Development of a Novel Electro-Chemiluminescent Sandwich Immunoassay for the Detection of CFTR Specific Antibodies

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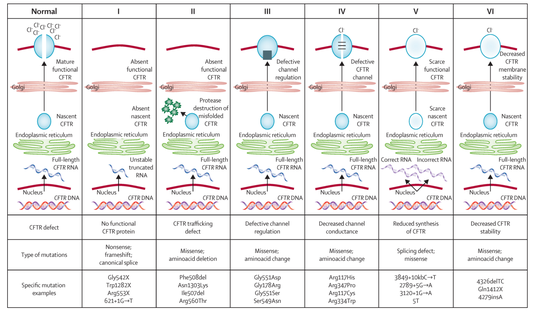
Introduction

Cystic Fibrosis (CF) is a rare, progressive, and life-threatening disease caused by mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR), a chloride ion channel protein located on epithelial cells that regulates fluid transport in the mucosal membranes of the airway. In people with CF (pwCF), mutations in the CFTR gene lead to a misfolded or dysfunctional protein, resulting in very little, if any, functional protein expression on the epithelial cell surface.



Elborn J. Cystic fibrosis. The Lancet, 2016; 388, 2519-2531.

This lack of expression provides a compelling argument for AAV-based gene therapy delivery of CFTR to lung epithelial cells in pwCF. Immune responses to transgenes, whether pre-existing or treatment-induced, have the potential to impact both the safety and efficacy of AAV-based gene therapy. Consequently, health authority guidance recommends clinical monitoring of emerging antibody response to the transgene-expressed protein in AAV gene therapy[2]. For multi-pass transmembrane proteins such as CFTR, anti-drug antibody (ADA) assays have proven to be very challenging to develop, primarily due to the difficulty in stably expressing recombinant CFTR *in vitro* in sufficient quantity. Leveraging a recently developed technology platform that allows for stable expression of CFTR within a cell membrane, we were able to develop what we believe is the first ADA assay against CFTR.

Methods

The CFTR ADA assay is an electro-chemiluminescent (ECL) sandwich immunoassay that employs a novel CFTR capture reagent, a commercially available mouse surrogate positive control (SPC) antibody, and a detection cocktail containing SULFO-anti-mouse, SULFO-anti-human kappa and SULFO-anti-human lambda antibodies. This configuration enables the detection of all major antibody subclasses, IgG, IgM and IgA, in human serum.

To establish the robustness and reliability of the assay, extensive assay optimization and characterization were performed. Key assay parameters including specificity, sensitivity, intra- and inter- assay precision, and selectivity were systematically evaluated to ensure that the assay is capable of accurately measuring an immunogenicity response. The resulting assay can reliably detect a humoral immune response to a CFTR transgene.

Critical Reagents


Biotinylated CFTR Nanodisc, KACTUS

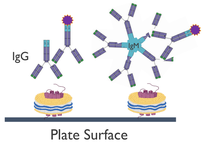

CFTR Nanodisc, KACTUS


Empty Nanodisc, KACTUS


Surrogate Positive Control (SPC) mouse monoclonal anti-CFTR Antibody, Abcam

ECL Sandwich Immunoassay Format

Biotinylated CFTR Nanodiscs are passively coated onto streptavidin coated plates and serve to capture CFTR-specific serum antibodies. Serum is washed away and captured antibodies are detected with SULFO-TAG conjugated anti-kappa, anti-lambda antibody (anti-mouse detection for the positive control). Format detects all antibody isotypes. IgG and IgM are shown.



