6.08 The CFTR modulator combination elexacaftor/tezacaftor/ivacaftor restores CFTR protein expression in circulating neutrophils of patients with cystic fibrosis

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Cystic Fibrosis (CF) is caused by mutations in the CF transmembrane conductance regulator (CFTR) gene and is characterized by sustained inflammation. Studies have demonstrated that neutrophils account for ~70% of the total cell count in CF bronchial lavage fluid. Neutrophil dysfunction in people with CF (PWCF) is due to lack of CFTR function and chronic inflammation, causing altered cell chemotaxis, oxidant production, degranulation and apoptosis. CFTR modulator therapy (elexacaftor/tezacaftor/ivacaftor (ETI)), improves FEV1 in PWCF. The aim of this study was to explore the impact of ETI on neutrophil CFTR protein expression. PWCF eligible for ETI-therapy (n=16, mean FEV=81%) were recruited, non-paired samples collected pre- (n=9) and 12 months post-ETI treatment (n=7). Neutrophil whole cell lysates were analysed for CFTR and NHERF1 expression, as NHERF1 mediates CFTR translocation to plasma membranes. Cytosolic [Ca^{2+}] and calpain activity were assessed fluorometrically. Markers of neutrophil adhesion (CD16b) and degranulation (CD66b) were assessed by flow cytometry.Compared to healthy control neutrophils, CFTR expression in CF neutrophils was significantly decreased ($p=0.04$). Significantly increased cytosolic activity levels of the Ca^{2+}-dependent cysteine protease calpain were detected in circulating CF neutrophils ($p<0.0001$), which lead to significantly decreased NHERF1 expression ($p=0.005$). ETI significantly decreased cytosolic Ca^{2+} levels ($p<0.0001$) and calpain activity ($p=0004$), with a corresponding increase in NHERF1 expression ($p=0.001$). Of importance, ETI increased neutrophil CFTR protein expression compared to the pre-therapy group ($p=0.0029$), and reduced the percentage of $CD16b^{+}/CD66b^{+}$ cells ($p=0.018$).

Our results conclude that ETI significantly increases CFTR expression in circulating blood neutrophils of PWCF and decreases markers of neutrophil activation. This is of particular interest as not all patients are currently eligible for ETI or other CFTR modulator therapy. ***Conflict of Interest:*** *None to declare*