# Development of a GLA NAb Assay with a Fully-human, Neutralising IgG4 Positive Control to Characterise Antibody Response in Fabry Disease Patients

Poster #211

Sujata Ravi, Erald Shehu, Jonathan H Foley, Olivia Allen, Sophie A Snow, Jey Jeyakumar, Rose Sheridan, Romuald Corbau

*Freeline, Stevenage, United Kingdom*

## INTRODUCTION

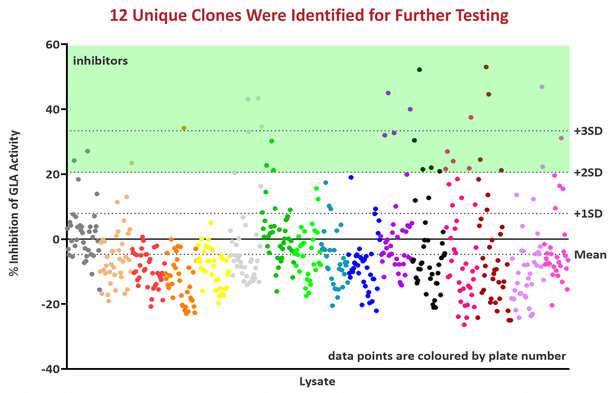
- Fabry disease is an X-linked lysosomal storage disorder caused by deficiency of the enzyme α-galactosidase A (GLA), which is responsible for the hydrolysis of the terminal alpha-galactosyl moiety from globotriaosylceramide (Gb3).[1]
- Current standard of care for the treatment of Fabry disease consists of the replacement of the deficient GLA enzyme using enzyme replacement therapy (ERT).[1] Migalastat is also available for the treatment of Fabry disease but can only be used by approximately 30% of patients who have specific mutations.[2] Due to the heterogenous nature of the disease, individual patient response to treatment may vary, and there is a need for more effective therapies.[1]
- Additionally, long-term treatment with ERT can result in the development of anti-drug antibodies (ADA) to recombinant human GLA.[3-5] Assessment and characterisation of anti-GLA antibody status is important during the development of gene therapy for Fabry disease to improve understanding of efficacy and safety.
- Currently, ADA titres are assessed using either enzyme-linked immunosorbent assay (ELISA) or activity-based neutralising antibody (NAb) assays. However, these methods employ poorly defined positive controls (e.g., patient or animal serum) that may result in intra-lab differences in sensitivity to GLA ADA status.
- To address the shortcomings of current assays, we developed a sensitive NAb assay featuring a recombinant, fully-human, neutralising IgG4 antibody as a positive control.

## METHODS AND RESULTS

### Screening of commercial antibody phage display library against GLA

- An *in vitro*, custom-made library of GLA-binding antibodies was developed from a phage display library.
- The anti-GLA library generation involved 3 rounds of selection following which hits were cloned and expressed as Fab fragments. Crude lysates were evaluated for GLA binding by indirect ELISA.
- The library was then screened for GLA neutralising activity, where lysates exceeding two standard deviations (SDs) of mean inhibition were considered 'hits.'
- From the top 20 hits of the primary screen, 12 unique clones were identified and purified as Fab2 antibodies for further testing (**Figure 1**).

**Figure 1.** Screening of Fab2 containing lysates from the library using a miniaturised (384-well plate) GLA assay.

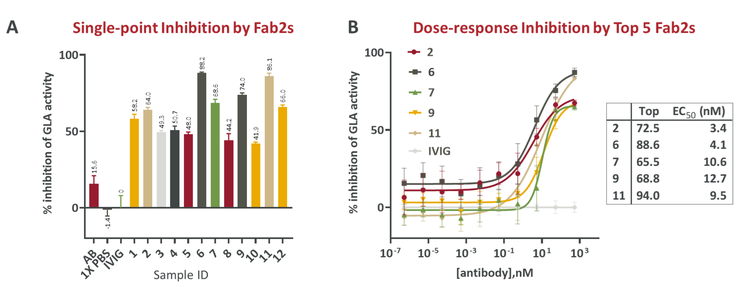



**Methods:** 594 Fab2 lysates were screened for GLA neutralising properties with *E. coli* lysate as a negative control. The percent inhibition of GLA activity in the presence of each lysate was plotted. The screen included 16 assay plates, and the lysates in each plate have been plotted using a different colour.

### Evaluation of GLA neutralising activity of purified Fab2 antibodies

- Percent inhibition of GLA activity (0.25 ng GLA or 12.5 ng/mL; equivalent to 25 nmol/h/mL) was determined in the presence of a fixed concentration of the of 12 neutralising Fab2 antibodies.
- All Fab2 antibodies resulted in the inhibition of GLA activity by 42-88% (**Figure 2A**).

