

**Exploring the role of CT densitometry: a randomised study of augmentation therapy in alpha-1 antitrypsin deficiency**

**A. Dirksen<sup>\*</sup>, E. Piitulainen<sup>#</sup>, D.G. Parr<sup>¶</sup>, C. Deng<sup>+</sup>, M. Wencker<sup>§</sup>, S.B. Shaker<sup>\*</sup> and R.A. Stockley<sup>f</sup>**

**AFFILIATIONS**

<sup>\*</sup>Gentofte Hospital, Copenhagen University, Hellerup, Denmark, <sup>#</sup>University Hospital, Malmö, Sweden, <sup>¶</sup>University Hospitals of Coventry and Warwickshire, UK, <sup>+</sup>Talecris Biotherapeutics Inc, Research Triangle Park, NC, USA, <sup>§</sup>Talecris Biotherapeutics GmbH, Frankfurt, Germany, <sup>f</sup>University Hospitals of Birmingham, UK

**CORRESPONDENCE**

Professor Asger Dirksen,  
Gentofte Hospital,  
Copenhagen University.  
DK-2900 Hellerup, Denmark  
Tel: 45 3977 7172  
Fax: 45 3977 7693  
E-mail: adi@dadlnet.dk

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**ABSTRACT:** Assessment of emphysema-modifying therapy is difficult, but newer outcome measures offer advantages over traditional methods. The EXAcerbations and CT scan as Lung Endpoints (EXACTLE) trial explored the use of CT densitometry and exacerbations for the assessment of the therapeutic effect of augmentation therapy in subjects with alpha-1 antitrypsin (AAT) deficiency.

Seventy-seven subjects (PiZ) were randomised to weekly infusions of 60 mg/kg human AAT (Prolastin®) or placebo for 2–2.5 yrs. The primary end-point was change in CT lung density, and an exploratory approach was adopted to identify optimal methodology, including two methods of adjustment for lung volume variability and two statistical approaches. Other end-points were exacerbations, health status and physiological indices.

CT was more sensitive than other measures of emphysema progression, and the changes of CT and FEV<sub>1</sub> were correlated. All methods of densitometric analysis concordantly showed a trend suggestive of treatment benefit (p-values for Prolastin® *versus* placebo ranged from 0.049–0.084). Exacerbation frequency was unaltered by treatment, but a reduction in exacerbation severity was observed.

In patients with AAT deficiency, CT is a more sensitive outcome measure of emphysema-modifying therapy than physiology and health status, and demonstrates a trend of treatment benefit from AAT augmentation.

**KEYWORDS:** Alpha-1 antitrypsin deficiency, computed tomography, emphysema, exacerbations, lung densitometry, lung function

Emphysema is believed to occur in individuals with alpha-1 antitrypsin (AAT) deficiency, primarily because of an inability to control neutrophil elastase (NE) released in the lung, thereby causing excessive tissue proteolysis and progressive alveolar destruction [1]. The hypothesis that emphysema progression could be reduced by redressing this imbalance has led to the development of plasma purification processes for AAT augmentation therapy for use in AAT deficiency in a number of countries.

A randomised placebo-controlled trial of AAT augmentation therapy with traditional physiological measures used as the primary end-point has not been undertaken because such a study is considered impractical [2, 3]; this therapy is therefore currently justified by clinical logic, proven biochemical efficacy [4] and data from well-controlled observational studies that are suggestive of treatment efficacy [5–8]. Forced expiratory volume in 1 s (FEV<sub>1</sub>) has been regarded as the “gold standard” outcome measure as a surrogate marker for emphysema progression; however, in order to detect a difference in FEV<sub>1</sub> decline of 15 mL/yr, it has been estimated that 494 subjects with a baseline FEV<sub>1</sub> of 35–79% predicted would be required per treatment arm over 3 yrs [2]. The change in computed tomography (CT) lung density has been shown to be a more sensitive and specific alternative measure than FEV<sub>1</sub> [9], and a randomised controlled efficacy study with change in lung density as a secondary end-point has been undertaken [10]. Although this latter study was not suitably powered to demonstrate a treatment effect, it did indicate that if CT scan was the primary outcome measure, a study of intervention in emphysema could be conducted over a shorter timescale in fewer patients than are needed for a physiological outcome.

