CONTROLLED RELEASE OF GENE EDITING TECHNOLOGY FOR THERAPEUTIC MANAGEMENT OF CYSTIC FIBROSIS SYMPTOMS

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Cystic fibrosis is one of the most common inherited lethal diseases, caused by mutations in the *CFTR* gene. Small-molecule therapies have revolutionised patient outcomes but are lifelong interventions and cannot correct all *CFTR* mutation variants.

We have demonstrated effective non-viral gene delivery to mice using peptide-nanocomplexes composed of plasmid (p)DNA and GET peptides. Glycosaminoglycan (GET) peptides bind and transduce cell membranes and we have generated mucus-penetrating formulations; allowing enhanced delivery and transgene expression to epithelium when aerosolised. Our formulations utilise endosomal-escaping strategies to deliver gene correction/augmentation strategies, presenting a ‘genetic cure’ which includes treatment of CF patients unaffected by current state-of-the-art therapies.

Our pDNA vector library (lacking CpG dinucleotides, reducing methylation silencing), modified with S/MAR sequences, allows for increases in long-term retention/expression viability. Additional ‘integration sequences’ have been included, enabling us to target stable integration into the ‘safe harbour’ AAVS1 locus. We have confirmed our system’s efficacy with fluorescent-protein encoding *ZsGreen1* and will confirm CF correction in patient-derived lung cells with *CFTR* transgene pDNAs.

Presently, we are comparing strategies: HDR (homology driven repair) and HITI (homology-independent targeted integration) via CRISPR. We are also exploring directed integration with Rep-mediated (exploiting viral mechanisms targeting P5IEE to the AAVS1 locus) systems and *Sleeping beauty* transposase.

Here we present the efficiencies and comparison of different systems. Repeat delivery/transfections using GET technology does not affect cell viability, so we can build integration. Ultimately, an aerosol-based strategy to progressively correct CF patients, converting transient gene expression into stable life-long genetic correction, may be viable.