Overcoming the Effect of Previous Enzyme Replacement Therapy on the Detection of Anti-transgene Protein Antibodies After Treatment with Gene Therapy

Abstract #727

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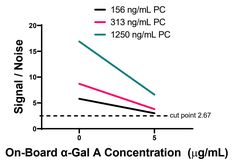
Introduction

- Many AAV gene therapies in the clinic target lysosomal storage disorders, such as Fabry disease, with patient populations that are being treated with enzyme replacement therapy (ERT). Patients often develop high levels of persistent anti-drug antibodies (ADA) to the recombinant enzyme products that may have an impact on later gene therapy treatments
- Ability to monitor relative ADA levels to assess the potential induction of tolerance or treatment-boosted ADA production for patients with pre-existing ADA is important in gene therapy clinical trials
- Unlike ERT, where washout collections are feasible for the ADA assessment, in gene therapy, the continuous expression of the transgene encoded lysosomal enzyme (e.g., alpha-galactosidase A [α-Gal A]) can interfere with the detection of ADA and assessment of changes in ADA levels
- To address this interference, novel assay methods incorporating an alkaline sample pretreatment step were developed to improve the enzyme drug tolerance of the anti-α-Gal A total antibody (TAb) and neutralizing antibody (NAb) assays supporting Fabry clinical trials

Challenges

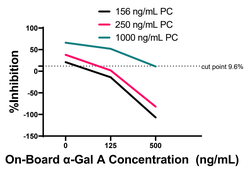
Decreased Antibody Response and Titer in the Presence of α-Gal A Impedes the Assessment of Immune Tolerance Induction and Treatment-Boosted ADA

Direct Binding ELISA to Detect TAbs

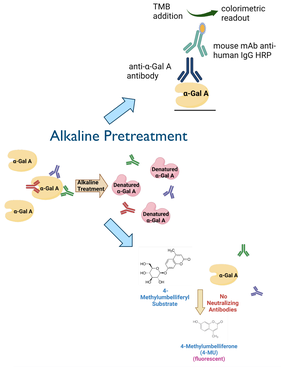


PC = monoclonal positive antibody control to α-Gal A

Enzymatic Assay to Detect NAbs



Methods



ELISA Assay to Detect Anti-α-Gal A Total Antibodies

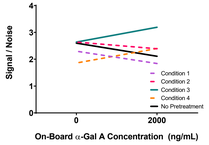
- Recombinant human α-Gal A is first coated onto a microtiter plate
- Human serum samples are pretreated with alkaline solution (pH>10), neutralized, and added to the coated plate
- Alkaline pretreatment expected to dissociate antibody:α-Gal A complex
- Mouse monoclonal anti-human IgG-HRP is used for the detection
- The presence of bound ADA will result in a colorimetric signal

Fluorescent Enzyme Inhibition Assay to Detect Anti-α-Gal A Neutralizing Antibodies

- Human serum samples are pretreated with alkaline solution (pH>10) and incubated with recombinant human α-Gal A in acidic (physiological pH) buffer overnight
- Alkaline pretreatment expected to dissociate antibody:α-Gal A complex
- The following day, samples are mixed with an artificial substrate 4-methylumbelliferyl α-D-galactopyranoside (4-MU-α-Gal)
- The presence of NAbs inhibits α-Gal A from cleaving the substrate to yield fluorescent 4-methylumbellioferone (4-MU)
- % inhibition = (1-(Sample/Negative Control))*100%

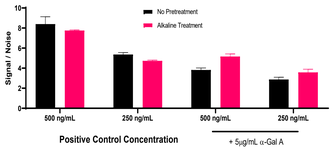
TAb Assay Optimization

Pretreatment Conditions Optimization



- In the presence of on-board α-Gal A at low ADA levels, Condition 3 (pH>10) improved the detection of TAbs without affecting assay performance
- Other tested conditions decreased the detection of TAbs
- Some conditions (e.g. lower pH, high temperature) interfered with the detection of TAbs in the absence of on-board α-Gal A (Conditions 1 and 4)

