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# Recombinant human DNase I in cystic fibrosis patients with severe pulmonary disease: a short-term, double-blind study followed by six months open-label treatment

P.I. Shah*, A. Bush*, G.J. Canny**, A.A. Colin+, H.J. Fuchs++, D.M. Geddes*, C.A.C. Johnson#, M.C. Light##, S.F. Scott*, D.E. Tullis†, A. De Vault++, M.E. Wohl+, M.E. Hodson*

*Recombinant human DNase I in cystic fibrosis patients with severe pulmonary disease: a short-term, double-blind study followed by six months open-label treatment. P.I. Shah, A. Bush, G.J. Canny, A.A. Colin, H.J. Fuchs, D.M. Geddes, C.A.C. Johnson, M.C. Light, S.F. Scott, D.E. Tullis, A. De Vault, M.E. Wohl, M.E. Hodson. ©ERS Journals Ltd 1995.*

**ABSTRACT: Chronic pulmonary infection is the major cause of morbidity and mortality in cystic fibrosis (CF). Recombinant human deoxyribonuclease (rhDNase) *in vitro* has been shown to dramatically reduce the viscoelasticity of the sputum from CF patients. Phase II and III clinical trials have shown the drug to be safe, and that patients with a forced vital capacity (FVC) of >40% predicted show an improvement in pulmonary function when receiving rhDNase. The current study evaluates the safety and efficacy of rhDNase in the most severly ill CF patients (FVC <40% predicted).**

**A double-blind, randomized, placebo-controlled trial in which patients received either 2.5 mg rhDNase twice daily or placebo for a period of 14 days followed by a 6 month open extension period (OEP) is reported. Seventy patients were recruited for the double-blind study, and 64 entered the OEP of whom 38 completed. During the OEP, all patients received 2.5 mg rhDNase twice daily.**

**In both the double-blind period and the OEP the drug appeared to be safe. During the double-blind study, forced expiratory volume in one second ($FEV_1$) and FVC improved in both groups but there was no statistically significant difference between the groups. In the OEP, there was mean improvement in percentage predicted $FEV_1$ and FVC, 9 and 18%, respectively, for all patients participating.**

**In conclusion, DNase is safe when administered in conjunction with a rigorous regimen of chest physiotherapy to severely ill patients (FVC <40% predicted) with CF. The double-blind, 14 day study showed no significant improvement in pulmonary function but some patients may have improved after longer administration of rhDNase.**

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*Dept of Cystic Fibrosis, Royal Brompton Hospital, London, UK. ++Genentech Inc., South San Francisco, CA, USA. ##University of California, San Diego, CA, USA. +Boston Children's Hospital, Boston, MA, USA. #Children's Hospital, Washington University, St Louis, MO, USA. †The Wellesly Hospital, University of Toronto, Toronto, Ontario, Canada. **Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada.

Correspondence: M.E. Hodson
Royal Brompton Hospital
Sydney Street
London
SW3 6NP
UK

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When cystic fibrosis (CF) was first described in 1938, 80% of patients died within a year of birth [1]. Nowadays, many are surviving to adult life and the median survival in the USA is now 28 yrs [2]. Most adults have lungs which are colonized by *Pseudomonas aeruginosa*, and the commonest cause of death is respiratory failure due to bronchopulmonary sepsis [3]. Accumulation of infected viscous secretions is the major cause of lung damage. Disintegrating inflammatory cells, in particular polymorphonuclear leucocytes, release deoxyribonucleic acid (DNA) and this is a major cause of the abnormal viscoelasticity of sputum from CF patients. Concentrations of DNA in infected sputum can be very high [4–6]. Human deoxyribonuclease I (DNase I) was cloned and sequencing of the expressed recombinant protein and the natural protein isolated from human urine indicated that the primary structure of recombinant (rh) and natural human DNase I is identical. Human DNase I has been shown to reduce the viscosity of CF sputum *in vitro* [7]. Two Phase I studies of aerosolized rhDNase carried out in hospital showed encouraging results [8, 9].

