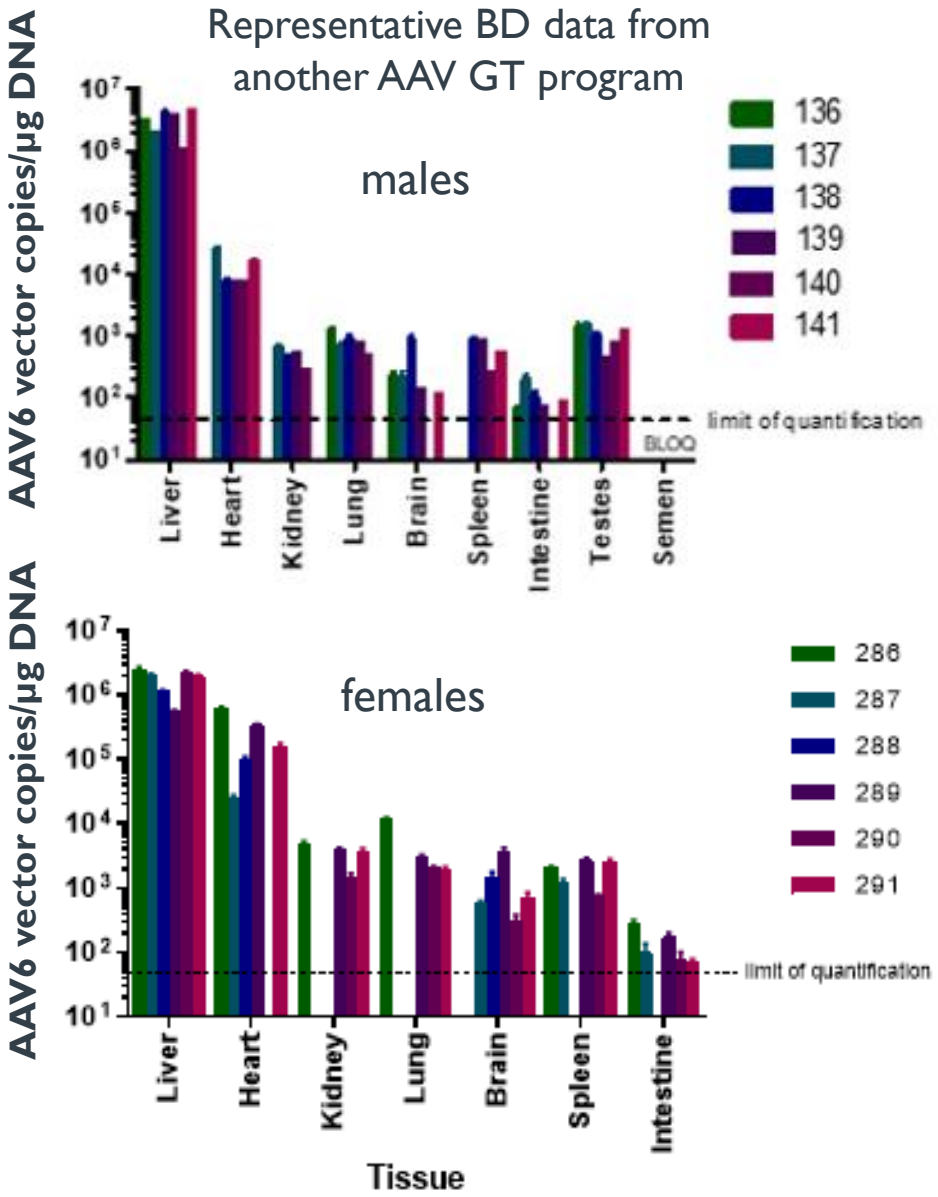
AAV6 biodistribution data in mice

- Highest average AAV6 vector copy levels were detected in liver
- Ovaries/testes vector copies were 1,000- to 10,000-fold lower than liver



ST-920 shedding data in mice

- Urine and semen – no measurable copies
- Feces – no measurable copies except 5 samples collected prior to Day 29 (study end)



GLP 3-month Study Demonstrated ST-920 Robust Safety Profile

No ST-920- or ST-920PC-related effects on:

- Morbidity or mortality
- Clinical observations
- Body weight and body weight gain
- Serum chemistry, hematology and urinalysis
- Organ weights and gross pathology
- Microscopic pathology

The ST-920 no-observed-adverse-effect level (NOAEL) was ≥ 1.5e14 vg/kg, the highest dose tested in mice

Supports Phase 1/2 and Phase 3 clinical dosing

- 3-fold safety margin based on vector dose compared to highest clinical dose (5E+13 vg/kg)
- Safety demonstrated ~2,200x - 4,100x higher plasma α-Gal A enzyme activity vs endogenous activity

ST-920 Nonclinical Program Supports Phase 1/2 and Phase 3 Studies

Key Findings

- Proof-of-concept (substrate clearance in plasma and peripheral tissues) demonstrated in Fabry mouse models
- Supraphysiological levels of α-Gal A activity observed in GLP 3-month mouse study (up to ~4,100x of background in male and ~2,200x background in female mice)
- No ST-920 and/or ST-920PC related safety findings at IV doses up to 1.5E+14 vg/kg in mice and 6E+13 vg/kg in monkeys
- NOAEL is the highest dose tested in mice, ≥1.5E+14 vg/kg, and ≥ 6E+13 vg/kg in cynomolgus monkeys

Overall Summary

- Nonclinical package supports initiation of STAAR Phase 1/2 and Phase 3 clinical studies

ST-920 Posters and Presentations

- **Isaralgagene civaparvovec (ST-920) gene therapy in adults with Fabry disease: Updated results from an ongoing Phase 1/2 study (STAAR)**

  Presenter: Robert J Hopkin, Cincinnati Children's Hospital

- **Anti-AAV6 antibody assay for patient enrollment supporting ST-920 phase 1/2 study for Fabry disease**

  Presenter: Liching Cao, Sangamo Therapeutics

- **A 3-month gene therapy single-dose IV administration pharmacology and safety study with ST-920 (isaralgagene civaparvovec) for Fabry disease in mice**

  Presenter: Kathleen Meyer, Sangamo Therapeutics

Acknowledgements

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