CORRESPONDENCE

A Phase I/IIa Safety and Efficacy Study of Nebulized Liposome-mediated Gene Therapy for Cystic Fibrosis Supports a Multidose Trial

To the Editor:

The vast majority of treatments for cystic fibrosis (CF) target the downstream consequences of the disease and are incompletely effective. The success of the CF transmembrane conductance regulator (CFTR) potentiator ivacaftor has illustrated the clinical benefits arising from restoration of CFTR protein function (1). This agent is applicable as a monotherapy for a minority of patients with specific, rare mutations. *CFTR* gene therapy, a mutation-independent alternative, has demonstrated proof of principle for gene transfer in animal models and human trials, but only one study (using a viral vector) has unsuccessfully assessed whether clinical outcomes can be improved (2). In preparation for a phase IIb clinical trial of repeatedly administered, nonviral, liposome-mediated *CFTR* gene transfer assessing clinically meaningful outcomes (3), the UK CF Gene Therapy Consortium (www.cfgenetherapy.org.uk) undertook a single-application safety and dose-ranging study (NCT00789867).

Some of the results of these studies have been previously reported in the form of abstracts (4, 5).

The chosen plasmid DNA expresses CFTR under the control of the human cytomegalovirus enhancer/elongation factor 1α sequence (6), a modified EF1a promoter aiming for extended duration of expression (7), and was rendered CpG-free to minimize a host inflammatory response (6). The cationic lipid, GL67A, was chosen on the basis of extensive preclinical testing (8). After informed consent, adult patients with CF received a single nebulized +/− nasal dose of pGM169/GL67A. Reconstitution and preparation of pGM169/GL67A was undertaken on the day, and doses were delivered in sealed negative-pressure cubicles after pretreatment with inhaled salbutamol (albuterol). Preplanned adjunctive therapies including ibuprofen, prednisolone, or paracetamol were administered to some patients.

The primary outcome of the clinical study was safety; assessment included examination, standard hematology/biochemistry, adverse events, spirometry, lung clearance index, chest computed tomography scan, gas transfer, bronchial biopsy histology, and immune markers. pGM169-specific DNA and mRNA were measured on nasal and lower airway brushings, with potential difference also measured bronchoscopically and nasally. For the latter, "responders" were defined as demonstrating chloride secretion 5 mV or more greater than their mean predose value, and greater than any of their predose responses.

A total of 35 subjects (Tables 1 and 2) received a nebulized dose (5 ml, n = 8; 10 ml, n = 10; 20 ml, n = 17) via an AeroEclipse II (Trudell Medical International, London, ON, Canada) breath-

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actuated nebulizer (9). Three subjects undertook slow delivery (~75 vs. 25 min for each 5 ml). Standard spray devices were used for nasal delivery (2 ml, n = 21). According to pre-/post-device weighing, a mean (SD) of 88.7% (2.9%) of expected nebulized dose and 94.5% (15.0%) of expected nasal dose was delivered. There were two serious adverse events: one occurred after the predosing bronchoscopy (swelling of the uvula related to intubation) and led to observation overnight in hospital, and the other was an episode of pancreatitis occurring around Day 10 after dosing (10 ml nebulized cohort). The subject was exocrine pancreatic sufficient and had likely experienced previous similar, but undiagnosed, episodes.

Overall, in the trial, 94.3% of subjects experienced at least one adverse event, the majority of which were mild to moderate in severity and resolved spontaneously or with standard antipyretics. The most common occurred on the day of dosing and largely resolved within 24–48 hours (Tables 1 and 2): Typically, within the first few hours after dosing, a mild, self-limiting influenza-like systemic response was seen, most frequently in the 20-ml patients. This was not affected by slow delivery or coadministration of ibuprofen or prednisolone but was clearly dose-related and reduced by paracetamol. Symptoms of headache and/or tiredness were reported by 82, 70, and 13%, and raised serum inflammatory markers were recorded in 100, 60, and 63% of the 20-, 10-, and 5-ml groups respectively, with dose-related trends in maximal values. No patient dosed with 5 ml had a temperature higher than 38°C (Table 2). A relatively asymptomatic, dose-related, restrictive drop in spirometry was also observed, with no change in respiratory rate or oxygen saturation. No patient dosed with 5 ml showed a more than 20% relative fall in FEV_1 (Table 2). The 20-ml group showed a small, significant ($P < 0.05$) mean (SD) drop in gas transfer (transfer factor for carbon monoxide corrected for alveolar volume and hemoglobin concentration) on Day 2 of 4.5% (6.0%), which returned to baseline values by Day 14. No changes were seen in the other cohorts. Two of the 20-ml patients had small areas of ground glass opacity reported on their Day 2 chest computed tomography scans, which resolved by Day 14. No significant changes were seen in endobronchial histology (20 ml; n = 10).