The first phase II clinical trial which was multicentre, double-blind and placebo-controlled, included 181 clinically stable patients. Their forced vital capacity (FVC) was more than 40% predicted at baseline and they were more than 8 yrs of age. These patients were randomized to receive either placebo or 0.6, 2.5 or 10 mg rhDNase twice daily. Patients were treated for 10 days. Administration significantly improved pulmonary function and well-being, and all three doses were well-tolerated. There was no increase in the number of serious respiratory adverse events. There was no evidence of anaphylaxis or drug allergy. There was a small increase in voice alteration, pharyngitis, dyspepsia and facial oedema [10].

In the UK Phase II study [11], 71 patients were randomized to receive placebo or rhDNase 2.5 mg twice daily

for 10 days. Administration of rhDNase significantly increased the forced expiratory volume in one second ($FEV_1$) by 13% ($p<0.001$) and FVC was increased by 7%, but this did not reach statistical significance. Quality of life, general well-being, CF-related symptoms and breathlessness all improved, but this did not reach statistical significance. There was no increase in the number of respiratory events, nor signs of anaphylaxis, drug allergy or antibodies to rhDNase. There was no increase in incidence of voice alteration, pharyngitis, dyspepsia or facial oedema.

These two studies concentrated on the 93% of the CF population who have an FVC of greater than 40% predicted. A further 7% of the CF population have end-stage lung disease with an FVC <40% predicted. Many of them are being prepared for lung transplantation. Results of transplantation are encouraging [12], but due to the shortage of donor organs many patients die on the long waiting lists.

This report is of double-blind, placebo-controlled, parallel design study of pulmonary function and safety of aerosol rhDNase in adults severely ill with CF over a 14 day treatment period and a 6 month optional extension period. This group of patients with a FVC of less than 40% predicted have a 50% chance of dying within 2 yrs even with the best available conventional medical treatment [13].

## Methods

Seventy patients with CF were recruited from three centres in the USA, one in Canada and one in the UK. The protocol was approved by the Institutional Review Boards at the participating hospitals and written consent was obtained. All patients had stable disease, *i.e.* FVC on Day 1 within 20% of screening FVC. They had a documented sweat sodium concentration of >70 $mmol \cdot L^{-1}$ and all were 5 yrs of age or more. Females of childbearing potential had a negative pregnancy test and were taking adequate contraceptive precautions. All patients were on a standard regimen of treatment and had no changes in antibiotics or admissions to hospital within 14 days of randomization. Patients had an FVC <40% predicted [14] for height, age and sex, and were able reproducibly to perform pulmonary function tests. Lung function was performed according to American Thoracic Society (ATS) guidelines and measurements were made at the same time each day for individual patients. Patients were excluded if they had severe hypercapnia, arterial carbon dioxide tension ($P_{a,CO_2}$) >10 kPa (>75 mmHg); severe hypoxaemia, with an oxygen saturation <85% on fractional inspiratory oxygen ($F_{I,O_2}$) greater than 0.5; respiratory acidosis, arterial pH<7.3; or were receiving assisted ventilation using an endotracheal tube. Patients with a history of haemoptysis requiring interventional therapy within 90 days prior to randomization, pneumothorax requiring a chest tube, focus of infection outside the respiratory tract, pregnant or lactating females, hospitalization for respiratory tract infection within 14 days of randomization, or use of other investigational drug or prior treatment with rhDNase were also excluded.

Patients were randomized to receive either 2.5 mg rhDNase or placebo twice daily for 14 days. Placebo contained 150 mM NaCl and 1.5 mM $CaCl_2$. During the double-blind phase, patients were followed for 28 days from the start of drug/placebo administration (table 1). All routine treatments, including physiotherapy, aerosol antibiotics, pancreatic enzymes, multivitamins, xanthines, bronchodilators and steroids, were maintained throughout the study. These patients were severely ill and, therefore, the first three doses of drugs were administered in hospital under medical supervision and vital signs were recorded every 4 h for the first 24 h. All patients received chest physiotherapy 30–60 min before inhalation of the study drug. The drug was administered using a Hudson T updraft II nebulizer and pulmo-aid compressor.