CT scanning has been validated as an *in vivo* measure of emphysema by studies that have shown good correlation with pathology [11–12], exercise capacity [13], health status [13] and lung function [14, 15]. Furthermore, while CT densitometry is a more sensitive measure of emphysema progression and a better predictor of mortality than lung function [16], CT lung

density decline also relates to progressive reduction in FEV<sub>1</sub> [14]. Although these studies have led to the acceptance of this relatively novel technique, subsequent technological advances and a continued lack of consensus on methodology make further exploratory studies necessary.

The aim of the current study (the EXACTLE [EXAcerbations and CT scan as Lung Endpoints] study) was to assess various surrogate outcome measures for the progression of emphysema with focus on CT lung densitometry. In order to make the comparison as realistic as possible, we decided to carry it out as part of a randomised, double-blind, placebo-controlled clinical trial of the effects of AAT augmentation therapy in patients with severe AAT deficiency. The primary outcome was change in whole lung density between the two treatment groups measured by CT. Also, different methods for lung volume correction were explored. Other outcomes included lung function, health status and exacerbations of chronic obstructive pulmonary disease (COPD).

## MATERIALS AND METHODS

### ***Patients***

Patients with severe congenital AAT deficiency (all with AAT serum concentration  $<11 \mu\text{M}$ ) were recruited from AAT registries in Denmark, the UK and Sweden. Inclusion and exclusion criteria are reported in the online data supplement. The study was approved by relevant local ethics review committees and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines, and all patients gave written informed consent.

### ***Study design***

This was a randomised, double-blind, placebo-controlled, parallel-group study conducted at three European centres (Copenhagen, Birmingham and Malmö). Eligible patients were randomly assigned to weekly infusions of *either* AAT (Prolastin® 60 mg/kg body weight) *or* placebo (2% albumin) (in permuted blocks of four with stratification according to country), for 24 months, with an optional extension to 30 months in subjects who agreed to continue in the study. After randomisation, patients were seen every week to receive study medication, check diary cards, record any unscheduled visits to a health care provider, and note the occurrence of any adverse events (AEs). CT scans were performed at baseline and at 12 and 24 months, and there were options for additional scans at 3 months and 30 months (see online supplement for details). Post-bronchodilator lung function and health status were assessed at baseline and at 6, 12, 18, 24 and 30 months.

### ***CT densitometry end-points***

The progression rate of emphysema was determined by the change in the 15th percentile density (PD15) derived from the CT voxel distribution histogram of the whole lung. (For details of the CT scanner protocols and image analysis please see the online data supplement).

The reproducibility of level of inspiration during scan acquisition is recognised to influence lung density and reduces the reproducibility of CT lung densitometry. Consequently, different methods of adjustment for variation in inspiratory level between scans were explored: two methods were used to standardise density values for total lung volume (TLV), measured from CT, and the data were analysed using two different statistical approaches resulting in four different analytical methods (fig. 1).

In Methods 1 and 3, PD15 was adjusted in individual patient scan series to a ratio of TLV divided by the individual patient's predicted total lung capacity (pTLC) ("Physiological Adjustment"; see the online data supplement for further details) [17, 18]. In Methods 2 and 4, adjustment was made by including log(TLV) as a covariate in the statistical model [9, 19] ("Statistical Adjustment"; see Statistical Analysis below for further details).

### ***Other end-points***

Lung function (FEV<sub>1</sub>; diffusing capacity of lung for carbon monoxide, DL<sub>CO</sub>; and transfer coefficient, K<sub>CO</sub>) was assessed by post-bronchodilator spirometry and the single-breath method according to European guidelines [17]. The frequency of exacerbations was collected using patient diary cards and case-report forms, and exacerbations were defined according to the criteria described by Rodriguez-Roisin [20]. Moderate exacerbations were those requiring treatment with antibiotics and/or systemic corticosteroids. Exacerbations requiring emergency

room treatment or hospitalisation were considered to be severe. Health status was assessed according to scores on the St George's Respiratory Questionnaire (SGRQ) [21].

### ***Statistical analysis***

Analysis of the study population included intent-to-treat (ITT) and modified intent-to-treat (mITT) populations. The ITT population included all randomised subjects. The mITT population comprised the ITT population excluding patients with less than two valid CT scans (baseline and 12 months or after). Prior to unblinding, a review panel assessed CT scan data to identify invalid scans due to technical issues (see the online data supplement). These values were excluded from further statistical analyses. All CT scan analyses were based on the mITT population, whereas analyses on other end-points used the ITT population.

