# DEVELOPMENT OF IN VITRO TRANSCRIBED mRNA THERAPEUTICS FOR CYSTIC FIBROSIS

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**Background:**

Cystic fibrosis (CF) is a recessive disease that affects approximately 10,000 people in the UK. The disease is caused by mutations in the gene encoding the cystic fibrosis transmembrane conductance regulator (CFTR). Absent/non-functional CFTR leads to an imbalance of sodium, chloride and bicarbonate ion transport, and production of thick, sticky mucus in the lung which results in chronic bacterial infection and inflammation. Gene replacement therapy with viral/non-viral vectors has been explored in the last 25 years but all failed to show significant clinical efficacy. *In vitro* transcribed (IVT) mRNA has emerged in the last few years as a new approach for protein replacement. IVT mRNA offers potential advantages of greater efficiency of expression as mRNA delivery is only required to the cytoplasm rather than the nucleus, and safety as there is no risk of genomic integration.

**Methods:**

We are developing CFTR IVT mRNA therapy for CF to replace to wild type CFTR protein using receptor-targeted nanocomplex (RTN) formulations. RTNs consist of liposomes, and lung epithelial cell specific receptor targeting peptides and nucleic acid. We have optimised the ability of RTNs to transfect primary cystic fibrosis bronchial epithelial (CFBE) cells at submerged culture, air-liquid interface (ALI) culture and mouse lung using luciferase and GFP reporter IVT mRNAs. CFTR mRNA was also delivered and the transfection efficiency was assessed by Western blot.

**Results:**

We first optimised the RTN formulation, comparing combinations of three different cationic liposomes and five peptides. As a result, we identified a novel formulation for mRNA delivery that achieved almost 100% cellular uptake efficiency, and 90% transfection efficiency, compared to a maximum of approximately 20% with a plasmid reporter. The optimised formulations were able to deliver luciferase and GFP mRNA in ALI cultured cells and mouse lungs. CFTR IVT mRNA was successfully delivered to a lung epithelial cell line. 16HBE14o-, primary normal epithelial cells (NHBE) and CF epithelial (CFBE) cells. In addition, we found co-delivery of the commercial drug, corrector of CFTR: Lumacaftor (VX-809) improved the expression or stability of CFTR protein in CFBE cells and non-CF 16HBE14o- cells in submerged culture. Moreover, CFTR protein expression was shown to be upregulated in ALI culture of CFTR cells transfected with RTN CFTR mRNA by Western blot.

**Conclusions:**

IVT mRNA of CFTR delivered by RTNs is a promising novel therapeutic for cystic fibrosis. In addition, the flexibility of lipoplex allows co-delivery of CFTR mRNA with Lumacaftor, which leads to significantly improved CFTR expression.