Efficacy variables were pulmonary function ($FEV_1$ and FVC), dyspnoea scores and quality of life questionnaires. Dyspnoea was evaluated using a visual analogue scale [15]. The patient was shown a 100 mm vertical line, in which the bottom of the line represented "no shortness of breath", and the top of the line represented "severe shortness of breath". The patient was asked to score the line to indicate the degree of breathlessness that he was experiencing. Patients also completed a quality of life questionnaire, which covered eight domains of health-related quality of life that were thought to be important in this patient population: cough, congestion, dyspnoea, physical functioning, emotional well-being, fatigue, staying in bed, and general health perception.

Safety variables were pulmonary function, adverse event review, laboratory evaluation and physical examination. Initial medical history, details of chest physiotherapy, inhaled bronchodilators usage and other medications were recorded. Patients underwent physical examination, including height, weight and temperature, routine haematology and biochemistry tests. Females had a pregnancy test.

Table 1. – Plan of study: double-blind protocol

| Day | -7 | 1 | 2 | 8 | 15 | 28 |
|---|---|---|---|---|---|---|
| Informed consent | X | | | | | |
| Vital signs | X | X | X | X | X | X |
| Height | X | | | | | |
| Weight | X | X | | X | X | X |
| History | X | | | | | |
| Physical examination | X | X | X | X | X | X |
| Dyspnoea score | | X | X | X | X | X |
| Quality of life questionnaire | | X | | X | X | X |
| Spirometry ($FEV_1$/FVC) | X | $X^1$ $X^2$ | $X^2$ | X | X | X |
| Chest radiograph | X | | | | | |
| Pulse oximetry | X | | | | | |
| Arterial or capillary blood gases | X | | | | | |
| Record of intercurrent events | X | X | X | X | X | X |
| Record all medications | X | X | X | X | X | X |
| Blood for biochemistry/ haematology | X | | | | X | |
| Serum antibodies to DNase | X | | | | X | X |
| Serum pregnancy test | X | | | | X | |

$X^1$: spirometry before study drug administration; $X^2$: spirometry 1–2 h after the first study drug administration. $FEV_1$: forced expiratory volume in one second; FVC: forced vital capacity; DNase: deoxyribonuclease.

FEV1 and FVC were recorded, as were dyspnoea scores and quality of life questionnaires. Patients commenced a diary of drug administration. Spirometry was repeated 1–2 h after the first dose on Day 1. All medications and intercurrent events were recorded. Follow-up visits were completed at Day 28. Serum and whole blood was obtained at baseline and Day 15 for full biochemical and haematological profile. Additionally, serum was assayed for the presence of antibody to rhDNase using a radioimmunoprecipitation assay (Genentech, San Francisco, CA, USA).

Follow-up visits were completed at Day 28, except for those patients who entered the 6 month open-label treatment phase of the study. These patients were readmitted on Day 15 of the study to commence treatment with rhDNase under medical supervision. Chest physiotherapy was performed 30–60 min prior to inhalation of rhDNase and vital signs recorded every 4 h. Spirometry was performed predose and 1–2 h postdose, on Days 15 and Days 16. Patients were then discharged to continue their treatment as out-patients. Their progress was evaluated at 12 further study visits over a 6 month period (table 2).

### *Methods of analysis*

The sample size was based on the change in FEV1 and made the following assumptions. A 12% increase from baseline to Day 15 (as seen in previous studies receiving rhDNase) constitutes a clinical benefit, whilst a 12% decrease would represent a significant safety concern in these patients. The standard deviation on the percentage change of FEV1 from baseline to Day 10 was 16 in a previous study. A sample size of 40 in each group would provide a 90% power to detect a difference of FEV1 of 12% at the 0.05 level of significance. The study was planned to randomize 80 patients but was concluded early for logistical purposes. The final sample size of 70 patients provides 95% confidence intervals, which clearly exclude changes as large as those proposed above.

