

Testing CFTR repair in cystic fibrosis patients carrying nonsense and channel gating mutations

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Background. In order to measure CFTR activity in a minimal invasive manner, we set up a method to assay functional CFTR and focused on leukocytes from healthy and CF donors. Leukocytes are recognized in the scientific literature as a key component of the pathogenetic events associated to cystic fibrosis and represent an easily accessible source of primary cells that might be exploited to monitor CFTR expression and activity.

Hypothesis and objective. The major aim of this project is to validate the sensitivity and specificity of a fluorescence reporter assay as a functional, minimally invasive test of CFTR channel activity in peripheral blood cells (PBMCs or monocytes).

Methods. The readout of this method is based on measurements of the residual amount of iodide left in the cell supernatant, after proper stimulation, quantified by the fluorescence of Halide Sensitivity Yellow Fluorescence Protein (HS-YFP).

Results. The specificity of the assay for CFTR activity is tested using two different CFTR inhibitors, CFTR-172 and PPQ-102, and downregulating CFTR (siRNA technology) in the acute monocytic leukemia MM6 cell line, highly expressing CFTR. The outcome was confirmed also in peripheral blood leukocytes collected by venipuncture (3-5 mL), performed in different centers and by different operators. Significant differences between WT and CF peripheral blood mononuclear cells (PBMC) and purified monocytes were recorded. Of special interest were the results obtained by the HS-YFP assay performed in PBMCs of a patient carrying the G1349D/F508del mutation and taking Ivacaftor, showing a significant increase in iodide transport at all time points (7) after the start of the treatment (running from October 2015 to September 2016). Furthermore leukocytes from 18 patients in a PTC clinical trial (flow cytometry for CFTR expression and HS-YFP assays available), 7 patients taking Orkambi, 4 patients in clinical trial 108/110 (VX

770+VX 661), 5 non-CF patients affected by COPD, 6 patients with recurrent pancreatitis were also tested by the HS-YFP CFTR assay. 14 S1251N CF patients before and after ivacaftor treatment were studied in Rotterdam confirming the ability of the VX770 potentiator to enhance S1251N CFTR activity in PBMCs in an acute fashion. We identified several assay variables that need further optimization among partners.

Spin-off for research and clinical purpose. HS-YFP assay might represent a minimally invasive, convenient and fast method to measure CFTR function in leukocytes. Monitoring CF patients taking CFTR correctors or potentiators with a simple blood test can represent a further step toward a personalized medicine approach in CF.

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