Strategy to Assess the Effect of Duvoglustat Co-administered with Alglucosidase Alfa Infusion on the Immune Response to Enzyme Replacement Therapy for Pompe Disease

Elfrida R. Benjamin[1], Xiaoyang Wu[1], M. Cecilia Della Valle[1], Eric Sjoberg[2], Sheela Sitaraman[1], Anthony Stevens[2], Nestor Gomez[1], Evan Katz[1], Farhana Pruthi[1], Jeffry S. Kelley[3], Brandon Wustman[2], Kenneth J. Valenzano[1], Barry J. Byrne[3,4], Carrolee Barlow[1,2], and David J. Lockhart[1,2]

[1]Amicus Therapeutics, 6 Cedar Brook Drive, Cranbury, NJ 08512 USA; [2]Amicus Therapeutics, 11099 North Torrey Pines Road, La Jolla, CA, 92037; [3]Powell Gene Therapy Center, University of Florida, Gainesville, FL; [4]Department of Pediatrics, University of Florida, Gainesville, FL

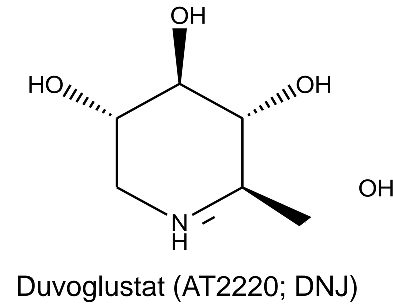
Introduction

Pompe Disease and ERT

- A lysosomal storage disorder caused by acid α-glucosidase (GAA) deficiency
- Enzyme Replacement Therapy (ERT) is currently the primary treatment
- However, the efficacy of ERT is adversely affected by the immune responses elicited by enzyme infusion
- A survey of literature indicates that ≥90% of Pompe patients can be expected to show immune responses at some point after treatment with ERT
- Studies of new ERT products/treatment strategies for the Pompe disease should include assessments of immunogenicity

Potential role for the pharmacological Chaperone AT2220 in Pompe ERT

- Duvoglustat (AT2220; 1-deoxynojirimycin) is a pharmacological chaperone that was shown to selectively bind to and increase the physical stability of rhGAA *in vitro*, and improve the pharmacological properties of the enzyme after co-administration with alglucosidase alfa *in vivo*
- Direct binding and stabilization of rhGAA by duvoglustat when co-administered with alglucosidase alfa may protect the enzyme in the circulation in a properly folded and monomeric form that may improve ERT tolerability and potentially mitigate immunogenicity



Duvoglustat (AT2220; DNJ)

Assays useful for assessing the effect of duvoglustat co-administered with alglucosidase alfa infusion on the immune response

- Anti-rhGAA antibody titer assay
- Measurement of the amount of infused rhGAA not bound by anti-rhGAA antibody (NBBA-rhGAA)
- Measurement of complement activation and cytokines

Anti-rhGAA Antibody Titer Assays

ELISA—Enzyme-linked Immunosorbent Assay, developed and performed at Amicus following a standard protocol. The minimum required dilution (MRD) was 1:100, plasma matrix concentration of 1% was kept constant for every assay dilution, and the assay used a 95% confidence interval floating cut point (FCP).

ECLIA—Mesoscale Discovery-based Electrochemiluminescence Assay, developed and performed at PRA International following a standard protocol (see http://www.mesoscale.com for details). The MRD was 1:100, plasma matrix concentration of 1% was kept constant for every assay dilution, and the assay used a 99.9% confidence interval FCP.