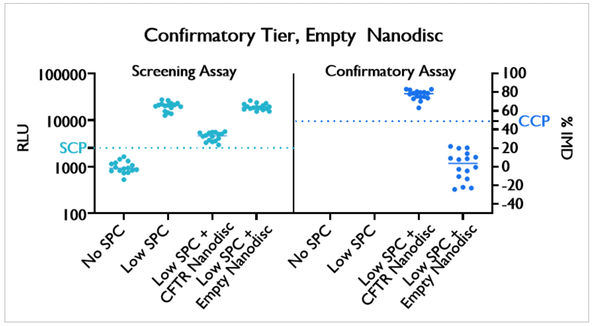
SULFO- anti-NHP/Hu IgG kappa, lambda

Test Article / SPC

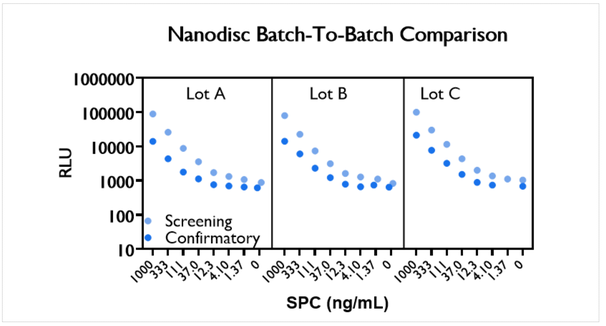
CFTR Nanodisc

Assay Characterization

The Nanodisc is a highly complex reagent, with CFTR embedded into a HEK cell membrane surrounded by proprietary polymers. This complexity results in the high potential for non-specific binding of antibodies (both anti-cell as well as anti-polymer) or other interfering molecules. Given this inherent complexity and potential for false positives, extensive characterization and reagent optimization were performed to ensure that the assay is specific to CFTR. Empty Nanodisc will be included in the confirmatory tier of the assay to ensure the specificity of the response.



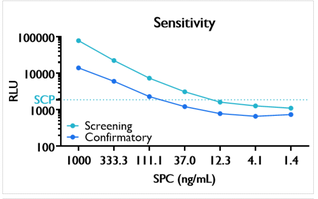
In the confirmatory tier of sample testing, CF and healthy individuals at low SPC do not confirm positive with Empty Nanodisc but do confirm as positive with CFTR Nanodisc.

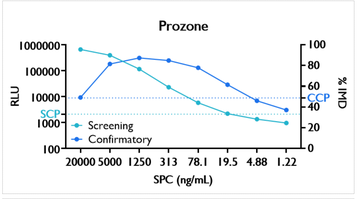


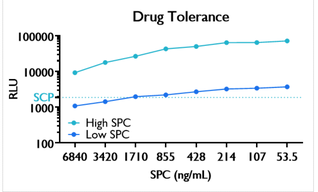
No differences were observed between three lots of CFTR Nanodisc (Lot A transiently transfected and Lots B and C stably transfected) in both the screening and confirmatory tiers.

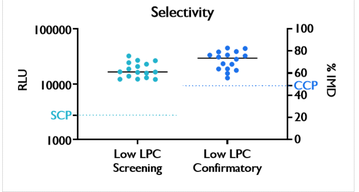
Results

Using an anti-CFTR SPC antibody, the assay demonstrated repeatability (inter- and intra-assay), strong drug tolerance in the presence of endogenous levels of circulating CFTR, and a sensitivity below the 100 ng/mL threshold recommended for ADA assays. The assay is highly selective, detecting and confirming both high and low levels of SPC in the presence of lipemic and hemolyzed samples. No prozone effect was observed in the screening assay at the concentrations tested, though prozone was observed in the confirmatory assay at high SPC concentrations, necessitating an addition dilution step in the titer assay for high signal samples. Collectively, the qualification established the assay as a highly sensitive, specific, and repeatable method for monitoring anti-CFTR immunogenicity in human serum.









Assay Parameter	ECL Sandwich Assay
Screening Cut Point Factor	2.41
Confirmatory Cut Point	48.8%
Screen LOD [SPC antibody]	31 ng/mL
Confirmation LOD [SPC antibody]	96 ng/mL
High SPC Inter-Assay Precision (%CV)	27%
Low SPC Inter-Assay Precision (%CV)	25%
High SPC Drug Tolerance [CFTR Nanodisc]	>6840 ng/mL
Low SPC Drug Tolerance [CFTR Nanodisc]	922 ng/mL
Matrix Interference	Hemolysis: Tolerated Hyper-lipidemia: Tolerated
Prozone	Screening: Absent Confirmatory: Present at high RLU

Conclusion

Similar to many large transmembrane proteins, recombinant CFTR protein is prone to misfolding and degradation, making it unstable, difficult to express, and yielding insufficient quantities for immunoassays. Due to difficulties with expression and stability, all previous attempts to develop a CFTR ADA assay, both internally and externally, have been unsuccessful. Our CFTR ADA assay overcomes these challenges by leveraging a recently developed technology platform that enables stable expression of CFTR within a cell membrane. This sensitive, repeatable, and drug tolerant assay enables clinical immunogenicity monitoring for transgene-expressed CFTR. Likewise, this assay format can be easily adapted to other difficult to express and multi-pass transmembrane proteins, making it an attractive new format to add to the immunogenicity repertoire.

References

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