Title: Cystic fibrosis epithelial cells are primed for apoptosis as a result of increased Fas

Dr. Sinéad 24225 Weldon s.weldon@qub.ac.uk ¹, Dr. Qiwei 24226 Chen qchen04@qub.ac.uk ¹, Dr. Irene 24227 Oglesby ioglesby@rcsi.ie ², Dr. Christine 24228 Wohlford-Lenane c-wohlford-lenane@uiowa.edu ³, Dr. Jennifer 24229 Bartlett jennifer-bartlett@uiowa.edu ³, Prof. Gerry 24243 McElvaney gmcelvaney@rcsi.ie ², Prof. Stuart 24253 Elborn s.elborn@qub.ac.uk ¹, Prof. Paul 24260 McCray, Jr paul-mccray@uiowa.edu ³, Dr. Catherine 24262 Greene cmgreene@rcsi.ie ² and Prof. Clifford 24272 Taggart c.taggart@qub.ac.uk ¹. ¹ Centre for Infection and Immunity, Queen’s University Belfast, United Kingdom ; ² Department of Medicine, Royal College of Surgeons in Ireland, Dublin, Ireland and ³ Department of Paediatrics, University of Iowa, Iowa City, United States.

Body: Apoptosis is a physiological process essential for homeostasis of epithelial organisation and function. Cystic fibrosis (CF) lung disease is characterised by chronic infection and inflammation and previous work suggests that apoptosis is dysfunctional in the CF airways with conflicting results. In addition, controversy exists regarding how CFTR misfolding contributes to apoptosis. In this study, we evaluated the relationship between CFTR mutation and apoptosis in CF airway epithelial cells. Basal activity of the executioner caspase, caspase-3, was significantly increased in CF tracheal and bronchial epithelial cell lines and primary bronchial epithelial cells compared to non-CF controls. In addition, activity of the upstream initiator caspase, caspase-8, was significantly increased in CF epithelial cells compared to controls, suggesting involvement of extrinsic apoptosis signalling, which is mediated by the activation of death receptors, such as Fas (CD95). Increased levels of Fas were observed in CF epithelial cells and bronchial brushings, reciprocal decreases in a selection of microRNAs predicted to target Fas were evident in the brushing samples, and neutralization of Fas significantly inhibited caspase-3 activity in CF epithelial cells compared to untreated cells. Furthermore, activation of Fas significantly increased caspase-3 activity and apoptosis in CF epithelial cells compared to control cells. Overall, these results suggest that CF airway epithelial cells are more sensitive to apoptosis via increased levels of Fas and subsequent activation of the Fas death receptor pathway. Further work will delineate the mechanism underlying increased Fas expression in CF epithelial cells.