

Plan of Work - MSc Thesis

Integrity of CFTR mRNA with PTC mutations and assessment of read-through therapies for Cystic Fibrosis

Background: A major challenge in current cystic fibrosis (CF) research is developing CF transmembrane conductance regulator (CFTR) mutation class specific therapies. Currently, six classes of mutations that may be susceptible to CFTR drug-based rescue are recognized. A significant proportion of the ~2000 potentially disease causing CFTR variants identified to date are Class I mutations, *i.e.*, those introducing an in-frame premature termination codon (PTC). Translation of an mRNA transcript bearing a PTC will produce a truncated and most likely non-functional protein, but this is largely avoided by an mRNA surveillance mechanism termed nonsense mediated decay (NMD). One potential treatment for PTC mutations is the use of read-through agents such as aminoglycoside antibiotics like gentamycin or tobramycin, which can promote insertion of near cognate amino acids in place of the PTC, thereby allowing translation of a full-length and potentially functional protein. However, NMD might reduce the effectiveness of any PTC read-through therapy by removing its substrate. We have found many PTC mutations in CFTR to be subject to similar levels of NMD, which reduce (by 60%-70%) but do not abolish PTC bearing mRNAs (Clarke et al, 2019).

Objectives: To investigate whether the reduced but consistent levels of PTC-bearing CFTR mRNAs we have detected represent: a) whole molecules that can be still be translated into full length CFTR protein following read-through, or b) a heterogeneous population of partially degraded mRNA that would in fact compromise the effectiveness of read-through approaches to PTC mutation correction.

Methodology: This proposal comprises a variety of strategies, and the following specific tasks:

- 1) Allele-specific quantitative PCR and Northern blot will first be used to determine the integrity of PTC bearing mRNAs and the extent of their partial degradation by NMD.
- 2) Western blot will be used to determine responses to read-through agents and the effects of the measured mRNA integrity on their efficacy, with or without inhibition of NMD.

The materials used in this project include: a) 16-HBE cell lines homozygously expressing some of the more common PTC mutations in CFTR (eg, Y122X, G542X, W1282X), and b) patient-derived materials (e.g., nasal epithelial cells or intestinal organoids) expressing the same mutations either in homozygosity or in *trans* with the most common CFTR (non-PTC) mutation, F508del. The latter will be used for validation of data obtained in the cell lines.

We expect the work proposed to further characterize the effects of PTC mutations on CFTR mRNA expression and provide crucial data for the implementation of rescue strategies specific for this as yet untreated class of mutations.

Reference:

Clarke LA, Awatade N, Felicio V, Silva IA, Calucho M, Pereira L, Azevedo P, Cavaco J, Barreto C, Bertuzzo C, Gartner S, Beekman J, Amaral MD (2019). The effect of PTC mutations on CFTR mRNA abundance in human nasal epithelium and intestinal organoids: a basis for read-through therapies in Cystic Fibrosis. *Human Mutation* **40**:326-334.

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