The results for pulmonary function are presented as mean percentage changes from baseline unless otherwise stated. Conversion of pulmonary function variables to mean percentage changes from baseline allowed an assessment of the effect of treatment in a population with heterogeneous values for pulmonary function as suggested by the ATS [16]. Analysis of variance was used to compare the mean percentage change in FEV1 from baseline to treatment period. The method of analysis was to compare the mean percentage change in FEV1 from baseline to Day 15. Percentage change from baseline is defined as:

$$\frac{\text{FEV1 Day 15 - FEV1 Baseline (Day 1 pretreatment)}}{\text{Baseline FEV1}}$$

Similar analysis was performed for FVC. Quality of life data were analysed using t-test or nonparametric methods depending on the normality of the distribution.

## Results

Seventy patients were recruited, 35 received placebo and 35 rhDNase. The baseline characteristics of the two groups are shown in table 3. There were no significant differences between them for the parameters shown or quality of life scores. Five patients did not complete the placebo-controlled part of the study. Two died, two withdrew consent, and one had a heart-lung transplant.

The two patients who died both received rhDNase. One died of haemoptysis having only received one dose of the drug, the other of respiratory failure. The second had been ventilated a number of times in the previous few months. Postmortem examination of the first patient revealed a large aspergilloma cavity in the apex of his lung. The clinicians felt that the deaths were not related to administration of rhDNase. No patient experienced acute adverse events. The overall incidence of adverse events, including intercurrent illness, was comparable in the treatment groups. Nineteen serious adverse events were reported during the double-blind portion of the study (11 in eight patients in the rhDNase group, and eight in three patients in the placebo group). There were 173 adverse events reported (82 in the rhDNase group and 91 in the placebo group). The adverse events reported

Table 2. – Plan of study for 6 month open extension period

| Day | 15 | 16 | 22 | 29 | 36 | 43 | 57 | 71 | 85 | 99 | 127 | 155 | 183 | 197 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Informed consent | X | | | | | | | | | | | | | |
| Vital signs | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Height | | | | | | | | | | X | | | | |
| Weight | X | | X | X | X | X | X | X | X | X | X | X | X | X |
| History | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Physical examination | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Dyspnoea score | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Quality of life questionnaire | X | | X | X | | X | | X | | X | X | X | X | X |
| Spirometry (FEV1/FVC) | $X^1$ $X^2$ | $X^2$ | X | X | X | X | X | X | X | X | X | X | X | X |
| Chest radiography | | | | | | | | | | | | | X | |
| Review patient calendar | | | X | X | X | X | X | X | X | X | X | X | X | X |
| Record of intercurrent events | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Record all medications | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Blood for biochemistry/ haematology | X | | | X | | | | | | X | | | X | |
| Serum antibodies to DNase | X | | | X | | X | | X | | X | X | X | X | X |
| Serum pregnancy test | X | | | X | | | | | | X | | | X | |

$X^1$: spirometry before study drug administration; $X^2$: spirometry 1–2 h after the first study drug administration. For further abbreviations see legend to table 1.

Table 3. – Baseline characteristics (Day 1): double-blind period

| | Placebo | rhDNase |
|---|---|---|
| Patients n | 35 | 35 |
| Age yrs* | 26±2 | 25±1 |
| | (9–48) | (5–41) |
| Sex M/F | 16/19 | 22/13 |
| Height cm** | 162±2 | 165±2 |
| Weight kg** | 48±2 | 50±2 |
| Oxygen saturation %** | 89±1 | 88±1 |
| Baseline $FEV_1$ % pred* | 21±1 | 22±1 |
| | (12–29) | (14–39) |
| Baseline FVC % pred* | 35±1 | 34±1 |
| | (16–45) | (20–46) |
| Baseline dyspnoea score* | 42.6±4.0 | 42.3±4.6 |
| | (0–96) | (0–99) |