In Methods 1 and 2 for the densitometric analysis (fig. 1), treatment differences (Prolastin<sup>®</sup> *versus* placebo) were tested by linear regression on time of PD15 measurement in a random coefficient regression model (Method 1 – TLC-adjusted 15th percentile of lung density from CT scan as the dependent variable, treatment, centre, treatment by time interaction as the fixed effects, and intercept and time as the random effects; Method 2 – 15th percentile of lung density from CT scan as the dependent variable, treatment, centre, treatment by time interaction as the fixed effects, logarithm of total lung volume as a time-dependent covariate, and intercept and time as the random effects). The estimated mean slope for each treatment group represented the rate of lung density change with respect to time. The tested treatment difference was the estimated difference in slope between the two groups, considered to be equivalent to the difference in the rates of emphysema progression.

In Methods 3 and 4, the first and last available CT scans only were used in an end-point analysis (main effect ANCOVA model), either with the Physiological Adjustment (Method 3) or by including log(TLV) as covariate in the model (Method 4). The effect variables and covariates

were as follows: Method 3 – change from baseline to the last CT scan measurement in TLC-adjusted PD15 lung density as the dependent variable, treatment and centre as fixed factors, and baseline measurement as covariate; Method 4 – change from baseline to the last CT scan measurement in PD15 lung density as the dependent variable, treatment and centre as fixed factors, change in logarithm of CT-measured TLV and baseline measurement as covariates.

Before unblinding, Method 1 was identified as the primary end-point and Methods 2, 3 and 4 were identified as secondary CT outcomes. For the other efficacy variables, either the random coefficient model or the Cochran-Mantel-Haenszel (CMH) test was employed. This was an exploratory study; for practical reasons we did not power the trial as a definitive study for proving a beneficial effect of augmentation therapy for any of the efficacy end-points. The statistical analyses were performed using PROC MIXED procedure in SAS version 9.13. The detailed description about the use of this procedure can be found at <http://support.sas.com/documentation>.

## **RESULTS**

### ***Patient disposition and baseline characteristics***

Patient disposition is summarised in figure 2. Of the 82 patients enrolled into the study from the three centres, 77 patients were randomised to Prolastin<sup>®</sup> (n=38) or placebo (n=39), and 71 patients (n=36, Prolastin<sup>®</sup>; n=35, placebo) were included in the mITT population. The number of patients in the ITT population who completed the study (either 24 or 30 months, fig. 2) was 67, and 10 patients (three in the Prolastin<sup>®</sup> group and seven in the placebo group) discontinued prematurely, resulting in a median of 127 weeks of exposure to Prolastin<sup>®</sup> and 108 weeks to placebo. In terms of the mITT population, the study was completed by 34 (94%) and 31 (89%) patients in the Prolastin<sup>®</sup> and placebo groups, respectively.



Overall, demographics and disease severity for patients at baseline were well distributed between the groups (table 1). There were some gender differences between the treatment groups, with more males in the Prolastin<sup>®</sup> treatment group and more females in the placebo group ( $p=0.021$ ). There were also gender differences between participating centres: in the UK there were more males enrolled into the study, and in Sweden there were more females. In Denmark, on the other hand, males and females were equally distributed. All patients fulfilled the physiological inclusion criteria, except for two patients with FEV<sub>1</sub> baseline values slightly below 25% predicted.

### ***CT densitometric progression***

A total of 15 scans were considered to be technically invalid, which resulted in six patients having fewer than two CT scans, and therefore these patients were excluded from the mITT population (for further details please see the online supplement). CT densitometry demonstrated a significant decline in both the Prolastin<sup>®</sup> and placebo groups over the course of the study ( $p<0.001$  for both groups), consistent with emphysema progression.

The mean decline in whole lung density as measured by PD15 was determined using each of the four exploratory methods (tables 2A and 2B). A difference in the decline of lung density between the Prolastin<sup>®</sup> and placebo groups, suggesting a trend towards beneficial treatment effect with Prolastin<sup>®</sup>, was seen consistently with all four analytical methods ( $p$ -values for treatment difference ranged from 0.049–0.084). Furthermore, the change in TLC-adjusted PD15 from baseline over the course of the study determined using Method 1 showed that the difference in lung density between the two treatment groups increased with time (fig. 3). Whereas there was a significant difference ( $p<0.001$ ) in the measured decline in lung density between the centres, no significant interaction between centre and treatment was found. This indicated that the variation between the centres did not have an impact on the differences in the treatment effect observed between the two groups.

