

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

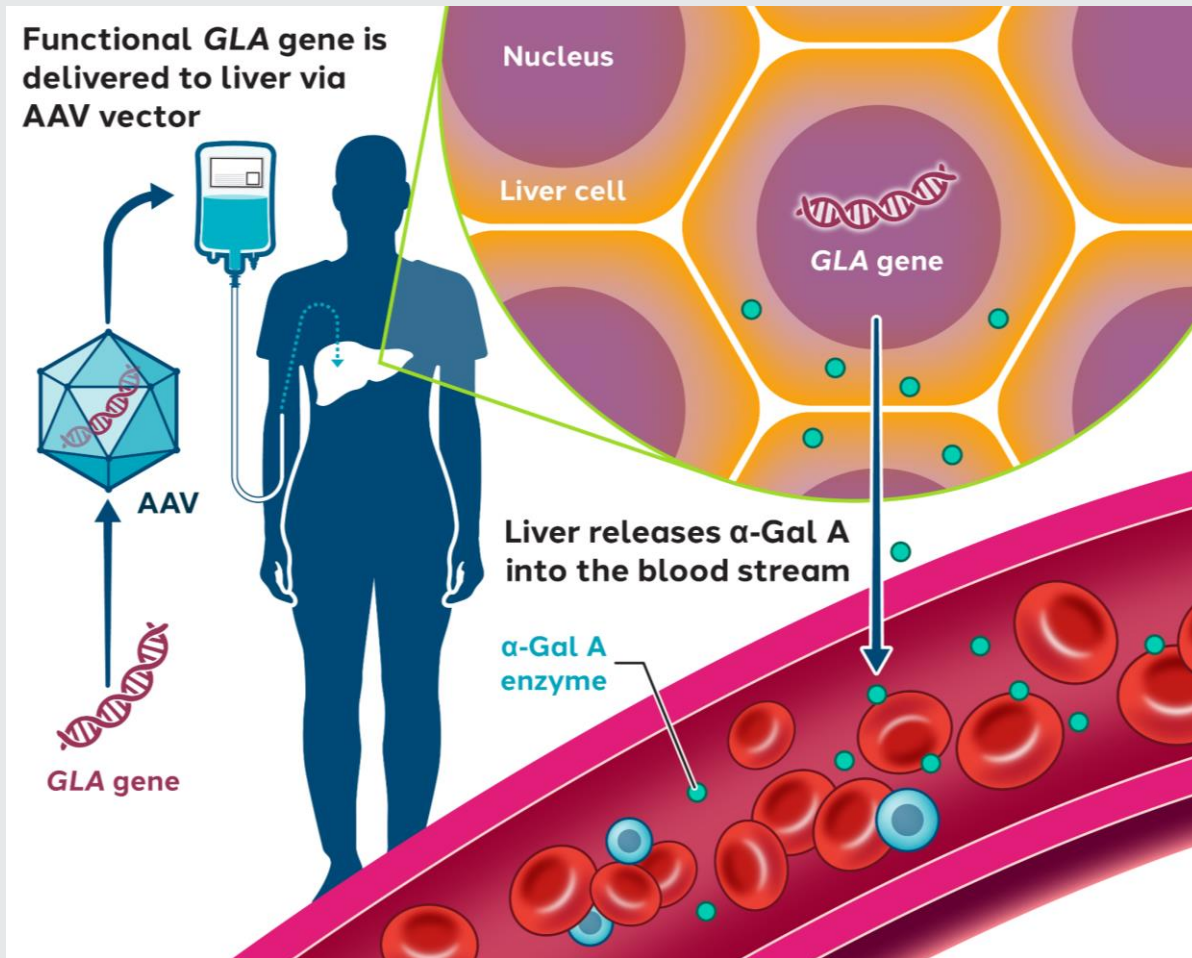
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Abstract No. 224