**Figure 2.** Neutralising performance of 12 Fab2 antibodies identified as hits from the primary screen.




**Methods:** Purified Fab2s were tested for neutralising activity at a single concentration. Acetate buffer (AB) was included as an assay control; 1xPBS and intravenous immunoglobulin (IVIG) were included as controls to account for effects of the antibody diluent and non-specific IgG protein.

**Methods:** Five Fab2s were tested in 10-point ten-fold dose response curves to determine antibodies with maximum efficacy and lowest $EC_{50}$s. Fab2 antibodies 2, 6, 7, 9 and 11 were tested in parallel with IVIG negative control.

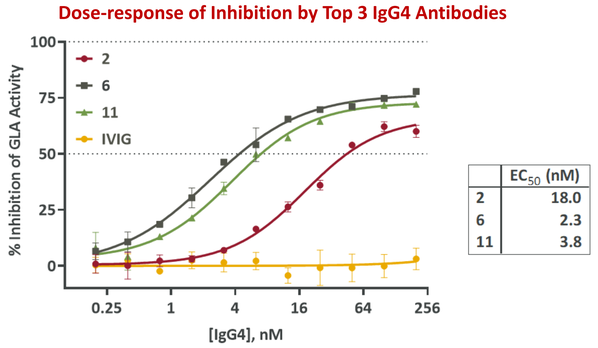
## METHODS AND RESULTS

- Five of the 12 Fab2 antibodies with the highest efficacy in Figure 2A were further characterised using 10-point dose response curves ranging from 526 to $0.526x10^{-6}$ nM of each Fab2 antibody (**Figure 2B**). Equivalent concentrations of IVIG were used as a negative control or 100% activity.
- Bivalent Fab2s 2, 6, and 11 were found to have the lowest $EC_{50}$ values of 3.4nM, 4.1nM and 9.5nM, respectively, and the highest neutralising efficacy of 72.5%, 88.6% and 94%, respectively (**Figure 2B**).
- These three Fab2 antibodies were converted into the corresponding IgG4 format for further evaluation as IgG4 is the predominant IgG subclass of ADAs reported in the literature.[6,7]

### Evaluation of the neutralising activity of monoclonal IgG4 antibodies

- The corresponding IgG4 antibodies 2, 6, and 11, generated by converting the Fab2 antibodies, were then tested for their ability to neutralise GLA in the presence of human serum (**Figure 3**).
- The neutralising efficacy of the Fab2 antibodies was maintained following IgG4 conversion and the three IgG4 antibodies neutralised GLA activity between 65-80% with $EC_{50}$ values of 18.0 nM, 2.3 nM, and 3.8 nM for Fab2 antibodies 2, 6, and 11, respectively.
- Antibody 6 displayed the highest inhibition of GLA, approximately 77% (**Figure 3**).

**Figure 3.** Dose response curves for IgG4 antibodies 2, 6 and 11.

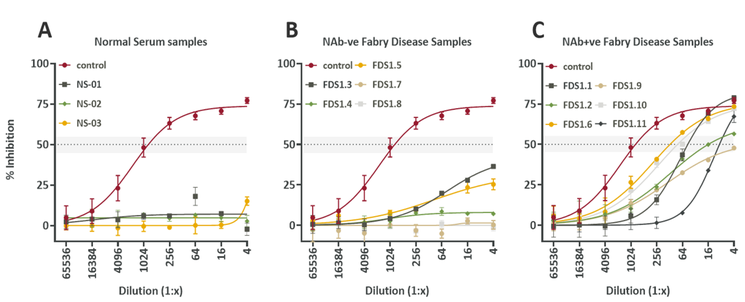



**Methods:** An 11-point dose response curve using a 2-fold dilution factor was carried out in parallel with IVIG as a negative control.

### Development and testing of the GLA NAb Assay

- We developed a dose-response based NAb assay to determine the sample titre at which 50% inhibition of GLA is observed (i.e., the NAb titre). IgG4#6 was used as the neutralising positive control.
- A 4-fold dilution series of patient serum samples starting at 1:4 was pre-incubated with a fixed amount of GLA (12.5 ng/mL or 25 nmol/h/mL). Percent inhibition of GLA in the presence of the sample dilution was calculated by normalising against 0% (100 nM IVIG in a mixed pool of normal serum with GLA enzyme) and 100% inhibition (100 nM IVIG in a mixed pool of normal serum without GLA enzyme) controls.
- The percent inhibition values resulting from the normalisation were fitted to a four-parameter non-linear regression curve, with variable slope and the titre at which 50% inhibition occurs interpolated.
- A sample was termed neutralising if the NAb titre was ≥4, the minimum required dilution in the assay.
- Fabry disease serum (FDS) samples positive for anti-GLA antibodies by ELISA were tested using the GLA NAb assay. Three normal serum samples, and 11 FDS samples were evaluated.