*: data are presented as mean±SEM, and range in parenthesis; **: data are presented as mean±SEM. rhDNase: recombinant deoxyribonuclease; M: male; F: female; % pred: percentage of predicted value. For further abbreviations see legend to table 1. There were no significant difference between placebo and rhDNase groups.

were similar in the groups and were consistent with CF. Table 4 details the adverse events related to the respiratory system. No patients had antibodies to rhDNase detected in serum samples at Days -7, 15 and 28. Changes in biochemical and haematological profile were not evident among rhDNase or placebo-treated patients.

Changes in $FEV_1$ are shown in table 5 and figure 1. The maximum mean increase in the placebo was 6% and in rhDNase 7%. The differences were not statistically significant. Changes in FVC are shown in table 5 and figure 2. The maximum mean improvement in the placebo group was 14% and in the rhDNase group 13%. The difference was not statistically significant. Dyspnoea scores and quality of life scores showed no significant differences between the two groups.

Of the 65 patients who completed the double-blind period, 64 elected to enter the open extension period (OEP). Thirty nine patients completed treatment but one was not able to attend for spirometry on Day 183. Twenty five patients were unable to complete the full 6 months treatment protocol. One patient was mandatorily withdrawn

Table 4. – Numbers of patients having adverse events related to the respiratory system

| Adverse event | Placebo n=35 | rhDNase n=35 |
|---|---|---|
| Cough increase | 6 | 2 |
| Dyspnoea | 7 | 11 |
| Haemoptysis | 3 | 2 |
| Pneumothorax | 1 | 0 |
| Laryngitis | 0 | 1 |
| Voice change | 3 | 1 |
| Rhinitis | 6 | 2 |
| Sputum increase | 7 | 6 |
| Wheeze | 1 | 0 |
| Hyperventilation | 1 | 0 |
| Pharyngitis | 3 | 2 |
| Sinusitis | 1 | 1 |
| Others | 4 | 7 |

rhDNase: recombinant deoxyribonuclease.

Table 5. – Mean percentage change from baseline in percentage predicted $FEV_1$ and FVC double-blind period

| | Placebo | | | rhDNase | | | |
|---|---|---|---|---|---|---|---|
| Study Day | Pts n | $FEV_1$ | FVC | n | $FEV_1$ | FVC | p-value |
| 1 | 34 | 0.5 (1.1) | 4.5 (2.2) | 35 | 0.2 (1.7) | 1.8 (1.9) | NS |
| 2 | 34 | 5.0 (1.6) | 13.6 (4.2) | 34 | 7.0 (2.2) | 10.6 (2.9) | NS |
| 8 | 34 | 6.0 (1.7) | 13.5 (3.6) | 32 | 6.4 (3.1) | 12.7 (3.6) | NS |
| 15 | 34 | 4.2 (2.2) | 13.7 (3.8) | 31 | 1.4 (2.1) | 8.8 (3.6) | NS |

Data are presented as mean, and SEM in parenthesis. NS: not significant; Pts: patients. For further abbreviations see legend to tables 1 and 3.

as per protocol due to pregnancy, four patients underwent lung transplantation, seven patients decided to discontinue treatment, one withdrew due to an adverse event, and one was lost to follow-up. Eleven patients died.

No patient experienced an acute adverse event. A total of 570 adverse events were recorded during 168 day OEP. Forty eight were serious (mainly exacerbations of respiratory infection). A hundred and sixty were severe and one of undetermined severity. None of the deaths were unexpected or attributed by the investigator to the drug. One patient withdrew due to an adverse event (a respiratory tract infection). The overall adverse event profile was consistent with that seen in patients with CF. There were no consistent changes from baseline in any of the laboratory profiles or physical examination.