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

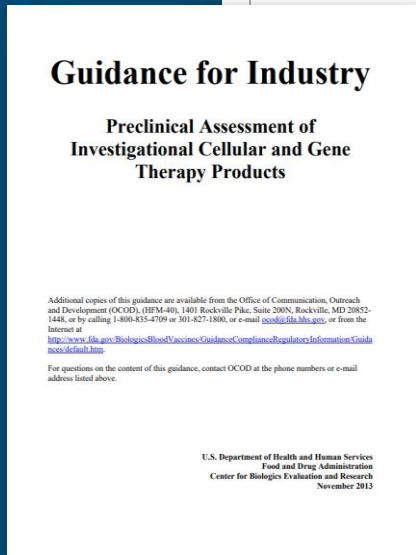
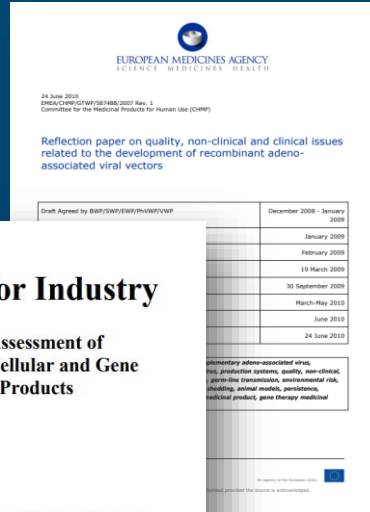
Elements and design of preclinical testing based on:

- 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)

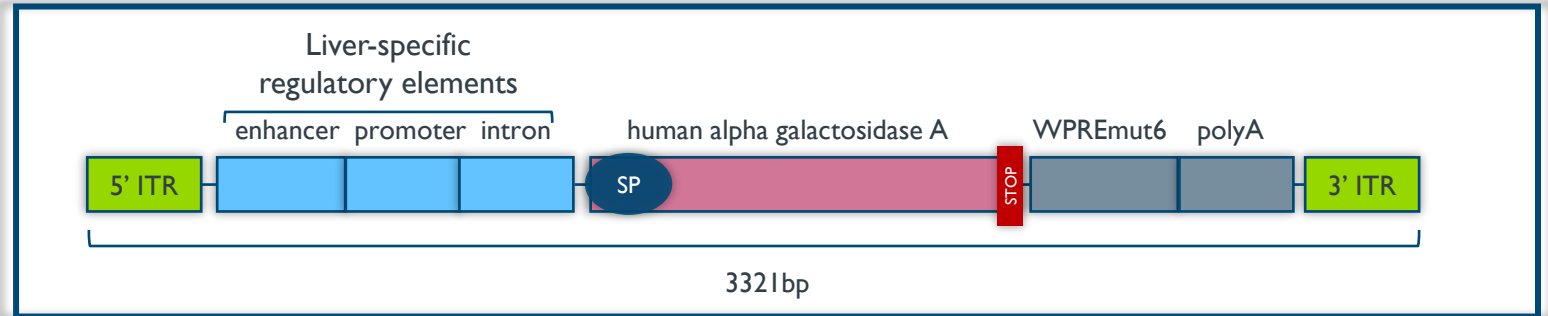


FDA Guidance Document: Preclinical Assessment of Investigational Cellular and Gene Therapy Products 2013

ST-920 Vector Construct



ST-920 - isaralgagene civaparvovec

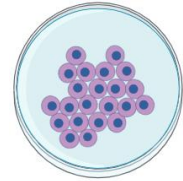


- AAV2 ITR & AAV6 capsid
- Liver-specific promoter elements
 - Enhancer and hepatic control region from human apolipoprotein E (ApoE) gene and human α -1-antitrypsin (hAAT) promoter
 - A modified chimeric intron (HBB-IgG) added to increase transgene expression
- Human codon optimized *GLA* cDNA transgene, 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

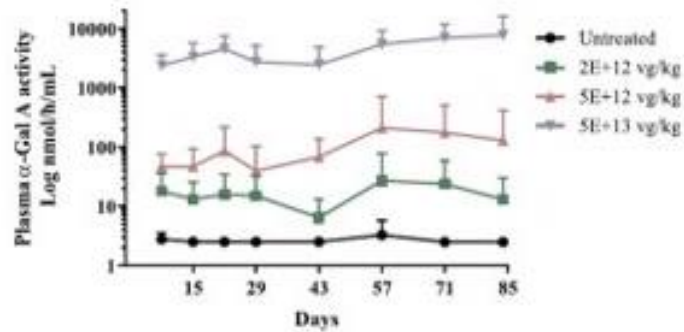


- Studies in transgenic mouse models of Fabry disease (GLAKO and GLAKO/Gb3)
- Plasma and tissue levels of α -Gal A and reduction of tissue lyso-Gb3
- Relationship between dose, plasma and tissue α -Gal A and substrate reduction
- Safety assessment

