A randomised, double-blind, placebo-controlled trial of repeated nebulisation of non-viral cystic fibrosis transmembrane conductance regulator (*CFTR*) gene therapy in patients with cystic fibrosis

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Scientific summary

Repeated nebulisation of non-viral CFTR gene therapy

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Background

Cystic fibrosis (CF) is a chronic, life-limiting disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene encoding a chloride ion channel active on the apical surfaces of epithelia. Although it is a multisystem disease, the major morbidity and mortality is as a result of lung disease. In the conducting airways, lack of normal CFTR protein function leads to airway surface dehydration and impairment of the body's primary innate defence system, mucociliary clearance. Bacterial infections ensue from early life and the aggressive inflammatory response that results ultimately leads to irreversible airway scarring in the form of bronchiectasis and respiratory failure. Approximately 95% of CF patients die of respiratory failure unless a transplant is performed. The median age of death in the UK currently is 29 years.

With the exception of one new drug, the small-molecule potentiator ivacaftor (Kalydeco®, Vertex Pharmaceuticals, Boston, MA, USA), there are no licensed therapies targeting the basic defect. This drug is currently suitable for only a minority of patients (4–5%) with particular, relatively rare, *CFTR* gene mutations. All the other clinically available treatments target downstream consequences of the disease rather than the cause, and at best delay, rather than prevent, the decline in lung function.

Gene therapy, whereby a normal copy of the *CFTR* gene is introduced into cells of the conducting airways, has been considered for some time to be an attractive option, as, unlike the small-molecule approach, it would be mutation independent. Viral vectors have proved problematic because of immune responses occurring on repeat application, but non-viral approaches do not suffer from the same problem. Proof of principle has been confirmed for non-viral gene therapy, although trials have largely been single application and outcomes have been molecular rather than clinical.

The UK Cystic Fibrosis Gene Therapy Consortium (UK CFGTC) comprises scientists, clinicians and allied health professionals from three sites in the UK: University of Edinburgh, Imperial College London and University of Oxford. The consortium has been working together for more than a decade with the aim of developing clinically applicable gene therapy for patients with CF. In our wave I programme, we have chosen the most optimal non-viral vector; designed a plasmid capable of long-duration expression, with limited proinflammatory potential; identified the optimal nebuliser delivery system; and tested the product in two preclinical animal models and a single-application safety and dose-ranging trial.

Objective

The primary objective of this trial was to determine the clinical efficacy of the formulation delivered to the airways over a period of 1 year in patients with CF.

Design

This was a randomised, double-blind, placebo-controlled Phase IIb trial of the *CFTR* gene–liposome complex, pGM169/GL67A. Randomisation was performed via InForm™ version 4.6 (Phase Forward Incorporated, Oracle, CA, USA) and was 1 : 1, except for patients in the mechanistic subgroups (2 : 1). Allocation was blinded by masking nebuliser chambers.

Setting

Data were collected as the clinical and scientific sites and entered onto a trial-specific InForm, version 4.6 database.

Participants

Eligible CF subjects were at least 12 years old and had mild to moderate lung disease, with forced expiratory volume in the first second (FEV_1) between 50% and 90% predicted. Subjects could have any combination of *CFTR* mutations. Exclusion criteria included infection with organisms related to an increased rate of disease progression or posing a cross-infection risk (meticillin-resistant *Staphylococcus aureus, Mycobacterium abscessus* and the *Burkholderia cepacia* complex).

Intervention

Following a successful screening visit, subjects received 5 ml of pGM169/lipid 67A (GL67A) (active) or 0.9% saline (placebo) at 28 (±5)-day intervals over 1 year. Based on previous trial data identifying cytosine–phosphate–guanidine (CpG) motifs within the bacterially derived deoxyribonucleic acid (DNA) as the most likely cause of mild flu-like responses, and with an aim to increase the duration of expression, our chosen formulation comprises a plasmid, pGM169, encoding the *CFTR* gene driven by a CpG-free human cytomegalovirus enhancer/elongation factor 1a (*hCEFI*) enhancer/promoter. The cationic lipid is made up of three components to optimise DNA binding, stability and gene transfer: (1) cholest-5-en-3-ol (3β)-, 3-[(3-aminopropyl)[4-[(3-aminopropyl)amino]butyl]carbamate] (GL67); (2) 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine (DOPE); and (3) 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy (polyethylene glycol 5000)] (ammonium salt) (DMPE-PEG5000) formulated at a 1 : 2 : 0.05 molar ratio. Components were mixed following stringent standardised operating procedures by unblinded trial pharmacists on the day of dosing. The formulation was nebulised via a breath-actuated nebuliser, the AeroEclipse® II (Trudell Medical International Europe Ltd, Nottingham, UK), which had been masked and locked to prevent unblinding of patients or trial team. A subgroup of patients also received doses to the nasal epithelium via a standard nasal spray device. Randomisation was 1 : 1 except for the mechanistic subgroups, which were randomised 2 : 1 in favour of active treatment to enrich for this group.

