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

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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 heterogeneous nature of the disease, individual patient response to treatment may vary, and there is a need for more effective therapies.3
• Additionally, long-term treatment with ERT can result in the development of anti-drug antibodies (ADA) to recombiant human GLA.4 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. 

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).

Evaluation of GLA neutralising activity of purified Fab2 antibodies

• Percent inhibition of GLA activity (0.25 ng GLA or 12.5 ng/mL or equivalent to 25 nmoles/h/mL) was determined in the presence of each lysate. The screen included 16 assay plates, and the lysates in each plate had been plotted using a different colour.

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). IgG486 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 nmol/h/mL in a mixed pool of normal serum with GLA enzyme) and 100% inhibition (100 nmol/h/mL 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.

Assay performance

• Key assay parameters namely EC50 and NAb titre of the positive control (IgG486), 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.

SUMMARY AND CONCLUSIONS

• 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.

References