Mean values of lung volume as assessed by CT remained almost unchanged over the course of the study (see table 2 in the online data supplement). However, there was wide variation in lung volume between scans in individual patients. A plot of change in lung density against change in lung volume from baseline is shown in figure 4. A significant decrease ( $p<0.001$ ) in lung density was observed for both treatment groups when there was no change in lung volume (*i.e.*  $\Delta TLV=0$ ). This indicated that a proportion of the loss of lung density was not secondary to progressive hyperinflation but was consistent with an absolute loss of lung mass, which was greater in the placebo group. These data were further supported by the observation that mean values of CT-measured lung weight decreased from baseline in both the Prolastin<sup>®</sup> and placebo groups during the study. Comparison of the slopes showed that there was a greater decrease in lung weight in the placebo group, although the treatment difference was not statistically significant (see table 3 in the online data supplement).

PD15 was a more sensitive measure of emphysema progression than conventional physiological and health status indices (table 3). Of the different exploratory methods used to analyse PD15, Method 4 was the most sensitive.

### ***Other efficacy end-points***

Values for FEV<sub>1</sub>, DL<sub>CO</sub> and K<sub>CO</sub> decreased slightly in both treatment groups during the study but, since these measures were less sensitive than CT, no significant differences were found between the groups (see online supplement for details).

The mean annual exacerbation rates in the Prolastin<sup>®</sup> and placebo groups were similar ( $2.55\pm 2.14$  and  $2.19\pm 1.33$ , respectively;  $p=0.265$ ), with a median frequency of 2.02 exacerbations per yr for both groups. A *post hoc* analysis, however, indicated that the Prolastin<sup>®</sup>

group had proportionately fewer severe exacerbations, in relation to the total number of exacerbations with known severity, than the placebo group (13/194 [6.7%] *versus* 21/155 [13.5%];  $p=0.013$ ). This concurred with there being fewer subjects in the Prolastin<sup>®</sup> group with at least one hospitalisation due to an exacerbation during the course of the study (6/38 subjects [15.8%] *versus* 11/39 [28.2%] in the placebo group;  $p=0.189$ ).

Patients showed markedly impaired health-related quality of life at baseline on the SGRQ (table 1). At study end-point (the last post-baseline measurement for each subject), mean SGRQ Total domain scores had increased (*i.e.* worsened) by 1.48 in the Prolastin<sup>®</sup> group and by 2.37 in the placebo group ( $p=0.695$ ).

### ***Relationship between CT densitometry and other outcome measures***

We found a statistically significant relationship between the progression of CT densitometry and the rate of decline in FEV<sub>1</sub> (fig. 5), but the correlation between loss of CT lung density and the rate of decline in K<sub>CO</sub> or health status indices was not statistically significant (data not shown).

### ***Safety***

All but one subject in each group experienced at least one AE. More than 90% of these AEs were classified as mild or moderate. There were 14 drug-related AEs in the Prolastin<sup>®</sup> group and 35 in the placebo group, which were reported in 28.9% and 38.5% of subjects in each group, respectively. This equates to an incidence of 0.004 and 0.009 drug-related AEs per infusion of Prolastin<sup>®</sup> and placebo, respectively. A total of 28 serious AEs (SAEs) occurred in 10 subjects (26.3%) in the Prolastin<sup>®</sup> group, and 40 SAEs were reported in 18 subjects (46.2%) in the placebo group. A listing of the numbers of subjects with SAEs is shown in table 4 in the online data supplement. One SAE was assessed as drug-related in the Prolastin<sup>®</sup> group

(psoriasis), and one in the placebo group (pulmonary embolism). Overall, two patients in the placebo group and none in the Prolastin® group withdrew owing to AEs.

## DISCUSSION

The current study was designed to prospectively explore the use of novel outcome measures as an alternative to traditional methods, such as FEV<sub>1</sub>, to assess the influence of AAT augmentation therapy on emphysema progression in AAT-deficient individuals. The data confirm the findings of previous studies [10, 22, 23] by demonstrating that CT densitometry is a more sensitive measure for the detection of emphysema progression than both physiological and health status indices. Consequently, it is predictable that the use of CT densitometry as the primary outcome measure in studies of emphysema-modifying therapy may be more likely to identify a treatment effect than traditional measures such as FEV<sub>1</sub>. The current study did identify a trend that was suggestive of a beneficial effect of augmentation therapy when assessed by CT densitometry, and furthermore this trend was not evident when assessed by FEV<sub>1</sub>. Similar findings have also been shown in a previous randomised placebo-controlled clinical trial [10] that was of comparable design. It is of interest that these two studies have generated data suggestive of an equivalent treatment effect when assessed by CT densitometry, whereas the reduction in the rate of decline in FEV<sub>1</sub> that had been demonstrated in previous observational studies [5–8] has not been reproduced. This latter finding, whilst disappointing, is both unsurprising and predictable since CT has been repeatedly shown to be more sensitive than other measures of emphysema progression [9, 22, 24], and power calculations would indicate that no treatment effect would be expected using FEV<sub>1</sub> in such a small sample size [2, 3].

