# Rescue of Rare CFTR Mutations by Novel CFTR Correctors

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**Background:** Cystic fibrosis (CF) is a monogenic disease caused by mutations in the gene encoding the CF transmembrane conductance regulator (CFTR) protein, an anion channel expressed at the plasma membrane (PM) of secretory epithelia. The p.Phe508del is the most prevalent CF-causing mutation and leads to CFTR protein misfolding (*i.e.,* class II CFTR mutation) that is retained by the endoplasmic reticulum quality control (ERQC), thereby precluding its processing and trafficking to the PM, being instead targeted for proteasomal degradation. Although recent therapeutic progress has been achieved in developing CFTR corrector drugs for p.Phe508del-CFTR, a considerable number of rare CFTR mutations (so-called 'orphan' mutations) remains without any effective causal therapy. Various CFTR mutations exhibit a primary defect similar to that of p.Phe508del-CFTR. However, despite mutations in the same class are expected to be treated by the same approach (*i.e.,* correctors for class II CFTR mutations), they might not be efficiently rescued by the same chemical compound. We have recently investigated the efficacy and the mechanism of action of novel correctors (PTI-801, ABBV-2222, FDL-169) in rescuing p.Phe508del-CFTR, but their effects on other class II CFTR mutations remains to be exploited.

**Objectives**: To investigate the potential ability of novel correctors (PTI-801, ABBV-2222, FDL-169) in rescuing processing, trafficking and function of rare class II CFTR mutations, namely p.Glu92Lys, p.Val232Asp, p.Ala455Glu, p.Ser492Phe, p.Leu1077Pro and p.Gly1249Arg.

**Methodology:** The techniques/assays used in this project include:

1) Site-directed mutagenesis and cloning to generate cell lines expressing the CFTR mutation under study;
2) Halide sensitive-yellow fluorescence protein (HS-YFP) assay on a plate reader to determine CFTR function;
3) Western blotting to determine CFTR protein level and pattern;
4) Immunofluorescence microscopy to determine CFTR localization in the cell;
5) Ion transport measurements in micro-Ussing chamber.

**Bibliography**:

1. Bacalhau M *et al* (2023) Identification of novel F508del-CFTR traffic correctors among triazole derivatives. *Eur J Pharmacol* 938: 175396.
2. Lopes-Pacheco M *et al* (2022) Rescue of Mutant CFTR Trafficking Defect by the Investigational Compound MCG1516A. *Cells* 11 (1): 136.
3. Silva IAL *et al* (2022) Advances in Preclinical In Vitro Models for the Translation of Precision Medicine for Cystic Fibrosis. *J Pers Med* 12 (8): 1321.
4. Lopes-Pacheco M *et al* (2021) Discovery of CFTR Modulator for the Treatment of Cystic Fibrosis. *Expert Opin Drug Discov* 16 (8): 897-913.
5. Lopes-Pacheco M (2020) CFTR Modulators: The Changing Face of Cystic Fibrosis in the Era of Precision Medicine. *Front Pharmacol* 10: 1662.
6. Botelho HM (2015) Protein Traffic Disorders: an Effective High-throughput Fluorescence Microscopy Pipeline for Drug Discovery. *Sci Rep* 5: 9038.
7. Pedemonte N, *et al* (2011) High-throughput Screening of Libraries of Compounds to Identify CFTR Modulators. *Methods Mol Biol* 741: 13-21.