AUDIT, RESEARCH AND GUIDELINE UPDATE

A randomised, double-blind, placebo-controlled phase IIB clinical trial of repeated application of gene therapy in patients with cystic fibrosis

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ABSTRACT

The UK Cystic Fibrosis Gene Therapy Consortium has been working towards clinical gene therapy for patients with cystic fibrosis for several years. We have recently embarked on a large, multi-dose clinical trial of a non-viral, liposome-based formulation powered for the first time to detect clinical benefit. The article describes the details of the protocol.

Cystic fibrosis (CF), a common inherited disease, is caused by mutations in the gene CF transmembrane conductance regulator (*CFTR*). This gene encodes the CFTR protein, which is located on the apical surface of the epithelial cells and has many functions, the most important of which is thought to be ion transport. Abnormal ion transport leads to accumulation of thick secretions in the airways, infection, inflammation and eventually irreversible lung damage. There is currently no treatment that has been demonstrated to halt the natural progression of the disease; all available successful therapies merely slow down the rate of decline in clinical condition, which still leads to premature death. The first therapy to target the CFTR defect directly has recently been approved in the USA and the European Union: treatment with ivacaftor (marketed as Kalydeco) resulted in significant improvements in lung function, exacerbation rate, weight gain and quality of life in patients with the G551D mutation.[1] Although this mutation is relatively rare, affecting 4%–5% of the global CF population, these results have proved the principles that direct targeting of CF at its root cause and restoring CFTR protein function can be clinically beneficial.

In contrast to the mutation-specific small molecule approach, gene therapy, which seeks to introduce a normal copy of the *CFTR* gene into the cells of the conducting airways, will be applicable for CF patients with any genetic defect. The many trials conducted to date have provided proof of principle for gene transfer to the airways based on molecular or electrophysiological measures.[2] Most of the clinical trials assessing gene therapy have been with a single dose and were not designed to assess clinical benefit. In one case where this was the focus with a recombinant viral vector, results were disappointing. In addition, there are clearly immune recognition barriers to repeated application of most viral vectors including adenovirus and adeno-associated virus. Such an issue has not been observed with non-viral vectors.

The UK Cystic Fibrosis Gene Therapy Consortium (UKCFGTC, Imperial College, University of Edinburgh and University of Oxford; http://www.cfgenetherapy.org.uk) was formed over a decade ago from the three UK groups that had conducted CF clinical gene therapy trials. We work cohesively on a structured programme to bring CF gene therapy to clinical reality. Our initial efforts (as part of the Wave 1 programme) led to the selection of the best, currently available, gene transfer agent (a CFTR-expressing plasmid complexed with a cationic lipid), which has recently been assessed in a single-dose phase I and IIa safety study (Evaluation of safety and gene expression with a single dose of pGM169 or GL67A administered to the nose and lungs of the individuals with CF. EudraCT reference: 2007-004050-85). In parallel, we have performed a number of non-interventional studies, including tracking the responsiveness of outcome measures to intravenous antibiotics for an infective exacerbation and a larger, longitudinal observational study (run-in study) of multiple outcomes (such as physiological, structural, inflammatory and patient reported) in almost 200 CF patients. Based on these studies, we have identified outcome measures as well as patient characteristics for a subsequent trial involving the application of multiple doses of the gene transfer vector.

As CF is a lifelong disease and because the respiratory epithelial cells have only a limited life span, we reason that any gene therapy with a chance of clinical success will require repeated application. As, to date, no viral gene transfer agents retain efficacy upon repeated administration in man due to immune responses, we have conducted extensive work to identify the best currently available non-viral gene transfer agent. Extensive preclinical studies of different non-viral vectors indicated that the cationic lipid formulation, GL67A, was the most effective agent.[3] GL67A has been aerosolised into healthy volunteers and CF patients.[4] In our recent single-dose trial, the plasmid (pGM169) incorporated novel features specifically designed for prolonged duration of expression including the hCEFI transcriptional element consisting of CpG-free versions of the

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human cytomegalovirus enhancer and human elongation factor 1 α promoter. Preclinical studies demonstrated sustained levels of expression (to at least 2 months) with this new promoter, which could permit less frequent dosing. In addition, the DNA usually used in gene therapy trials is rich in unmethylated CpG dinucleotides, which are likely recognised by cellular defence mechanisms in humans and to which an inflammatory response is mounted. Such a response is thought to have contributed to the flu-like illness, reported in our previous trial in the group receiving DNA or lipid but not lipid alone.[4] The new plasmid has been rendered entirely CpG free in an attempt to reduce such an inflammatory response.[5] Data from the single-dose study indicate that, using such a CpG-free plasmid, a dose can be achieved at which such inflammatory responses are negligible.

Here, we describe the protocol of our current phase IIB clinical trial, a double-blind placebo-controlled parallel group study. This trial is being undertaken by the UKCFGTC incorporating the universities of Oxford, Edinburgh, Imperial College London and their associated National Health Service centres (NHS Lothian and Royal Brompton and Harefield NHS Foundation Trust). The aim of the trial is to assess the clinical benefit of repeated doses of nebulised, non-viral CFTR gene therapy over a 12-month period with forced expiratory volume in 1 second (FEV_1) as the primary outcome measure.

Inclusion and exclusion criteria are as shown in table 1.