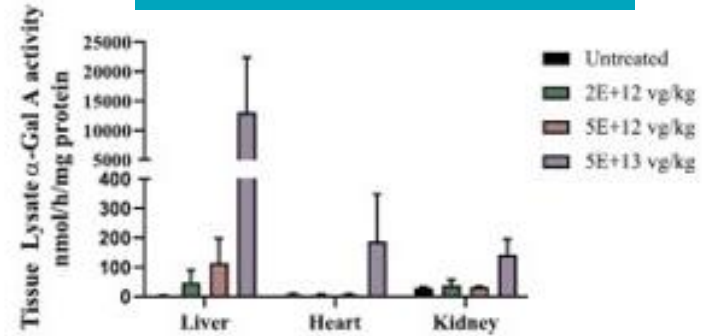
ST-920 Gene Therapy is Produced in Liver and Pharmacologically Effective in Affected Tissues in Fabry Mouse Models

3-Month GLAKO Mouse Study Results

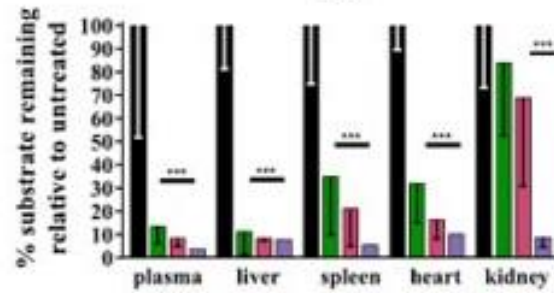
D Plasma α -Gal A activity



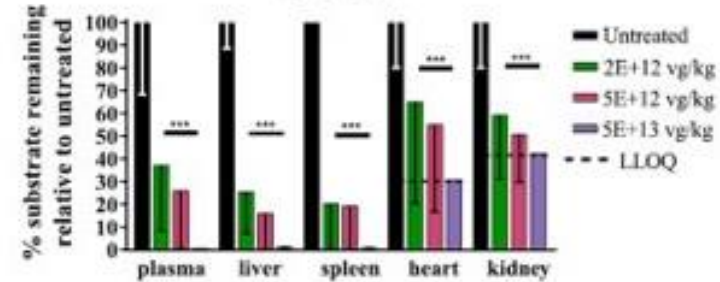
E Liver α -Gal A activity



F Tissue Gb3



G Tissue Lyso Gb3