Main outcome measures

The primary end point was the relative change in percentage predicted (based on Stanojevic reference ranges) FEV_1 over the 12-month period. Secondary clinical outcomes included other physiological tests [spirometric values, lung clearance index (LCI) assessed by multibreath washout using sulphur hexafluoride as a tracer gas, exercise testing and activity monitoring], structural changes on a computed tomography (CT) scan and a disease-specific, validated quality-of-life questionnaire. We also assessed inflammatory markers, infection burden and a large number of additional safety measures and collected data on adverse events (AEs). A mechanistic study was performed in two subgroups: one group of patients underwent bronchoscopies pre and post dosing, during which CFTR function was assessed with lower airway potential difference (PD) and samples were obtained for transgene DNA and messenger ribonucleic acid (mRNA) quantification as well as histology; the second group underwent similar measures in the nose after additional nasal dosing.

Results

We recruited 136 patients into the intention-to-treat cohort; the active and placebo groups were well matched at baseline with regard to age, sex, lung function severity and *CFTR* mutation class. The per-protocol cohort was predefined as those patients receiving at least 9 monthly doses of trial formulation; it consisted of 54 patients receiving placebo and 62 receiving gene therapy. The uneven split relates to the 2 : 1 randomisation in the mechanistic subgroup. There was a significant ($p = 0.046$) treatment effect (TE) of 3.7% [95% confidence interval (CI) 0.1% to 7.3%] in the primary end point of relative change in percentage FEV_1 at 12 months. There were also significant TEs in secondary end points, including forced vital capacity (FVC) ($p = 0.031$) and gas trapping on CT scans ($p = 0.048$); supportive, non-statistically significant changes were seen in the majority of other outcomes. Effects were noted by 1 month and were irrespective of sex, age or *CFTR* mutation class. Subjects with a more severe baseline FEV_1 had a FEV_1 TE of 6.4% (95% CI 0.8% to 12.1%) and larger responses in most other outcomes. However, the milder group also demonstrated trends towards a TE in the small airway measure, LCI, confirming that this group of patients may still benefit. The active group showed a significantly ($p = 0.032$) greater bronchial chloride secretory response; overall, there were no significant changes in nasal PD but some actively treated patients demonstrated improved chloride secretion. Plasmid DNA was detectable in the majority of samples from both upper and lower airways, although mRNA was not detectable; this assay is known to lack sensitivity. The formulation was safe with no evidence of immune responses and no differences in treatment-attributable AEs seen between the placebo and active groups.

Conclusions

The UK CFGTC has conducted the first trial of non-viral *CFTR* gene therapy designed specifically to detect clinical benefit. The trial formulation was designed to (1) permit repeated application over a period of time sufficient to determine change in clinically relevant outcomes; (2) minimise inflammatory responses by removing all CpG motifs; and (3) lead to extended duration of expression with a non-viral, humanised promoter. Monthly application of the pGM169/GL67A gene therapy formulation was associated with a significant improvement in the primary outcome, FEV_1. There were also significant improvements in FVC and gas trapping on CT scans, with supportive signals in other outcomes. Signals were larger in patients entering the trial with lower lung function, although they were apparent across the spectrum of disease severity. Evidence for *CFTR* expression was seen in the lower airway with changes in bronchial PD. The formulation did not lead to the generation of host immune responses and was confirmed as safe. The approach is of relevance to CF patients, independent of their underlying *CFTR* gene mutation.

Limitations

Although encouraging, the difference in FEV_1 between groups was modest and was not accompanied by detectable improvement in the quality of life of patients. The molecular assays appear to lack efficacy, and, although supportive changes were observed in bronchial PD, the mechanistic subgroups were underpowered.

Future work

The almost doubled benefit in patients in the more severe half of the group, based on $FEV_1 < 70\%$, is probably, in our opinion, related to increased proximal airway drug deposition. This provides encouraging support for improved outcomes if higher doses could be delivered. We will seek to explore this in a future trial, by increasing each dose, decreasing the dosing interval or, possibly, by maximising transgene-derived CFTR function with the coadministration of a potentiating drug. The consortium is also developing a pseudotyped lentivirus that leads in preclinical testing to high-level gene expression.

Trial registration

This trial is registered as ClinicalTrials.gov NCT01621867.

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