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

Poster #048

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Introduction

- Fabry disease is a progressive, multi-organ, lysosomal storage disease caused by pathogenic mutations in the *GLA* gene leading to deficiency of the lysosomal enzyme alpha-galactosidase A (α-Gal A) and accumulation of globotriaosylceramide (Gb3) in organs.
- ST-920 is an investigational gene therapy comprised of a recombinant AAV2/6 vector containing the cDNA encoding α-Gal A under the control of a liver-specific promotor. Patients with pre-existing antibodies to AAV6 are excluded from the ongoing Phase 1/2 STAAR study (NCT04046224).
- A validated lab-developed anti-AAV6 antibody cell-based transduction inhibition (TI) assay (Cao et al., 2023) was utilized for enrolling patients in the ongoing STAAR study.
- Implementing a cell-based TI assay supporting clinical studies is challenging. Challenges include the clinical relevance of the determined enrollment cutoff, frequent testing due to the concern of potential patient seroconversion, and the variability of a cell-based assay.
- The poster aims to address these challenges from the cutoff determination to implementing various strategies for long-term assay monitoring to provide consistent assay performance supporting patient enrollment in the STAAR study.

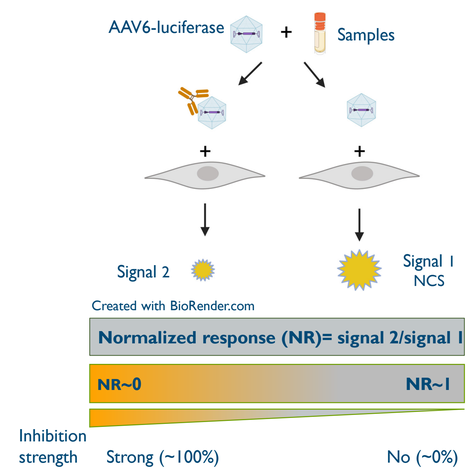
Methods

AAV6 Cell-based TI assay Support Patient Enrollment

Day 1
- Seed 20k human U-87 MG HTB-14 (ATCC) cells per well.

Day 2
- Dilute samples and controls 5-fold and mix 1:1 with AAV6-luciferase, incubate at 37°C 5% CO_2 for 30-40 mins.
- Remove culture media, add 50 µL culture media, add 50 µL of AAV6-luciferase/sample or QCs mix in duplicate, incubate at 37°C 5% CO_2 for 24h (±2h).

Day 3
- Add 100 µL One-Glo reagent/buffer mixture (Promega), room temperature for 10-15 mins and read luminescence.
- Calculate NR:Ratio of luminescent signals from test samples to the mean signal of normal control serum (NCS).
- Cutoff NR=0.34
  - Positive if NR<0.34
  - Negative if NR≥0.34



AAV6 *In Vivo* Mouse Passive Transfer Support Cutoff Determination

Day 1
- Administer 100 µL or 200 µL human serum samples via intravenous injection (IV) to naïve C57B/6 mice (n=5-10/group).
- Two hours later, IV administer 200 µL AAV6 encoding human Factor 9 cDNA, 6E+10 vg/mouse.

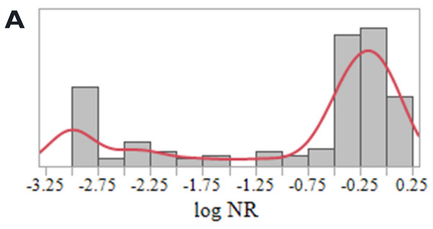
Day 7 or Day 10
- Measure plasma hFIX levels by ELISA using VisuLize FIX® antigen kit per the manufacturer procedure.

Statistical Cutoff Determination

- Data set was statistically analyzed at B2S Life Sciences. The cutoff was determined using a linear mixed-effects Analysis of Variance (ANOVA) model. Estimates for parametric and nonparametric cutoffs at 0.1%, 1%, and 5% error rates were determined using the log-transformed NR values.
- Tukey's biweight procedure was used to calculate robust estimates of the mean and standard deviation (SD) of all log-transformed NR values.
- Parametric cutoff = $10^{[\text{Biweight Mean}+\text{Biweight SD}*t_{\alpha, n-1}]}$. $t_{\alpha, n-1}$ is the α percentile of the t-distribution with degrees of freedom equal to the number of log-transformed NR values minus 1.
- Nonparametric cutoff = empirical percentile for the log-transformed NR values followed by an inverse log transformation.
- Minimum significant ratio (MSR) commonly used in potency assays to characterize the reproducibility of potency estimates was used to define the response range for each of the calculated cutoff values.
  - MSR = $10^{[2*\text{intrarun SD}]}$. Intrarun SD was estimated from the ANOVA model.