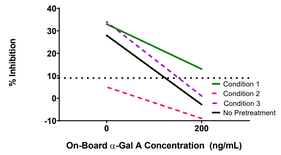
Effect of Pretreatment With Positive Control



- Sample pretreatment partially dissociated the α-Gal A: ADA complexes without affecting PC performance
- Sample pretreatment improves drug tolerance effectively, especially at lower ADA concentrations which is important as low ADA levels may be of clinical relevance

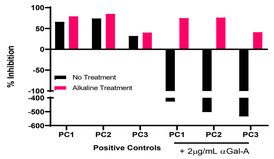
NAb Assay Optimization

Pretreatment Conditions Optimization



- In the presence of on-board α-Gal A, condition 1 (pH>10) improved the detection of NAbs with minimal effect on assay performance
- Other tested conditions either behaved similarly to the no pretreatment or negatively impact the assay performance (e.g. Conditions 2 and 3)

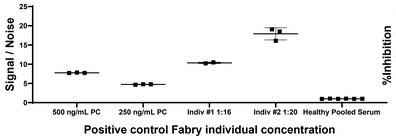
Effect of Pretreatment With Positive Control



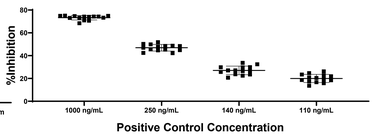
- Sample pretreatment is effective with various rabbit mAb PCs without compromising assay performance
- In α-Gal A:ADA complexes, NAbs that had undergone sample pretreatment show similar levels to PCs without α-Gal A, demonstrating the effectiveness of the pretreatment

Assay Precision

Anti-α-Gal A Total Antibody (TAb) Assay



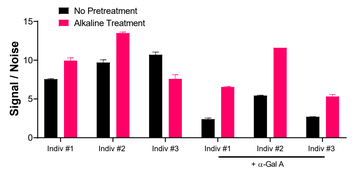
Anti-α-Gal A Neutralizing Antibody (NAb) Assay



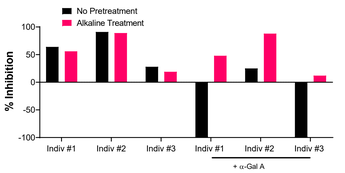
- The inter-assay precision is <20% for both TAb and NAb assays across tested PCs, Fabry patients (Indivs #1 and #2), and healthy pooled human serum

ADA Positive Fabry Patient Samples

Anti-α-Gal A Total Antibody (TAb) Assay



Anti-α-Gal A Neutralizing Antibody (NAb) Assay



- Circulating α-Gal A negatively impacts the detection of both TAbs and NAbs, and especially for NAbs. False negative results were observed for Individuals #1 and #3 in the presence of on-board α-Gal A without pretreatment
- An improvement in ADA detection using the novel pretreatment approach in both TAb and NAb assays was demonstrated using sera from Fabry patients with ADAs against α-Gal A ERT

Conclusion

- Sample pre-treatment with alkaline buffer dissociated α-Gal A:ADA immune complexes and selectively denatured circulating α-Gal A, thereby leaving ADA intact and free for detection in our TAb and NAb assays
- Alkaline treatment removed enzyme interference with positive control antibodies as well as with Fabry patient samples with pre-existing ADA to ERT
- Our novel approach demonstrated an improvement in circulating enzyme tolerance to μg/mL levels in serum for both the TAb and NAb assays, with elimination of enzyme interference in the NAb assay to the highest α-Gal A level tested
- Due to the similar nature and functional pH of lysosomal enzymes, we believe this method is applicable for antibody detection for other lysosomal storage diseases and may be invaluable in determining the impact of pre-existing anti-ERT immunogenicity on AAV gene therapy measurements

Acknowledgments

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