Consistent with the proposed excretion route for lipids, small but significant serum creatinine rises within the normal range could be detected 8 hours after dosing in the 20- and 10-ml groups, but not the 5-ml cohort; there were no other biochemical changes. Bilirubin rose on Day 1 in all dosing groups, as with creatinine, remaining within the normal range, and normalized by Day 2. There was no evidence of immune responses based on double-stranded DNA antibodies or human CFTR-specific T cells.

Lung clearance index, a sensitive marker of pulmonary dysfunction (10), was included as a safety assay. Fourteen 20-ml patients with paired predosing and 28-day postdosing values showed a small but significant increase (i.e., a deterioration; Figure 1A). In contrast, and unexpectedly, on *post hoc* analysis, 11 of 14 patients in the lower-dosing groups (5 and 10 ml) showed a small but significant improvement (Figure 1A).

With respect to bronchial samples, 10 patients (all 20 ml) had paired pre- and postdosing bronchoscopies. pGM169-specific DNA was detected in all bronchial brushing samples at levels ~1000-fold

Table 1. Baseline Demographics

	All Nebulized Subjects	20 ml	10 ml	5 ml
Number of patients	35	17	10	8
Percentage male	65.7	70.6	60	62.5
Age, yr, median (range)	27.4 (16.4–61.6)	26.7 (17.3–50.1)	33.2 (16.4–61.6)	32.6 (24.3–46.4)
FEV_1% predicted at recruitment, mean (SD)	79 (14)	78 (11)	77 (13)	84 (18)
Percentage homozygous p.Phe508del	57.1	58.8	40	75
Pancreatic insufficient, %	82.9	88.2	80	75
Nebulized antipseudomonal antibiotics, %	80	82.4	70	87.5
rhDNase, %	40	29.4	60	37.5
Hypertonic saline, %	22.9	23.5	20	25
Daily (long-term) azithromycin, %	65.7	70.6	70	50
Insulin, %	17.1	23.5	10	12.5
Inhaled steroids, %	45.7	35.3	70	37.5

Definition of abbreviation: rhDNase = recombinant human DNase.

higher than in the nasal samples. pGM169-specific mRNA was detected in 2 of 10 postdosing samples. Paired bronchoscopic potential difference measurements were interpretable for 8 of 10 patients. There was a trend toward an increase in chloride secretion (Figure 1B) but no changes in sodium-related parameters.

With respect to nasal samples, pGM169-specific DNA was detected in all 15 brushing samples taken between Day 2 and Day 14 after dosing and in two of six samples at Day 28. pGM169-specific mRNA was detected in 3 of 21 postdosing samples, with all positive samples being observed at either Day 14 (n = 2) or Day 28 (n = 1). In keeping with previous published data, there were no changes in sodium parameters on nasal potential difference. In contrast, 6 of 16 subjects (37.5%) demonstrated a "response" in terms of chloride secretory capacity. Responses were seen most commonly in the zero chloride perfusion phase and at the 14-day point; they were of sustained duration in one subject (Figure 1C).

These data were important in informing the design of the phase IIb trial. Thus, based on these findings, 5 ml was selected as

the optimal dose, with paracetamol being used as an adjuvant to minimize the risk of unblinding. Although well-tolerated, the adverse effects of the 20-ml doses were considered prohibitive for use in a repeated administration trial. We consider that the efficient delivery of large volumes of viscous fluid into the airways led acutely to both the influenza-like and restrictive responses, analogous to those seen after bronchoalveolar lavage, and masking the effect of plasmid DNA CpG depletion. At lower volumes, the latter effect was "revealed," allowing safe dosing of 5 ml. The unexpected improvement in lung clearance index after only one administration at the lower doses was intriguing; larger numbers and longer follow-up are needed to confirm or refute this finding. The variable responses both in molecular and CFTR functional terms underscore the technical challenges inherent in these assays and the limited sensitivity to low levels of gene expression (11). The clean safety profile and encouraging improvements in a sensitive measure of airway health lend support to progression to a phase IIb multidose trial designed to detect clinical improvements after prolonged administration. ■