Cutoff Determination

1A



1B

False Positive Error Rate	Cutoff Type	Cutoff Estimate	Range of Individual Sample Results Incorporating MSR
5%	Parametric	0.593	0.47 - 0.75
5%	Nonparametric	0.621	0.49 - 0.79
1.0%	Parametric	0.511	0.40 - 0.65
1.0%	Nonparametric	0.560	0.44 - 0.71
0.1%	Parametric	0.431	**0.34** - 0.55

1C

	S1	S2	S3	S4*	S5*	S6*	S7	S8	S9	S10	S11	S12	S13
NR	0.002	0.004	0.28	0.33	0.33	0.41	0.61	0.68	0.74	0.84	0.92	0.99	1.11
Mean FIX, ng/mL	0	0	109	170	219	107	123	171	251	350	268	424	197
SD	N/A	N/A	72	155	286	93	140	220	248	290	195	378	220
%CV	N/A	N/A	66	91	131	87	113	128	99	83	87	89	112
NR Group and Range	Group 1 NR < 0.1			Group 2 NR 0.1 to < 0.5				Group 3 NR 0.5 to < 0.9				Group 4 NR > 0.9	
Mean FIX (ng/mL) per AAV6 TI NR Range	0			151				224				281	
%Reduction	100%			46%				20%				N/A	

(**1A**) Histogram distribution of 158 serum samples from evaluation of healthy individuals. An initial NR< 0.7 (> 30% inhibition) was used to remove potential positive samples; 52 samples remained.

(**1B**) 52 samples were tested in 3 independent experiments. Estimates for parametric and nonparametric cutoff at 0.1%, 1%, and 5% false error rates were determined using the log-transformed values. Range of individual sample results were computed incorporating MSR of 1.27.

(**1C**) 13 individual human serum samples, with AAV6 TI assay results ranging from 0.002 to 1.11, were tested in three separate mouse passive transfer studies. C57BL/6 mice (6 mice per donor except S11 which had 5 mice). Plasma hFIX levels were measured by ELISA at Day 7* or Day 10 post-dosing. %Reduction in hFIX level was calculated (Group 1 NR<0.1, Group 2 NR 0.1 to <0.5, and Group 3 NR 0.5 to <0.9) relative to the mean hFIX level from animals treated with sera with NR>0.9 (Group 4). NR>0.33 on average still produced levels of hFIX (>150 ng/mL, 3% of FIX activity in healthy individuals per literature) and supports selection of the statistically determined NR of 0.34 as the clinical cutoff (NR of 0.34) for clinical studies using AAV6 vector.

Enrollment & Transgene Expression

2A

QC	PS-QC1	PS-QC2	PS-QC3	QC-ADK6[2]
Mean NR	0.004	0.203	0.375	0.532
Number of runs	64	64	64	13
%CV	60.9	23.3	17.0	9.9
Acceptance criteria	CV ≤ 25%, replicates for NCS and duplicates for samples/QCs except QC1 due to high TI, NR of PS-QC1<PS-QC2<PS-QC3, mean PS-QC2<0.34 and 0.34≤QC-ADK6<0.613[2]			
# of analyst/duration[1]	5 analysts and duration >3.5 years			

PS-QC: pooled serum Quality Control (QC) ; NCS: normal control serum; QC-ADK6: monoclonal ADK6 in NCS

2B

Screen[1]	NR < 0.34 (Excluded)	% Positive
72	21	29

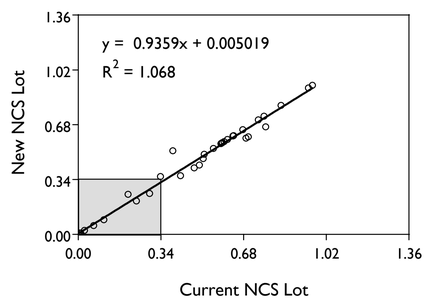
[1]Data cutoff date: September 19, 2023
[2]Added QC to monitor assay performance in 2022 and incorporated as acceptance criteria in 2023

(**2A**) Clinical testing quality control performance.