**Figure 4.** Testing of anti-GLA+ve Fabry disease samples in the GLA NAb assay.




**Methods:** 60 µL of each sample was diluted 4-fold in mixed-pool negative serum and tested in duplicate. Each assay plate consisted of the IgG4#6 as a positive control, and two test samples. Seven assay plates were tested in three runs within one occasion.

- The three normal serum samples NS-01, NS-02 and NS-03 previously confirmed negative for anti-GLA antibodies using a lateral flow immunochromatographic ELISA (Synthera Technologies, Japan) were also found to be negative for neutralising anti-GLA antibodies using the GLA NAb assay demonstrating assay specificity towards GLA NAbs (**Figure 4A**).
- Of the 11 anti-GLA positive sera, 2 samples, FDS 1.7 and 1.8 demonstrated 0% neutralisation of GLA activity at all dilutions. FDS 1.3, 1.4, and 1.5 demonstrated modest neutralisation of GLA at lower dilutions but 50% inhibition of GLA activity did not occur at any tested dilution, and therefore, these samples were reported as negative for GLA NAbs by the assay (**Figure 4B**).
- All remaining samples (FDS 1.1, 1.2, 1.6, 1.9, 1.10 and 1.11) surpassed 50% inhibition at one or more test dilution (**Figure 4C**) and NAb titre values were interpolated for each sample as detailed in **Table 1**.

**Table 1.** GLA NAb titre values interpolated for Fabry disease samples using the NAb assay.

| Sample ID | Description | ADA titre by ELISA | NAb titre |
|---|---|---|---|
| **Control** | IgG4 #6 | N/A | 1117 |
| **NS-01** | Normal | N/A | 0 |
| **NS-02** | Normal | N/A | 0 |
| **NS-03** | Normal | N/A | 2 |
| **FDS1.1** | Fabry Disease | 800 | 60 |
| **FDS1.2** | Fabry Disease | 800 | 31 |
| **FDS1.3** | Fabry Disease | 800 | 0 |
| **FDS1.4** | Fabry Disease | 800 | 0 |
| **FDS1.5** | Fabry Disease | 800 | 0 |
| **FDS1.6** | Fabry Disease | 1600 | 189 |
| **FDS1.7** | Fabry Disease | 400 | 0 |
| **FDS1.8** | Fabry Disease | 200 | 0 |
| **FDS1.9** | Fabry Disease | 6400 | 7 |
| **FDS1.10** | Fabry Disease | 1600 | 126 |
| **FDS1.11** | Fabry Disease | 200 | 11 |

### Assay performance

- Key assay parameters namely $EC_{50}$ and NAb titre of the positive control IgG4#6, and RFU values of the 0% inhibition and 100% inhibition wells were trended to analyse assay performance.
- Precision across three assays within one occasion (repeatability), and across occasions (intermediate precision), is summarised in **Table 2.**

**Table 2.** Performance of GLA NAb assay

| | Control NAb titre (1:x) | Control $EC_{50}$ (1:x) | 0% Inhibition (RFU) | 100% Inhibition (RFU) |
|---|---|---|---|---|
| **Repeatability (%CV)** | 18 | 20 | 6.8 | 7.5 |
| **Intermediate precision (%CV)** | 30.6 | 33.7 | 18.2 | 10 |

## SUMMARY AND CONCLUSIONS

- From a custom *in vitro* phage display library against GLA, we screened and developed a unique GLA neutralising IgG4 antibody, which is neither described in the literature nor commercially available from antibody suppliers.
- We developed a sensitive semi-quantitative GLA NAb assay to determine the titre of GLA neutralising antibodies in Fabry patient serum, enabling further characterisation and monitoring of ADA-positive Fabry patients during ERT or gene therapy.
- Early development and qualification data suggest that the assay performance was acceptable and accurate in detecting NAbs in Fabry disease samples, supporting its further development and validation as a robust, standardised assay for use in gene therapy trials.

### References

**1.** van der Veen S, et al. *J Inherit Metab Dis.* 2020;43:908–921. **2.** Lenders M, Stappers F, Niemietz C, et al. *J Med Genet.* 2019;56:548–556. **3.** Linthorst GE, et al. *Kidney International.* 2004;66:1589-1595. **4.** Wilcox WR, et al. *Am Soc Hum Gen.* 2004;75:65-74. **5.** Bigger BW, Saif M, Linthorst GE. *Best Pract Res Clin Endocrinol Metab.* 2015;2:183-194. **6.** Lenders M, et al. *J Allergy Clin Immunol.* 2018;141:2289-2292. **7.** van der Veen S, et al. *Mol Gen Metab.* 2019;126:162-168.

E-Poster Presented at the WORLD*Symposium* 2021 Virtual Scientific Meeting, 11 February 2021.