Table 2. Postdosing Responses

	Time Point of Maximal Effect	20 ml	10 ml	5 ml	*P* Value
Systemic					
Fever, % of group >38°C	6-8 h	93.3	50	0	0.0003
Maximum temperature, mean (SD)	6-8 h	38.6 (0.5)	38.0 (0.7)	37.4 (0.3)	0.0002
Blood leukocytes, mean (SD)	8 h	15.8 (3.2)	14.1 (4.5)	12.8 (3.8)	0.22
Blood neutrophils, mean (SD)	8 h	13.9 (3.4)	11.3 (4.1)	9.8 (3.5)	0.06
Serum C-reactive protein, median (IQR)	Day 2	59 (36-73)	51 (25-88)	38 (5-78)	0.4
Pulmonary					
FEV_1, % of group with >20% relative drop		66.6	33.3	0	0.02
FEV_1 relative % drop, mean (SD)	~6 h	24.6 (9.3)	17.5 (7.8)	16.8 (4.0)	0.08
FVC relative % drop, mean (SD)	~6 h	20.7 (2.9)	13.7 (2.2)	14.7 (2.2)	0.22
FEV_1/FVC ratio, absolute %	~6 h	−3.1	−2.7	−1.7	0.85

Definition of abbreviation: IQR = interquartile range.
Postdosing responses reported only for patients not receiving adjunctive treatments (20 ml, n = 15; 10 ml, n = 6; 5 ml, n = 6).
Results for two 20-ml patients receiving prednisolone and ibuprofen, respectively, lay within group spread. Data from six patients receiving paracetamol (10 ml, n = 4; 5 ml, n = 2) showed a trend to a reduction in systemic responses, but as numbers are small they have not been subjected to formal statistical analysis. Paracetamol was included in the future protocol to mitigate against the possibility that a patient might experience an influenza-like response and become unblinded. Three-group data were compared with analysis of variance, Kruskall-Wallis, or Fisher's exact tests, as appropriate.
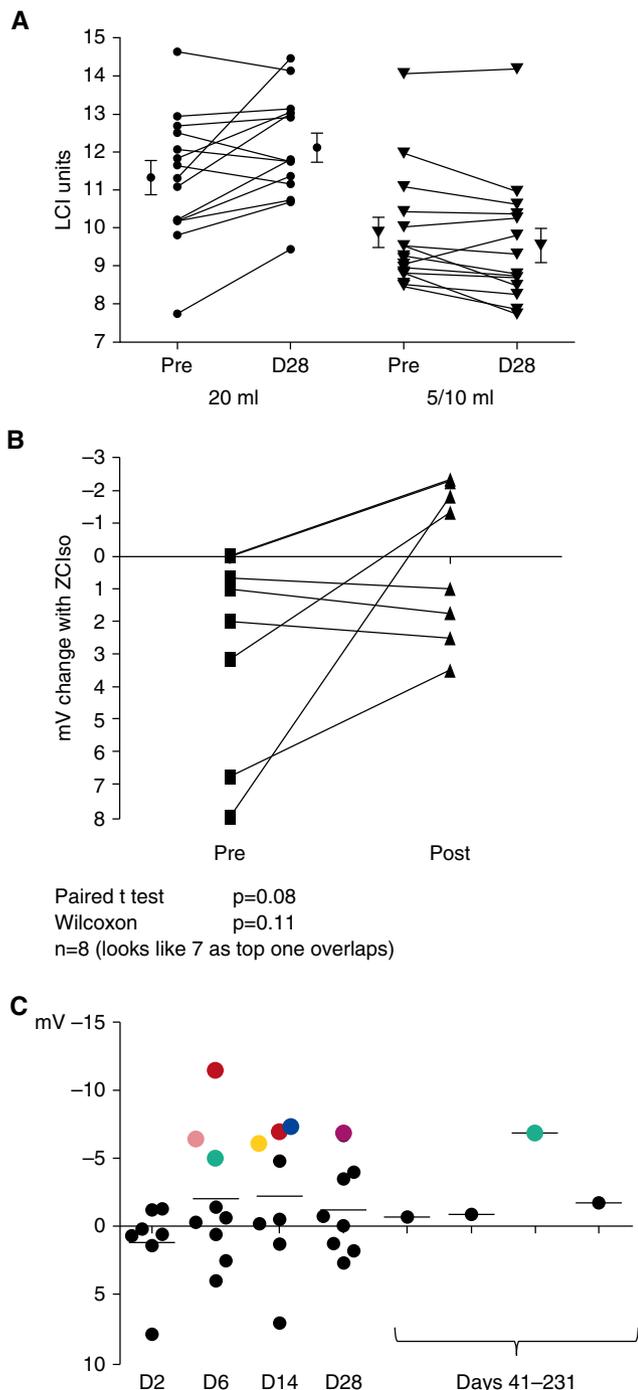
Figure 1. (*A*) Lung clearance index (LCI) increased (worsened) by a mean (SEM) of 0.75 (0.3) units in the 20-ml group (*P* = 0.03), whereas it decreased (improved) in the 5-/10-ml patients by 0.32 (0.1) (*P* = 0.04). The difference in change between the groups was significant (*P* = 0.003). (*B*) Because of the invasive nature of the procedure, bronchoscopic responses were assessed only in a subset of the 20-ml cohort. Chloride secretion was assessed in the lower airway by change in response to a zero chloride solution containing isoprenaline (isoproterenol) (ZCIso). Of the eight subjects with available pre- and post-treatment measurements, there was a trend (*P* = 0.08) toward non–cystic fibrosis (more negative) responses. (*C*) Nasal potential difference was measured in the group receiving nasal dosing. Sixteen subjects had scorable pretreatment chloride responses