Samples: Period 1 and 2 pre-infusion plasma samples from 25 subjects with late-onset Pompe disease enrolled in the clinical study AT2220-010 (for details see ClinicalTrials.gov identifier: NCT01380743)

Results:

Subject ID	ELISA Titers for Pre-infusion samples[1]		ECLIA Titers for Pre-infusion samples[2]	
	Period 1	Period 2	Period 1	Period 2
01	102400	51200	102400	204800
02	800	1600	100	100
03	12800	12800	51200	25600
04	800	800	200	200
05	100	100	Negative	100
06	800	1600	200	100
07	800	400	100	100
08	1600	1600	100	100
09	3200	6400	6400	3200
10	102400	102400	204800	204800
11	6400	6400	6400	12800
12	12800	12800	51200	51200
13	204800	204800	12800	25600
14	3200	3200	6400	1600
15	12800	12800	51200	102400
16	100	100	200	100
17	6400	12800	6400	3200
18	1600	1600	Negative	100
19	800	800	800	400
20	3200	3200	200	100
21	25600	25600	25600	51200
22	819200	819200	204800	204800
23	6400	6400	3200	6400
24	200	200	100	200
25	Negative	Negative	Negative	100

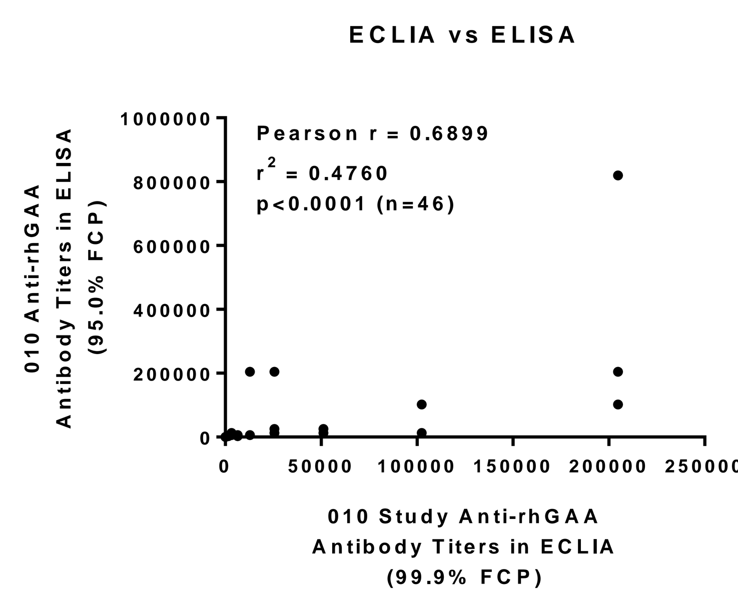
[1]:titers were determined from 3 independent assays using the averaged sample signals and the averaged floating cut-point (at 95% confidence interval) of all 3 assays

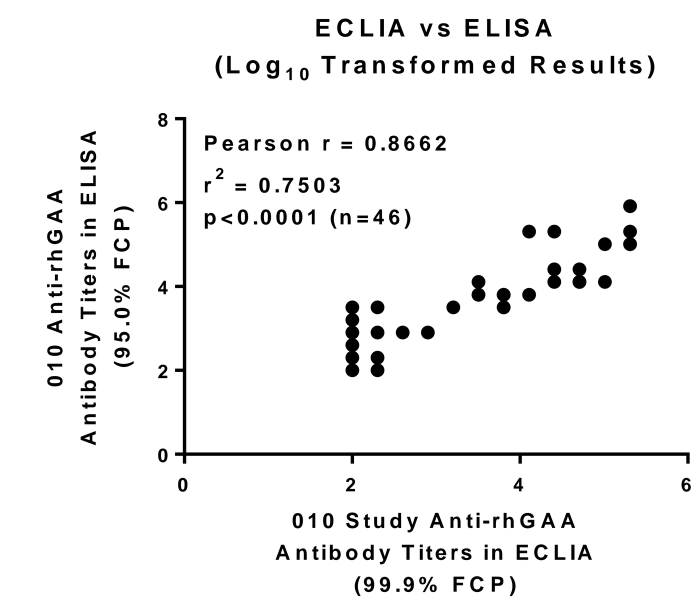
[2]: titers were determined from a single assay using a 99.9% confidence interval floating cut-point

Conclusions:

- In the ELISA, 48 of the 50 (96%) Period 1 and 2 samples showed positive titers (≥100) ranging from 100 to 819,200; two samples (Period 1 and 2 samples from the same subject) were negative in both assays
- In the ECLIA, 47 of the 50 (94%) samples showed positive titers (≥100) ranging from 100 to 204,800; 3 samples all from Period 1 were negative
- In both assays, for each subject, the titers are either identical or within one assay dilution step between Period 1 and Period samples, indicating no significant change in titer across the two periods