The patients who participated in the OEP (n=64) demonstrated mean improvements in percentage predicted $FEV_1$ and FVC at their last visit of 9% and 18%, respectively. Patients who completed the OEP (n=38) demonstrated mean improvements in percentage predicted $FEV_1$ and FVC of 11% and 23%, respectively, at the end of the

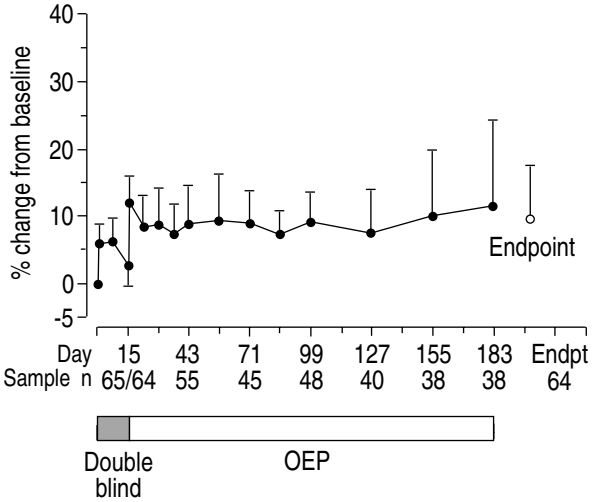


Fig. 1. – Mean (and 95% confidence interval) change from baseline in percentage of predicted $FEV_1$. Endpoint is the mean of the last measurement of all patients who entered the study. During the double-blind period half the patients received placebo and half rhDNAse and during the open extension all were receiving rhDNase. $FEV_1$: forced expiratory volume in one second; OEP: open extension period; rhDNase: recombinant deoxyribonuclease.

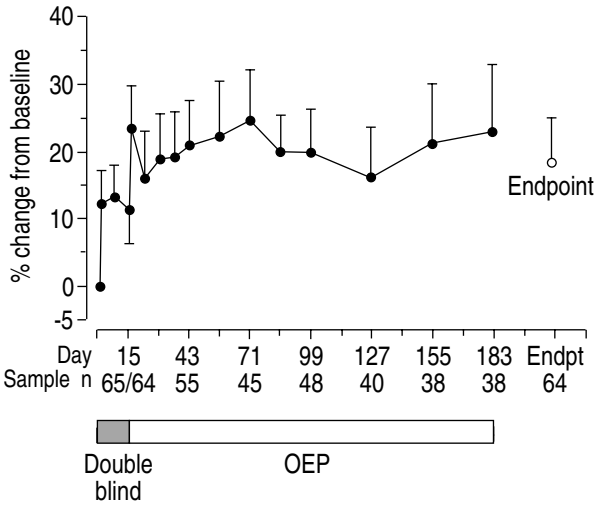


Fig. 2. – Mean (and 95% confidence interval) change from baseline in percentage of predicted FVC. Endpoint is the mean of the last measurement of all patients who entered the study. During the double-blind period half the patients received placebo and half rhDNase and during the open extension all were receiving rhDNase. FVC: forced vital capacity. For further abbreviations see legend to figure 1.

study (figs 1 and 2). The completers had a mean baseline FVC of 36% predicted compared to 32% for the patients who did not complete. There was some non-statistically significant improvement in the quality of life and dyspnoea scores.

## Discussion

Both in the double-blind study and the OEP, DNase appeared to be safe. There may be a slight concern about the increase in dyspnoea (table 4), although this did not reach statistical significance. It is possible that these patients with low body weight who have been chronically ill for a long time are very weak, and unable to cough up their sputum, which rhDNase liquefies. Some of the sputum may progress lower down the airway causing increased breathlessness. Previous studies have shown that rhDNase is effective and safe in patients with an FVC above 40% predicted, and this study has shown that in the severely ill patients with an FVC below 40% predicted the drug appears safe when used in conjunction with a rigorous regimen of chest physiotherapy.