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(zero chloride solution) and at least one post-treatment value. The figure shows the post-treatment values as a delta from the mean pretreatment. A more negative value indicates change toward non–cystic fibrosis. Responders (a post-treatment value at least 5 mV more negative than the mean pretreatment and greater than any of the [maximum 3] pretreatment values), are shown in color, with measures from the same patient being coded in the same color.

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Effects of Extracorporeal CO_2 Removal on Inspiratory Effort and Respiratory Pattern in Patients Who Fail Weaning from Mechanical Ventilation

To the Editor:

In patients with acute respiratory failure, severe respiratory acidosis is managed by either invasive or noninvasive ventilation (NIV). Recent data suggest that mechanical ventilation may be supported by extracorporeal CO_2 removal (ECCO2R) to manage the severe respiratory acidosis consequent to superprotective ventilatory strategies in patients with acute respiratory distress syndrome (1) or failure of NIV in patients with acute hypercapnic respiratory failure (2, 3).

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In patients who fail weaning from mechanical ventilation, the increase in respiratory muscle effort may quickly lead to respiratory muscle fatigue and pump failure (4). This prospective physiological pilot investigation aimed to examine the hypothesis that sharing CO_2 extraction between the minute ventilation provided by the respiratory muscles alone and the extracorporeal flow provided by ECCO2R in the absence of respiratory acidosis may result in a substantial reduction of respiratory muscle effort in patients who fail weaning from mechanical ventilation. Clinical trial registered with www.clinicaltrials.gov (NCT 02259335).

Methods

Four consecutive patients with chronic obstructive pulmonary disease ($FEV_1 = 24 \pm 17\%$ predicted) invasively ventilated for 9 ± 3 days for acute hypercapnic respiratory failure refractory to NIV were studied after giving written informed consent. The local institutional review board approved the protocol.

Inclusion criteria were at least two unsuccessful T-piece weaning trials and persistence of hypercapnia during invasive mechanical ventilation. Exclusion criteria are reported in the online supplement. On the morning of the study, the patients underwent a T-piece weaning trial. If they did not pass the trial, the patients were reconnected for a minimum of 2 hours to the ventilator, until they restored the previous gas exchange. Afterward, they repeated the trial with the addition of the ECCO2R (ProLung; Estor, Pero, Italy), started just 5 minutes before the suspension of ventilation. At the end of the latter trial, they could be extubated under ECCO2R if the predefined extubation criteria were fulfilled. The ECCO2R was continued until all the following criteria were stable for at least 12 hours: respiratory rate lower than 25 breaths/min, pH higher than 7.35, Pa_{CO_2} lower than 20% of baseline, and absence of signs of respiratory distress.

Changes in esophageal, gastric, and transdiaphragmatic pressures and pressure at the airway opening were measured as previously described (5). Inspiratory pulmonary resistance and elastance were assessed using Mead and Whittenberger's technique (6). Dynamic intrinsic positive end-expiratory pressure was measured (5), and the pressure time integrals of the diaphragm and esophageal pressures per minute were calculated (7). Inspiratory work of breathing performed by the patient was computed from esophageal pressure/tidal volume loops as previously described (8). Equipment dead space was 134 ml.

The CO_2 removal rate was calculated on the basis of internal measurements of CO_2 percentage and flow in the hollow-fiber membrane sweep gas.

Arterial blood gases together with the dyspnea score using the Borg scale were taken at the beginning and at the end of both T-piece weaning trials.

Results

All four patients who failed the first T-piece trial (49 ± 6 minutes) were able to successfully pass the second one when connected to the ECCO2R circuit and were extubated. All but one remained without mechanical ventilation for 48 hours; ECCO2R was suspended after 52 ± 3 hours. One patient needed to be reintubated after 4 hours for stridor and glottic edema. All patients tolerated the procedure well, but one required a change in the catheter site after 10 hours as a result of the catheter occlusion. Mean blood flow through the extracorporeal circuit was 320 ± 56 ml/min with a CO_2 removal of 78 ± 18 ml/min. Table 1 shows