Notwithstanding the relative insensitivity of FEV<sub>1</sub> to detect emphysema progression, the current data however also demonstrate that CT densitometric progression is related to the rate of decline in FEV<sub>1</sub> as previously shown in an observational study [14]. Although there are

differences in the scanning protocol, in the population demographics and in the use of augmentation therapy in half of the subjects in the current study, the regression equations derived from these two datasets are very similar, suggesting that a unit change in lung density may be equated with a recognisable effect in terms of FEV<sub>1</sub>. The correlation observed between densitometric and physiological declines may seem counter-intuitive when the treatment effect that is suggested by differences in the decline of densitometric indices is not evident in the decline in FEV<sub>1</sub>. However, this is not an inconsistent finding but rather a reflection of the lower sensitivity of FEV<sub>1</sub> arising from poor reproducibility (as evidenced by the greater standard error of this measure, table 3), and, although FEV<sub>1</sub> is less sensitive than CT densitometry in monitoring emphysema progression, it does correlate with CT in cross-sectional studies [12, 14].

Whilst the two confirmatory findings discussed above are of critical importance in further validating the methodology of CT densitometry, the primary purpose of the EXACTLE trial was to investigate alternative approaches to the analysis of CT densitometric data and to resolve several issues for which there remains a lack of consensus. We used PD15 as the CT end-point for the following reasons. Firstly, the sensitivity of the voxel index method is threshold dependent whereas the effect on sensitivity of using different percentiles is less evident with the percentile density method [9]. Consequently, the rate of decline in PD15 is more consistent across a wide spectrum of disease severity, whereas the progression of -950 HU increases with greater disease severity [14]. Secondly, PD15 has been gradually accepted as the most sensitive parameter for CT lung densitometry [9, 14, 19]. The workshop on quantitative computed tomography scanning in longitudinal studies of emphysema [25] recommended that the primary end-point should be a significant shift in the 15<sup>th</sup> percentile of lung density. However, a broad range of data was collected in order to allow a flexible analytical approach that would be able to take account of interim developments in a rapidly changing field. Some of these approaches will be addressed in a further paper.

The principal clinical confounder in densitometric reproducibility is the level of inspiration during scan acquisition, and controlling or adjusting for this factor is the key to improving physiological variability. The current study addressed this issue by incorporating two methods for volume adjustment. The inverse relationship that exists between lung volume and lung density [18, 19] enables density values to be *a priori* adjusted by a physiological adjustment (Methods 1 and 3). As an alternative method, an *a posteriori* statistical adjustment was performed by the inclusion of the logarithmically transformed TLV as a covariate in the statistical model (Methods 2 and 4). The two statistical methods of analysis that were applied to these data were the mixed-effects, random coefficient model (Methods 1 and 2) and an end-point analysis (Methods 3 and 4). The former is a comprehensive statistical method that takes into account all measurements from baseline to end-point and allows for variation in the rate of progression of the disease between patients within the same treatment group. The latter, however, may be preferred in some situations (such as regulatory submission) to show the treatment effect between two groups in change from baseline to the end of the study. Due to the significant difference in the measured decline in lung density between the centres, all statistical analyses included the centre as a fixed effect. The significant centre effect seen in the EXACTLE study implies that in future studies the centre should be included in the statistical model.

Use of the above methods of volume adjustment improved the reproducibility of CT densitometry, and statistically significant lung density loss was demonstrated in both treatment groups, consistent with emphysema progression. The decline in the group receiving AAT augmentation therapy was less than that in the placebo group, and this was consistently evident with all four methods (p-values for treatment difference, Prolastin® *versus* placebo, ranged from 0.049–0.084). When the method of volume correction used was similar to that of the previous randomised placebo-controlled clinical trial of AAT augmentation [10] (namely, Method 2), a comparable treatment effect was observed. The similarity between the findings of the two studies when using a comparable analytical method would suggest not only consistency of treatment effect, but also robustness of CT densitometry as an outcome measure.