Comparison Between the ELISA and ECLIA Titer Results





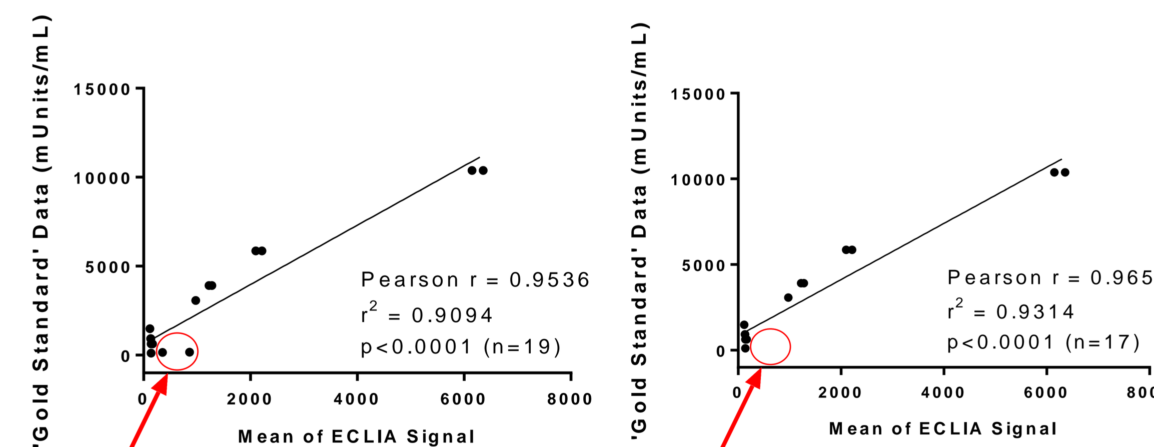
- Titer values (or Log_{10} transformed values) of 46 AT2220-010 pre-infusion samples with positive titers in both ELISA and ECLIA were compared between the assays
- Untransformed titer values and Log_{10} transformed titer values of Period 1 pre-infusion samples were significantly correlated between the ECLIA and the ELISA
- The Pearson r correlation coefficient was higher (0.8662) with the correlation analysis of the Log_{10} transformed values than with the correlation analysis of the untransformed titer values (0.6899)

Conclusions:

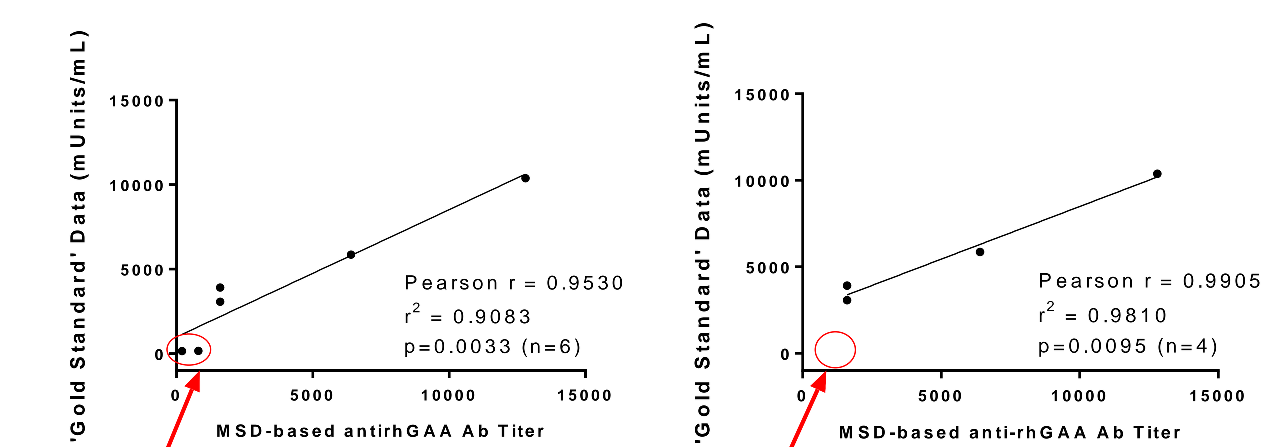
- The titer values in the ELISA were generally higher than the values in the ECLIA in subjects with low anti-rhGAA Ab titers (i.e., between 100-400 by the ECLIA vs between 100-3200 by the ELISA)
  - It is possible, although not proven, that the ELISA may be more sensitive than the ECLIA for low affinity anti-rhGAA antibodies and/or for low concentrations of anti-rhGAA antibodies; this may be due to the inherent difference in methods, the difference in confidence intervals used to calculate the floating cut points, and/or the difference in the range of antibody isotypes that are detectable with the two methods
- Samples with medium range titer values (800-6400) in the ECLIA showed the same range in the ELISA
- Samples with high titer values (>6400) in one assay are generally consistently high in the other assay although they may show more than one-dilution step difference