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

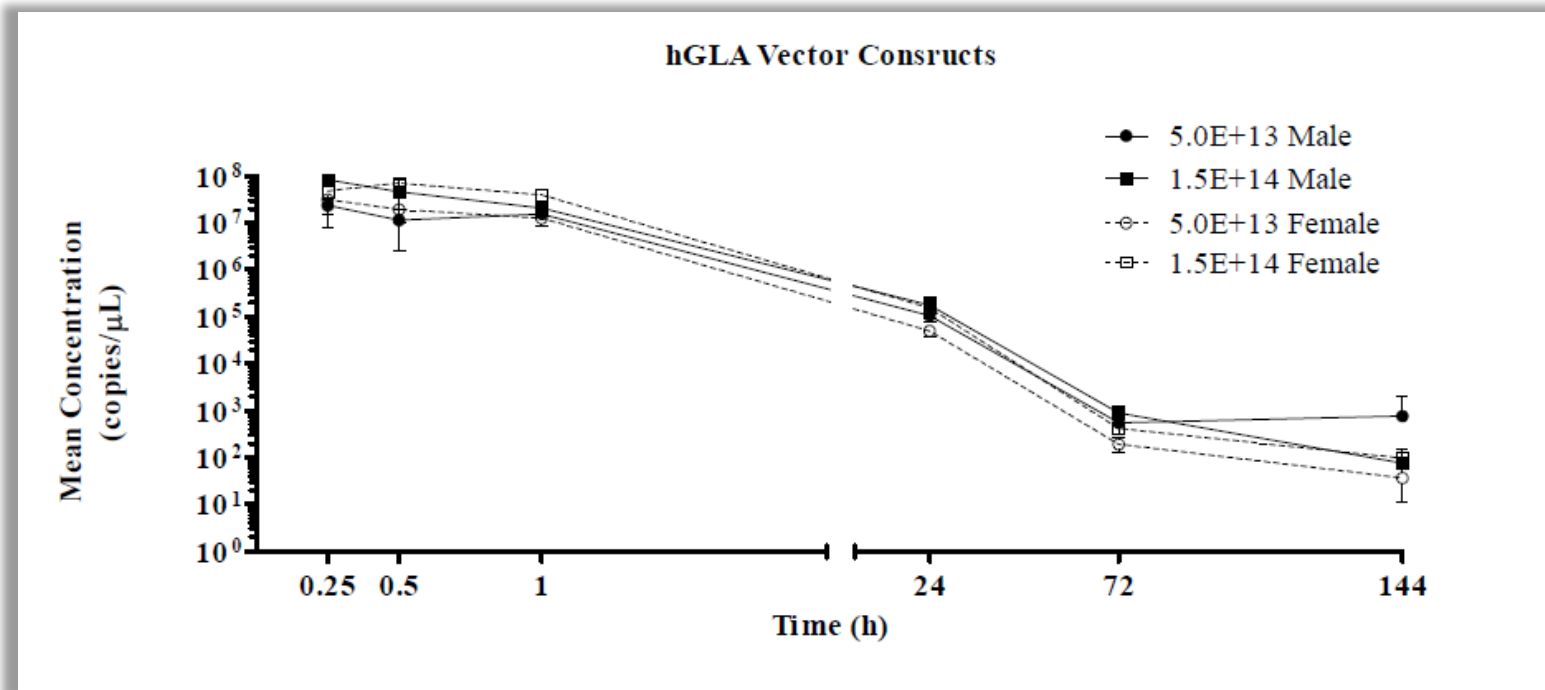
Group No.	Test Material	AAV Dose ^a (vg/mouse)	AAV Dose ^a (vg/kg)	No. of Animals			
				Main Study Cohort		TK/BD Study Cohort	
				Males	Females	Males	Females
1	Vehicle Control	0	0	10	10	5	5
2	ST-920 (Mid dose)	1.25E+12	5.0E+13	10	10	12	12
3	ST-920 (High dose)	3.75E+12	1.5E+14	10	10	12	12