A linear relationship between the rate of change in lung density and the rate of change in lung volume, as identified in a recent observational study, was seen in both treatment arms; this enabled densitometric values to be adjusted for lung volume using statistical modelling, as described previously [19]. In addition, the intercepts for both treatment groups ( $\Delta TLV=0$ ) were significantly different from zero ( $p<0.001$ ), consistent with absolute loss of lung mass, also shown in the recent observational study [19]. However, there was a difference in the intercept between the active and placebo arms, which is consistent with a protective effect of augmentation therapy, as hypothesised in the observational study [19]. This overall observation is of fundamental importance in understanding the therapeutic effects of AAT augmentation therapy and would refute the potential dissenting argument that modification of densitometric progression might only reflect a reduction in lung compliance. Furthermore, it provides evidence to support the protease-antiprotease hypothesis of proteolytic destruction without complete regeneration of tissue in the pathogenesis of emphysema [26].

With respect to the other outcome measures, no significant difference was observed between the two groups in the change in scores for the Total domain of the SGRQ. Additionally, only insignificant differences were seen with regard to decline in  $FEV_1$ ,  $DL_{CO}$  or  $K_{CO}$ . This last observation is not unexpected in view of the small size of the patient cohort in the present study. However, the CT densitometric progression identified in the present randomised placebo-controlled trial may be compared with the findings of previous observational studies that used lung function measures of assessment, and which suggested clinical efficacy of augmentation therapy [5–8]. An approximation of the clinical relevance of the treatment effect in the present study may be made by reference to previously published studies [14, 24]. A treatment effect of 1.472 g/L as demonstrated using Method 4 could equate approximately to a difference in  $FEV_1$  decline of 30 mL/yr [14], which would be comparable to the treatment differences of 21 and 27 mL/yr that were seen in individuals with moderate to severe lung function impairment in the previous observational studies [5, 7].

This is the first study to prospectively investigate the effect of augmentation therapy on exacerbations as a secondary end-point. Exacerbations are a major cause of morbidity and mortality in general COPD [27], and are related to the decline in lung function as measured by vital capacity and  $DL_{CO}$  [22] in AAT deficiency. These episodes are associated with greater neutrophilic inflammation and more free elastase activity in AAT deficiency compared with usual COPD [28], and retrospective data have suggested that exacerbations in AAT deficiency were less frequent once augmentation therapy was started [29]. The average annual exacerbation rate in the present study was similar in both the treated and placebo groups and was comparable with results for patients with AAT deficiency from the UK Registry who were not receiving augmentation therapy [30]. The number of patients included was too small to draw any conclusion on the treatment effect on the rate of exacerbations. In addition, it remains possible that there is a qualitative change in the episodes. HILL et al. demonstrated a greater degree of inflammation in exacerbations in patients with AAT deficiency compared with those in patients with no AAT deficiency [27]; it is therefore likely that the episodes in patients who received AAT augmentation therapy may be associated with less inflammation, although further studies will be needed to specifically address this issue. While the absolute number of exacerbations was no different in the current study, the severity as defined by health care utilisation was different, with fewer hospital admissions being required for subjects receiving AAT augmentation therapy. In the study, augmentation therapy was generally shown to be well tolerated, with a favourable safety profile in accordance with findings from previous studies [6].

In conclusion, disease progression was demonstrated in both treatment groups using CT densitometry as a surrogate marker of emphysema. CT densitometry was more sensitive than other outcome measures in detecting disease progression and was also related to the decline in  $FEV_1$ . Trends towards a deceleration of emphysema progression were observed for AAT augmentation therapy and were consistently seen using the different analytical approaches that were explored in this study. In future studies concerning the analysis of time-dependent non-



linear treatment effects, Method 1 would be preferred, although it is less sensitive and thus requires more patients to detect treatment differences. In order to demonstrate the superiority of one treatment over the other, Method 4 appears to be the most sensitive and requires the smallest number of patients in a trial. Furthermore, the legal and ethical obligation to minimise exposure to ionising radiation in the course of clinical studies is also of paramount importance when considering the design of studies that utilise CT lung densitometry [31]. These results indicate that CT densitometry, performed as part of a multicentre study, is a technically feasible outcome measure in therapeutic trials of emphysema-modifying therapy.