There was no significant improvement in pulmonary function in this group of very sick patients in the short-term, double-blind period. However, it is interesting that there was some improvement in pulmonary function in both groups, but the rhDNase group did not reach the level of improvement seen in previous studies. The improvement seen in both groups initially may have been due to a number of factors: admission to hospital and very active chest physiotherapy; participation in the clinical trial; or close follow-up of the patients. Another possible explanation for the fact that the rhDNase group did not reach the level of improvement seen in previous studies may be that severely ill patients need a longer period of treatment for a significant improvement to occur. The results of the open-label phase may suggest a gradual improvement in pulmonary function over approximately 3 months. However, not all patients completed this study. Improvement in FVC was considerably greater than improvement in $FEV_1$. The improved mucus clearance may allow recruitment of more lung areas and, therefore, greater increases in FVC.

The short-term, double-blind study of 14 days duration showed no significant improvement in pulmonary function. Double-blind studies of at least 3 months duration in severely ill patients are now being undertaken.

## References

1. Andersen DH. Cystic fibrosis of the pancreas and its relation to coeliac disease: a clinical and pathological study. *Am J Dis Child* 1938; 56: 344–399.
2. FitzSimmons SC. The changing epidemiology of cystic fibrosis. *J Pediatr* 1993; 122: 1–9
3. Penketh ARL, Wise A, Mearns MB, Hodson ME, Batten JC. Cystic fibrosis in adolescents and adults. *Thorax* 1987; 42: 526–532.
4. Chernick WS, Barbero GJ. Composition of tracheobronchial secretions in cystic fibrosis of the pancreas and bronchiectasis. *Paediatrics* 1959; 24: 739–745.
5. Potter J, Matthews LW, Lemm J, Spector JS. Composition of pulmonary secretions from patients with and without cystic fibrosis. *Am J Dis Child* 1960; 100: 493–495.
6. Matthews LW, Spector S, Lemm J, Potter JL. Studies on pulmonary secretions. *Am Rev Respir Dis* 1963; 88: 199–204.
7. Shak S, Capon DJ, Hellmiss R, Marsters SA, Baker CL. Recombinant human DNase I reduces the viscosity of cystic fibrosis sputum. *Proc Natl Acad Sci USA* 1990; 87: 9188–9192.
8. Aitken ML, Burke W, McDonald G, Shak S, Montgomery AB, Smith AL. Recombinant human DNase inhalation in normal subjects and patients with cystic fibrosis: a Phase I study. *J Am Med Assoc* 1992; 267: 1947–1951.
9. Hubbard RC, McElvaney NF, Birrer P, *et al.* A preliminary study of aerosolised recombinant human deoxyribose I in the treatment of cystic fibrosis. *N Engl J Med* 1992; 326: 812–815.
10. Ramsey BW, Astley SJ, Aitken ML, *et al.* Efficacy and safety of short-term administration of aerosolised recombinant human deoxyribonuclease in patients with cystic fibrosis. *Am Rev Respir Dis* 1993; 148: 145–151.
11. Ranasinha C, Assoufi B, Shak S, *et al.* Efficacy and safety of short-term administration of aerosolised recombinant human DNase I in adults with stable stage cystic fibrosis. *Lancet* 1993; 342: 199–202.
12. Madden BP, Hodson ME, Tsang V, Radley-Smith R, Khaghani A, Yacoub MY. Intermediate term results of heart-lung transplantation for cystic fibrosis. *Lancet* 1992; 339: 1583–1587.
13. Kerem E, Reisman J, Corey M, Canny GJ, Levison H. Prediction of mortality in patients with cystic fibrosis. *New Engl J Med* 1991; 326: 1187–1191.
14. Knudson RJ, Lebowitz MD, Holberg CJ, Burrows B. Changes in the normal maximal expiratory flow-volume curve with growth and ageing. *Am Rev Resp Dis* 1983; 127: 725–743.
15. Gift AG. Validation of a vertical visual analogue scale as a measure of clinical dyspnoea. *Rehab News* 1989; 14: 323–325.
16. American Thoracic Society lung function testing. Selection of reference values and interpretive strategies. *Am Rev Respir Dis* 1991; 144: 1202–1218.