- 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

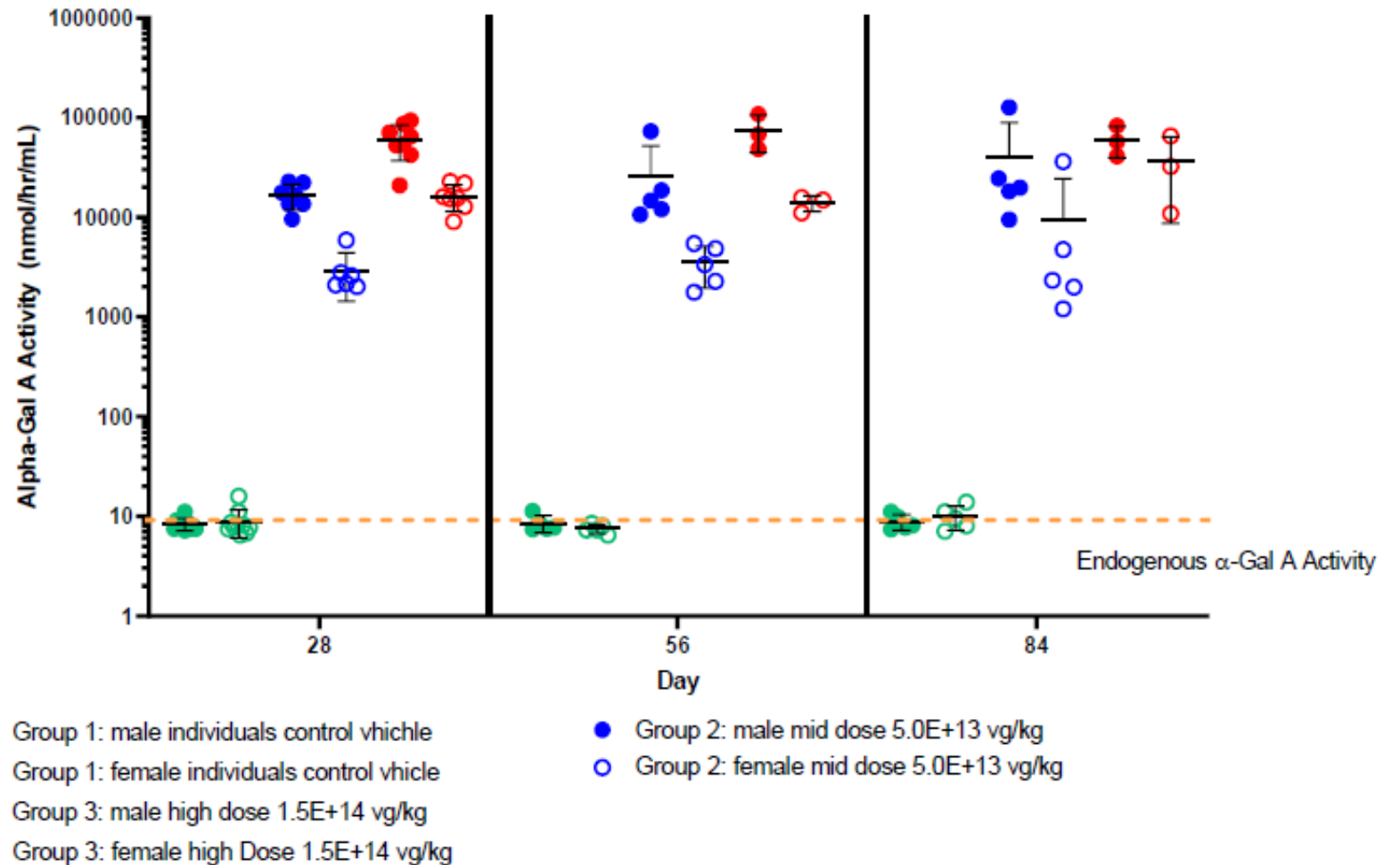


Dose (vg/kg)	Gender	AUC(0-t) (h*copies/μL)	AUC(0-t)/D	C0 (copies/ μL)	Cmax (copies/ μL)	Tmax (h)	Tlast (h)	CL (μL/h/kg)	Vd (μL/kg)	T _{1/2} (h)
5.0E+13	Male	203,000,000	0.00000406	47,300,000	23,300,000	0.25	144	NR	NR	NR
1.5E+14	Male	307,000,000	0.00000205	151,000,000	83,100,000	0.25	144	488,000	5,030,000	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