SUBGROUPS, RANDOMISATION AND STRATIFICATION

The trial seeks to enrol 130 subjects in total. In addition to the main protocol, patients are invited to participate in one or both subgroups designed to provide gene expression data. Correlations between gene expression data and clinical outcomes may be of significant benefit for future trial design.

Table 1 Inclusion and exclusion criteria

Inclusion criteria|	Exclusion criteria
Confirmed diagnosis of cystic fibrosis (sweat testing or genetic analysis)	Infection with *Burkholderia cepacia* complex organisms, MRSA or *Mycobacterium abscessus*
Men and women aged 12 years and above	Recent acute upper respiratory tract infection;
FEV_1 of between 50 and 90% predicted	Significant nasal pathology*
Clinical stability at enrolment	Chloride secretory response on nasal PD of >5 mV (nasal cohort only)*;
Prepared to take effective contraceptive precautions	Previous spontaneous pneumothorax without pleurodesis†
Able and willing to withhold rhDNase treatment for 24 h before and after the gene therapy dose.	Recurrent severe haemoptysis†
	Current smoker
	Significant comorbidity (moderate or severe CF liver disease, significant renal impairment, significant coagulopathy†)
	Receiving second-line immunosuppressant drugs‡
	Pregnant or breastfeeding.

*Applies to nasal subgroup only.
†Applies to bronchoscopic subgroup only.
‡Examples: methotrexate, intravenous immunoglobulin.
CF, cystic fibrosis;FEV_1, forced expiratory volume in 1 second; MRSA, methicillin-resistant *Staphylococcus aureus;* PD, potential difference; rhDNase, recombinant human deoxyribonuclease.

Nasal subgroup: patients will receive, in addition to the nebulised dose, nasal doses via nasal spray. They will undergo functional (nasal potential difference) and molecular (mRNA on nasal brushings) assessment of CFTR expression. The use of the nose facilitates more frequent, non-invasive assessment.

Bronchoscopic subgroup: patients will undergo two flexible bronchoscopies at which lower airway potential difference will be measured, brushings will be taken for measurement of vector-specific mRNA levels and endobronchial biopsies will be assessed for evidence of remodelling, lipid accumulation and inflammation.

Subjects agreeing to participate in either of these two groups are randomised in a 2:1 fashion to enrich for actively treated subjects. The remaining subjects are randomised on a 1:1 basis to active treatment or placebo. Randomisation is stratified for centre (Edinburgh hospitals or Royal Brompton), age and FEV_1.

FORMULATION, BLINDING AND ADMINISTRATION

Five ml pGM169 (13.25 mg) and GL67A (75 mg) or placebo (0.9% saline) are mixed and placed in an AeroEclipse II Breath Actuated Nebuliser (Trudell Medical Instruments, London, Canada) by an unblinded pharmacist. Nebuliser units are then locked and masked with tape to prevent either patient or study team members from identifying the contents. Doses are delivered to patients in cubicles designed to comply with local NHS requirements. Pre-dosing bronchodilator is administered to limit any bronchoconstriction that could be induced by the hypotonic gene therapy formulation. Paracetamol is administered post dosing to limit symptoms of any mild inflammatory response. Nasal subgroup patients receive 2 ml volumes of the pGM169/GL67A formulation administered via a pump action spray device during short breaks in nebulisation.

DOSING

Subjects receive 12 doses of nebulised study drug at intervals of 28±5 days. An adaptive design was included to allow the early identification of cumulative side effects under which 20 subjects (10 active treatments and 10 placebos) would receive three doses before any further subjects were dosed. In addition to the trial visits undertaken by the entire cohort, this subgroup was seen on day 2 following each dose. Clinical examination findings, lung function, gas transfer and systemic inflammatory markers (the clinical trial team remaining blind to the day 2 values of the latter) were to be reviewed by the Data Monitoring and Ethics Committee (DMEC): satisfactory safety data would allow further recruitment to be initiated. The adaptive design of the protocol allowed for the possibility that, should side effects be deemed unacceptable, a second cohort of 20 patients would receive three doses of the formulation at a 2.5 ml dose, followed by DMEC review in a similar fashion.

OUTCOME MEASURES

The primary outcome measure is change in the per cent predicted FEV_1; 60 patients per treatment arm provides 80% power to detect a relative change from a baseline of 6% based on data from our run-in study. Major secondary outcomes include lung clearance index measured by multiple-breath washout, selected CT markers of airway disease and the validated questionnaire, CFQR. We are also assessing other spirometric markers, gas transfer, exercise capacity, activity monitoring, serum markers (inflammation and transgene-specific immune responses) and sputum markers (24-hour weight, microbiology, cell counts and soluble inflammatory markers). Data on adverse events and

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frequency of additional antibiotics for increased respiratory symptoms will be collected throughout.

REGULATORY APPROVAL

The trial has been approved by the Gene Therapy Advisory Committee and the Medicines & Healthcare products Regulatory Agency (GTAC184/http://www.clinicaltrials.gov/ct2/show/NCT01621867) is sponsored by Imperial College London and managed by the Imperial Clinical Trial Unit. Patients are currently being recruited at the clinical sites: Western General Infirmary and Royal Hospital for Sick Children, Edinburgh and Royal Brompton Hospital, London and referred in from a number of patient identification centres.

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Competing interests None.

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Provenance and peer review Not commissioned; internally peer reviewed.

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