Antibody Purification For Use in Cystic Fibrosis Translational Pharmacology

Victoria Hung, Hellen Kim, Marko Pregel, Ryan Tyler
Rare Disease Research Unit, Pfizer, 610 Main St., Cambridge, MA 02139, USA

BACKGROUND AND SIGNIFICANCE

Background
- One of the most common lethal genetic diseases in Caucasians, with almost 60,000 affected people worldwide.
- Caused by a deficiency in the function of CFTR, a chloride channel regulating epithelial ion transport and homeostasis.
- Disease morbidly is caused by chronic lung inflammation and infection.
- Most common mutation in patients is ∆F508: 80% have one copy
- ∆F508 destabilizes CFTR and causes an overall reduction of channel density at the cell surface due to:
  - Increased ER degradation.
  - Increased rate of endocytosis and lysosomal degradation of active chloride channel.

Key Observations:
- Compounds that increase ∆F508del CFTR plasma membrane density (“correctors”) correlate with an increase in total CFTR protein levels. Levels of CFTR have been detected with flow cytometry in both human nasal epithelial cells and in leukocytes.
- Some of the best characterized monoclonal anti-CFTR antibodies are only available in ascites. The presence of contaminating serum/ascites may affect their ability to be used in clinical samples by measuring changes in total ∆F508del CFTR.

Question: Can flow cytometry be used to assess corrector efficacy in clinical samples by measuring changes in total ∆F508del CFTR?

Challenge: Some of the best characterized monoclonal anti-CFTR antibodies are only available in ascites. The presence of contaminating proteins in ascites do not make them amenable to the development of clinical assays.

Goal of Project:
- Establish methods to purify mAbs from ascites containing anti-CFTR mAb UNC 596 (Univ. of North Carolina).
- Establish flow cytometry assays using recombinant cell lines to assess mAb reactivity.
- Assess if purified mAbs exhibits better flow cytometry assay profile to help drive decision on whether to pursue further clinical assay development.

MATERIALS AND METHODS

Step 1: Affinity Protein G Spin Column Purification of UNC 596 CFTR Antibody

Step 2: Coomassie Staining of SDS-PAGE Gel

Step 3: Staining of Total CFTR in HEK293 Stable Cells
  - HEK293 WT CFTR cells were used as positive control staining of CFTR while parental HEK293 cells were used as negative control.

RESULTS

Initial Assessment of mAb UNC 596:
- Significant contamination with serum proteins
- mAb concentration not suitable for AKTA purification

Affinity Pro-G Spin Column Purification of UNC 596

Flow Cytometry Staining Optimization of Total CFTR Using UNC 596 Antibody in HEK293 Cells

Secondary Antibody Titration for Optimal Staining Dilution

CONCLUSIONS
- The protein purification process yielded only 25% of the final product meaning an efficient purification would require large amounts of starting material.
- Using mAb UNC 596, we demonstrated that purification of the IgG increases the fold change over background in flow cytometry (2.7 vs. 4.2-fold) compared to the original ascites. However, it may not be the best antibody to use for total CFTR flow cytometry because there are commercially available purified mAbs that exhibit a 7-10 fold difference in our assays.
- The significant increase in signal-to-noise in flow cytometry of purified UNC 596 indicates that purification of mAbs from sera/ascites may also better their flow cytometry profile.

REFERENCES

ACKNOWLEDGEMENTS
I would like to thank both Hellen Kim and Ryan Tyler for their guidance and invaluable mentorship, as well as the Pfizer Fibrosis group. I would also like to thank Vanessa Barnett for her expertise and guidance with our protein purification process and Daren Ferguson for the gift of his purified IgG. I am grateful to both Pfizer and Northeastern University for being able to participate in a great immersive learning experience.