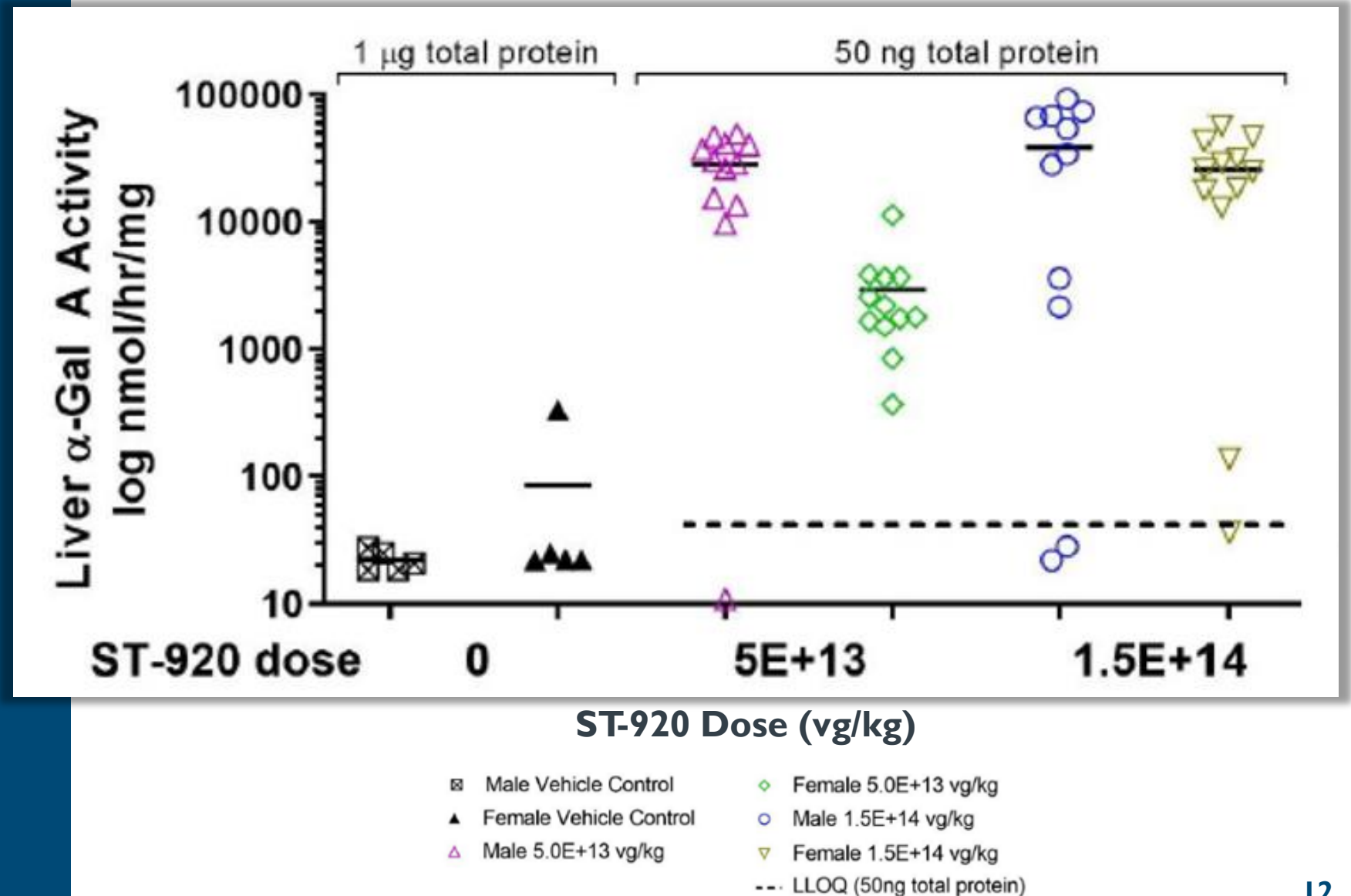
Plasma α -Gal A Activity in Mice



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

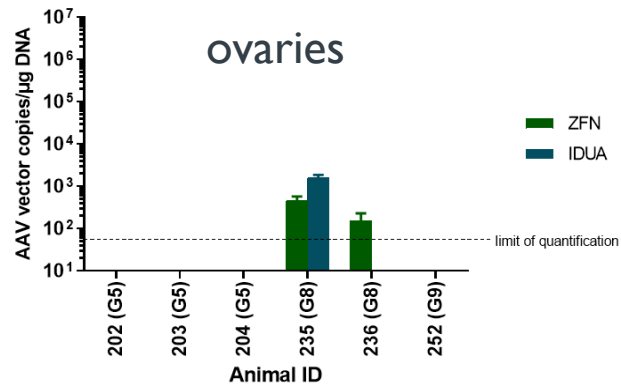
Liver α -Gal A Activity in Mice



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

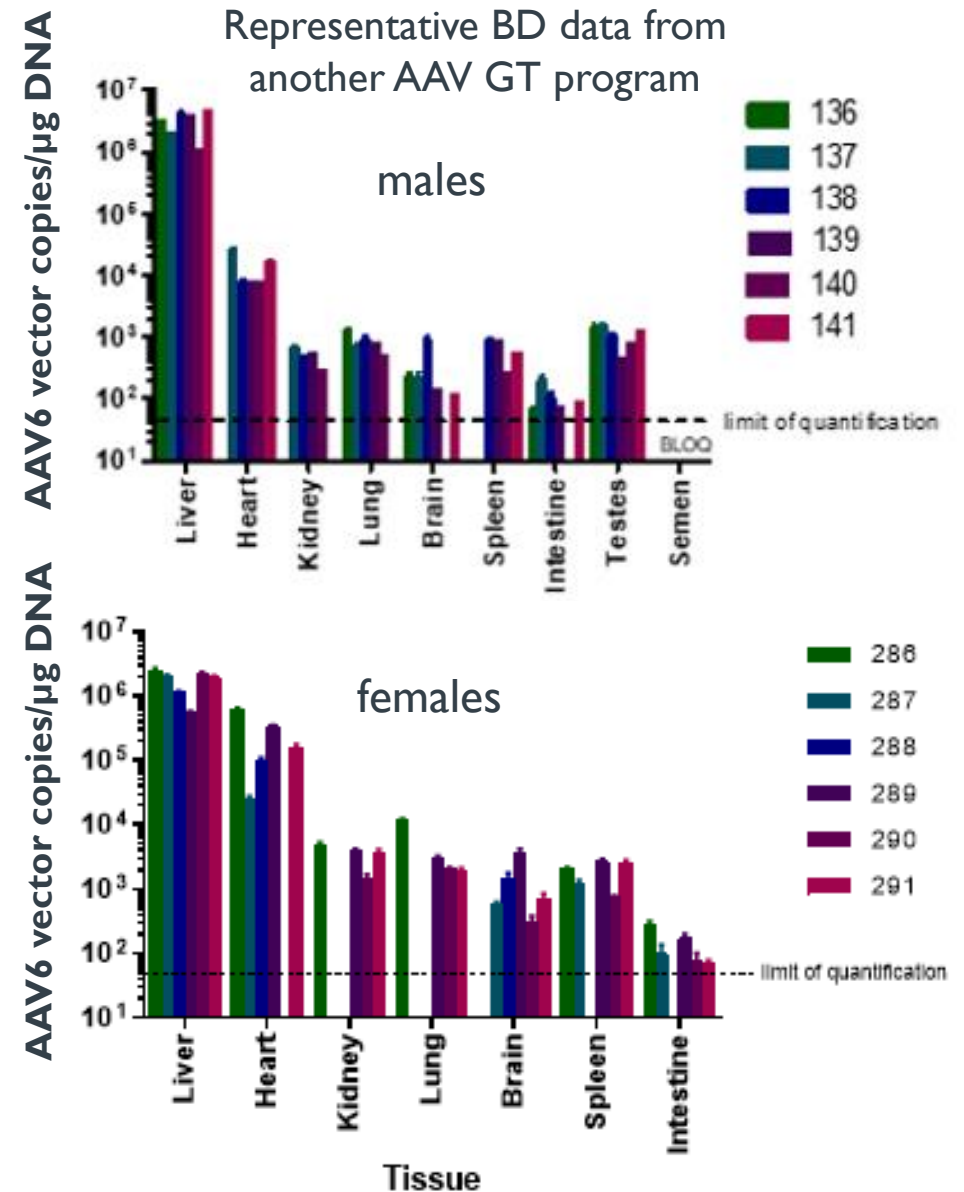
AAV6 biodistribution data in mice

- 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 $\geq 1.5 \times 10^{14}$ 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 (5×10^{13} vg/kg)
- Safety demonstrated $\sim 2,200\times$ - $4,100\times$ higher plasma α -Gal A enzyme activity vs endogenous activity

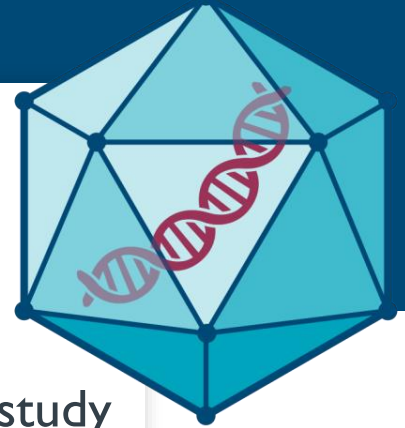
ST-920 Nonclinical Program Supports Phase 1/2 and Phase 3 Studies

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, $\geq 1.5E+14$ vg/kg, and $\geq 6E+13$ 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 I/2 study (STAAR)**

Presenter: Robert J Hopkin, Cincinnati Children's Hospital

- **Anti-AAV6 antibody assay for patient enrollment supporting ST-920 phase I/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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