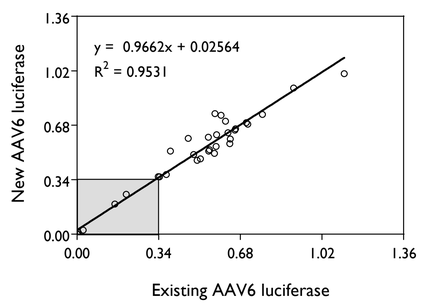
(**2B**) 72 unique subjects screened with 21 excluded. Of the remaining subjects, 24 were dosed as of data cutoff date. The 1st 22 subjects had ≥ 4 weeks post-treatment plasma α-Gal A activity data. All 22 subjects demonstrated α-Gal A expression (WORLD*Symposium*™ oral presentation Feb 7, 2024 and poster #145).

Strategy to Bridge Reagents Using Donors

3A



3B



(**3A**) 30 donors and 6 controls were used to demonstrate concordance results when switching NCS lots. 100% concordance observed. 9 samples in the gray box are seropositive samples.

(**3B**) After selecting the appropriate MOI of new AAV6 luciferase, 30 donors and 6 controls were used to demonstrate concordance results. 100% concordance observed. 9 samples in the gray box are seropositive samples.

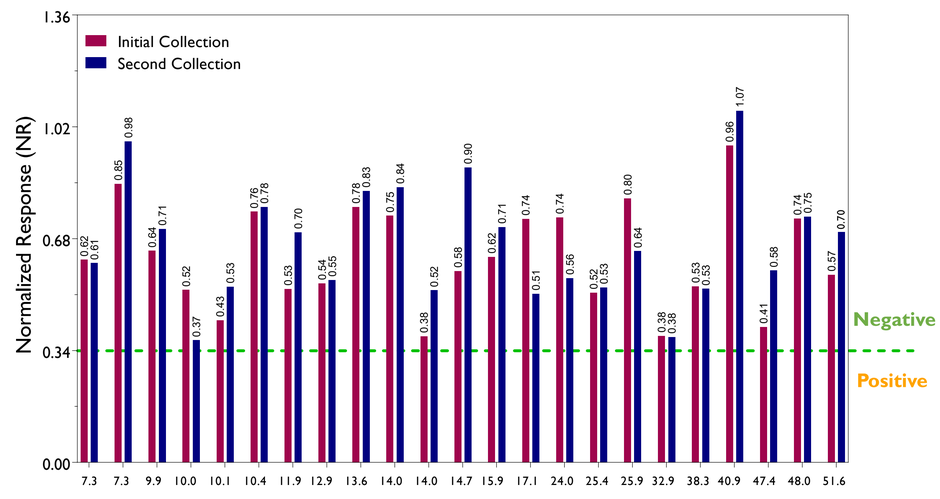
Seroprevalence & No Observed Seroconversion

4A

Population	Total Tested	NR<0.34	Seropositivity
Healthy Adults	120	48	40%
Healthy Pediatrics	62	12	19%
Fabry (Procured)[1]	54	13	24%
Fabry (Ongoing STAAR study)	72	21	29%
Overall	308	94	31%
Overall (adults)	246	82	33%

[1]In collaboration with Dr. Michael L. West, Canadian Fabry Disease Initiative Registry

4B



23 screened subjects: Weeks between two collections

(**4A**) Collectively, a total of 246 serum samples from Fabry patients, healthy adult and pediatric populations were evaluated for seroprevalence, which ranged from 19% to 40% with an overall seropositivity of 31%, 33% and 19% for adult and pediatric, respectively.

(**4B**) As of data cutoff date September 19, 2023, no seroconversion was observed for 23 screened subjects with two samples collected ranging from 7.3 to 51.6 weeks apart.

Summary & Conclusions

- A lab-developed anti-AAV6 cell-based transduction inhibition (TI) assay was validated to support patient enrollment for the Phase 1/2 STAAR study.
- The enrollment cutoff was determined statistically using samples collected from healthy donors and clinically validated as transgene expression was demonstrated in the ongoing STAAR study (WORLD*Symposium*™ oral presentation Feb 7, 2024 and poster #145).
- Seroprevalence for AAV6 ranged from 19% to 40% with an overall seropositivity of 31%, 33%, and 19% for adult and pediatric populations, respectively.
- Seroconversion has not been observed for 23 subjects retested in the STAAR study with the longest duration of 51.6 weeks between collections.
- Controls flanking the cutoff help to control the assay and to enable long-term stable assay performance monitoring.
- Strategy of using donors to demonstrate concordance between reagent lots allow reagent bridging without the need to redefine the cutoff.

Reference

Cao L, et al. Gene Ther 30, 150–159 (2023). https://doi.org/10.1038/s41434-022-00353-2

Acknowledgments

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