Comparison of ECLIA Results to the ELISA Results from An Independent Lab ('Gold Standard')

Comparison of 'Gold Standard' to ECLIA Signals



Comparison of 'Gold Standard' to ECLIA Titer Values



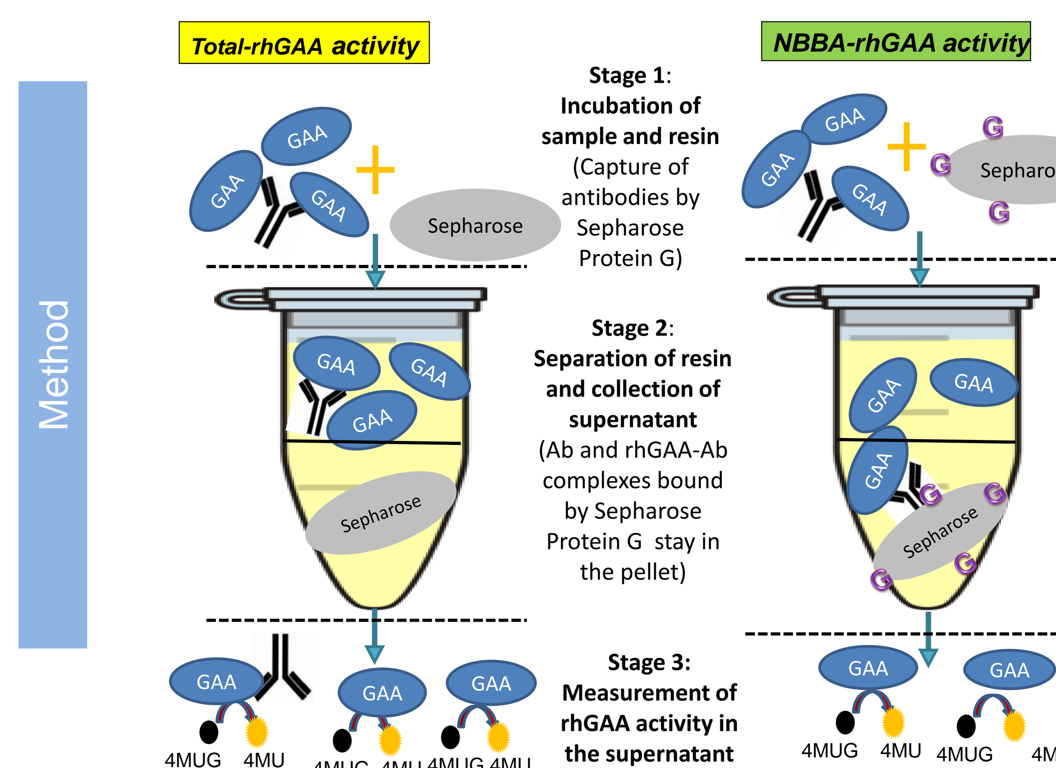
- The two graphs above show linear regression of the ECLIA signal data and the 'Gold Standard' data, either with the inclusion of the two samples in 10% FBS in PBS (left) or without (right)
- The Pearson r values (0.9536 and 0.9651) were high (perfect is a value of 1.0), indicating a strong correlation between these two sets of results