**Clinical trial registration information:** Registered in ClinicalTrials.gov as “Antitrypsin (AAT) to Treat Emphysema in AAT-Deficient Patients”; ClinicalTrials.gov Identifier: NCT00263887.

The trial was conducted between November 2003 and January 2007.

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**TABLE 1** Patient baseline demographic characteristics (ITT population)

	<b>Prolastin® (n=38)</b>	<b>Placebo (n=39)</b>	<b>p- value</b>
<b>Age yrs, mean±SD (range)</b>	54.7±8.4	55.3±9.8	0.749
<b>Sex n (male/female)</b>	25/13	16/23	0.021
<b>Smoking status n (never/ex-smokers)</b>	4/34	4/35	0.949
<b>Caucasian race n</b>	38	39	N/A
<b>Body Mass Index kg/m<sup>2</sup> (mean±SD)</b>	24.3±3.2	24.3±3.5	0.928
<b>AAT levels µM, mean± SD (range)</b>	4.6±1.6	4.6±1.7	0.898
<b>Genotype ZZ and/or phenotype PiZ</b>	38	39	
<b>FEV<sub>1</sub> L (mean± SD)</b>	1.44±0.60	1.35±0.62	0.542
<b>FEV<sub>1</sub>% predicted (mean± SD)</b>	46.3±19.6	46.6±21.0	0.873
<b>DL<sub>CO</sub> mmol/min/kPa (mean± SD)</b>	4.73±2.09	4.72±1.70	0.956
<b>DL<sub>CO</sub>% predicted (mean± SD)</b>	50.7±19.5	52.2±15.2	0.721
<b>K<sub>CO</sub> mmol/min/kPa/L (mean± SD)</b>	0.82±0.32	0.86±0.24	0.565
<b>K<sub>CO</sub>% predicted (mean± SD)</b>	55.3±21.0	56.5±14.8	0.816
<b>SGRQ Total score (mean± SD)</b>	41.9±17.9	46.1±17.2	0.335
<b>PD15 lung density g/L (mean± SD)<sup>#</sup></b>	47.98±19.07	45.48±16.95	0.288
<b>TLC-adjusted PD15 lung density g/L (mean± SD)<sup>#</sup></b>	54.55±17.37	53.90±15.97	0.823
<b>Lung weight g (mean± SD)<sup>#</sup></b>	956.40±140.64	946.09±224.12	0.750
<b>Lung volume L (mean± SD)<sup>#</sup></b>	7.46±1.60	7.27±1.78	0.557

ITT: intent-to-treat; N/A: not applicable; AAT: alpha-1 antitrypsin; FEV<sub>1</sub>: forced expiratory volume in 1 s; DL<sub>CO</sub>: diffusion capacity of the lung for carbon monoxide; K<sub>CO</sub>: carbon monoxide transfer coefficient; SGRQ: St George's Respiratory Questionnaire; PD15: 15th percentile density; TLC: total lung capacity. <sup>#</sup>: for the CT densitometric analyses, the modified ITT (mITT) population was used (n=36, Prolastin®; n=35, placebo).

**TABLE 2A** Changes in TLC-adjusted and statistically adjusted 15th percentile lung density (g/L) using slope analysis (mITT population)

Statistic	Method of adjustment			
	1 (Physiological) <sup>#</sup>		2 (Statistical) <sup>#</sup>	
	Prolastin <sup>®</sup> (n=36)	Placebo (n=35)	Prolastin <sup>®</sup> (n=36)	Placebo (n=35)
Change from baseline (mean±SD):				
Month 12 (n=34, 33)	-1.765±3.986	-2.217±3.703	-0.742±5.298	-1.026±5.536
Month 24 (n=35, 32)	-2.827±5.009	-4.209±3.447	-2.386±5.253	-3.810±4.062
Month 30 (n=18, 16)	-3.252±4.498	-4.707±4.512	-2.408±4.296	-3.462±4.691
Random coefficient regression model: mean slope <sup>+</sup>				
(95% CI)	-1.384	-2.241	-1.114	-1.814
	(-2.023–	(-2.905–	(-1.618–	(-2.339–
	-0.745)	-1.577)	-0.611)	-1.289)
Estimated treatment difference in mean slope 95% CI	0.857 (-0.065–1. 778)		0.700 (-0.028–1.427)	
p-value for treatment difference <sup>\$</sup>	0.068		0.059	

TLC: total lung capacity; mITT: modified intent-to-treat; <sup>#</sup> : The fixed and random effect variables are given in the statistical analysis section; <sup>+</sup> : average annual loss of lung density (g/L) measured by PD15 of lung density; <sup>§</sup> : Prolastin<sup>®</sup> treatment minus placebo in mean slope.