- The two graphs above show linear regression of the titer values with a 0.1% false positive cut point and the 'Gold Standard' data, either with the inclusion of the two samples in 10% FBS in PBS (left) or without (right)
- The Pearson r values (0.9530 and 0.9905) were high (perfect is a value of 1.0), indicating a strong correlation between these two sets of results

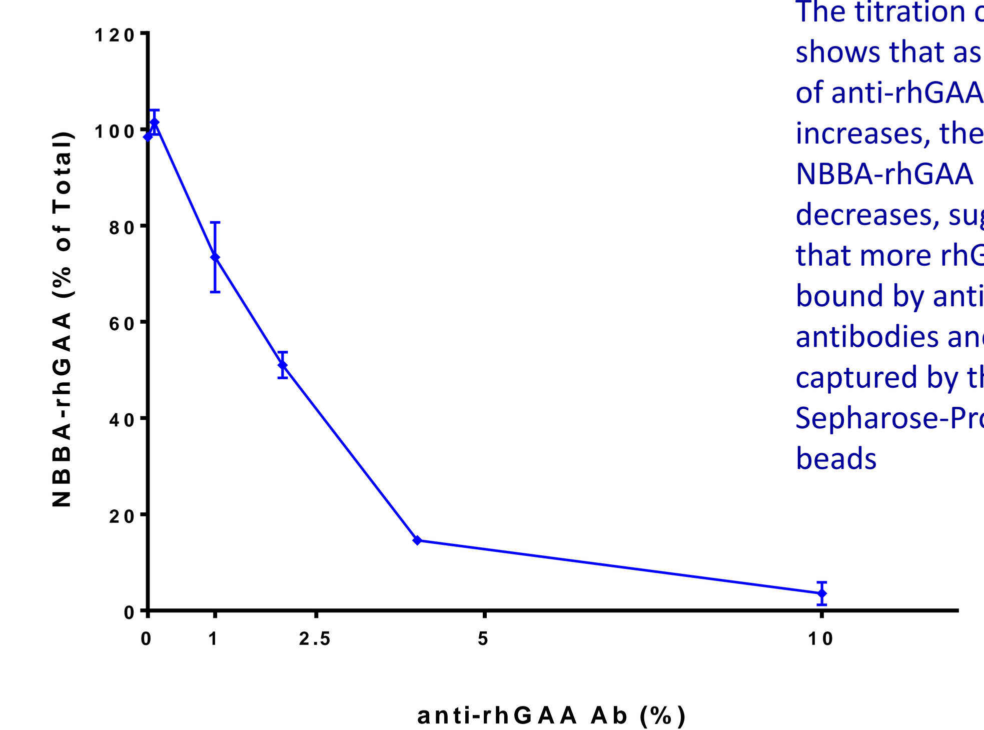
*: The two samples in the red circle were in the matrix of 10% fetal bovine serum in PBS while the ECLIA has not been validated for use in such sample matrix

The NBBA-rhGAA Assay Measuring the Amount of Infused rhGAA Not Bound by Anti-rhGAA Antibody

Method



Assay Development Data



The titration curve shows that as the % of anti-rhGAA Ab increases, the level of NBBA-rhGAA decreases, suggesting that more rhGAA is bound by anti-rhGAA antibodies and captured by the Sepharose-Protein G beads

Conclusion:

- The percentage of NBBA-rhGAA measured in this assay showed a concentration-dependent reduction in proportion to increasing levels of anti-rhGAA antibodies. This suggests that the assay may be useful for measuring NBBA-rhGAA levels in samples from patients co-administered AT2220 and ERT.

Summary

- **Assays to assess the immunogenicity of new ERT products for the treatment of Pompe in future clinical studies have been developed**
- **The assays developed so far include the anti-rhGAA antibody titer assays: ELISA and ECLIA, and the NBBA-rhGAA assay**
- **In addition, the formation of circulating immune complexes after the start of infusion can lead to classical pathway-dependent complement activation and/or cytokine release that may underlie infusion-associated reactions that are problematic for some Pompe patients. Therefore, assays assessing circulating immune complexes recognized by C1q and cytokines may be developed for future clinical studies.**

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