**TABLE 2B** Changes in TLC-adjusted and statistically adjusted 15th percentile lung density (g/L) using end-point analysis (mITT population)

Statistic	Method of adjustment			
	3 (Physiological) <sup>#</sup>		4 (Statistical) <sup>#</sup>	
	Prolastin <sup>®</sup> (n=36)	Placebo (n=35)	Prolastin <sup>®</sup> (n=36)	Placebo (n=35)
Change from baseline to last CT scan (mean±SD)	-3.387±4.621	-4.822±3.813	-2.895±4.739	-4.124±4.147
Change from baseline to last CT scan (LS mean [SE])	-3.202 (0.657)	-4.798 (0.671)	-2.645 (0.526)	-4.117 (0.539)
Estimated treatment difference between changes from baseline (95% CI)	1.596 (-0.220–3.412)		1.472 (0.009–2.935)	
p-value for treatment difference <sup>+</sup>	0.084		0.049	

TLC: total lung capacity; mITT: modified intent-to-treat; CT: computed tomography; LS mean: least squares mean; CI: confidence interval. <sup>#</sup> :

The effect variables and covariates are given in the statistical analysis section; <sup>+</sup> : Prolastin<sup>®</sup> treatment minus placebo.

**TABLE 3** Sensitivity indices for CT parameters compared with lung function and quality-of-life end-points (mITT population)<sup>#</sup>

Outcome measure	Unit	Mean change	Standard error (SE)	Sensitivity index <sup>†</sup>	F-test*
<b>CT parameters</b>					
TLC-adjusted PD15 (Method 1)	g/L/yr	-2.24	0.333	6.7	n.s.
Statistically adjusted PD15 (Method 2)	g/L/yr	-1.81	0.263	6.9	n.s.
TLC-adjusted PD15 (Method 3) <sup>+</sup>	g/L	-4.80	0.671	7.2	n.s.
Statistically adjusted PD15 (Method 4) <sup>+</sup>	g/L	-4.12	0.539	7.6	-
<b>Lung function tests</b>					
FEV <sub>1</sub>	mL/yr	-23	10.4	2.2	P<0.01
DL <sub>CO</sub>	mmol/min/kPa/yr	-0.37	0.058	6.4	n.s.
K <sub>CO</sub>	mmol/min/kPa/L/yr	-0.036	0.0075	4.8	P<0.05
<b>SGRQ</b>					
Overall	Unit/yr	0.81	0.800	1.0	P<0.01
Symptoms domain	Unit/yr	-0.09	1.577	0.06	P<0.01
Activity domain	Unit/yr	2.58	0.890	2.9	P<0.05
Impacts domain	Unit/yr	-0.15	0.776	0.2	P<0.01

CT: computed tomography; TLC: total lung capacity; PD15: 15th percentile density; FEV<sub>1</sub>: forced expiratory volume in 1 s; DL<sub>CO</sub>: diffusion capacity of the lung for carbon monoxide; K<sub>CO</sub>: carbon monoxide transfer coefficient; SGRQ: St George's Respiratory Questionnaire. <sup>#</sup>: the results are based on estimate for the placebo group only; <sup>¶</sup>: ratio of mean change divided by SE; <sup>+</sup>: results based on mean change from end-point analysis from baseline to last available measurement.

\* Ratio of outcome measure as compared to statistically adjusted PD15 (Method 4) – the most sensitive approach

## Figure legends

**FIGURE 1.** The four methods that were used for densitometric analysis (see Methods section in main text for descriptive account).

**FIGURE 2.** Flow of participants through each stage of the randomised trial. Planned study end (completion of the study) was Month 24 for those who did not enter into the extension period and Month 30 for those who entered into the extension period. ITT: intent-to-treat population.

**FIGURE 3.** Change in TLC-adjusted 15th percentile lung density (PD15; g/L) over the course of the study using Method 1 for densitometric analysis (modified intent-to-treat population; Prolastin<sup>®</sup> [●] and placebo [○]). The error bars indicate standard error of mean (SEM). Estimated treatment difference in mean slopes (annual change in lung density) = 0.857 (p=0.068).

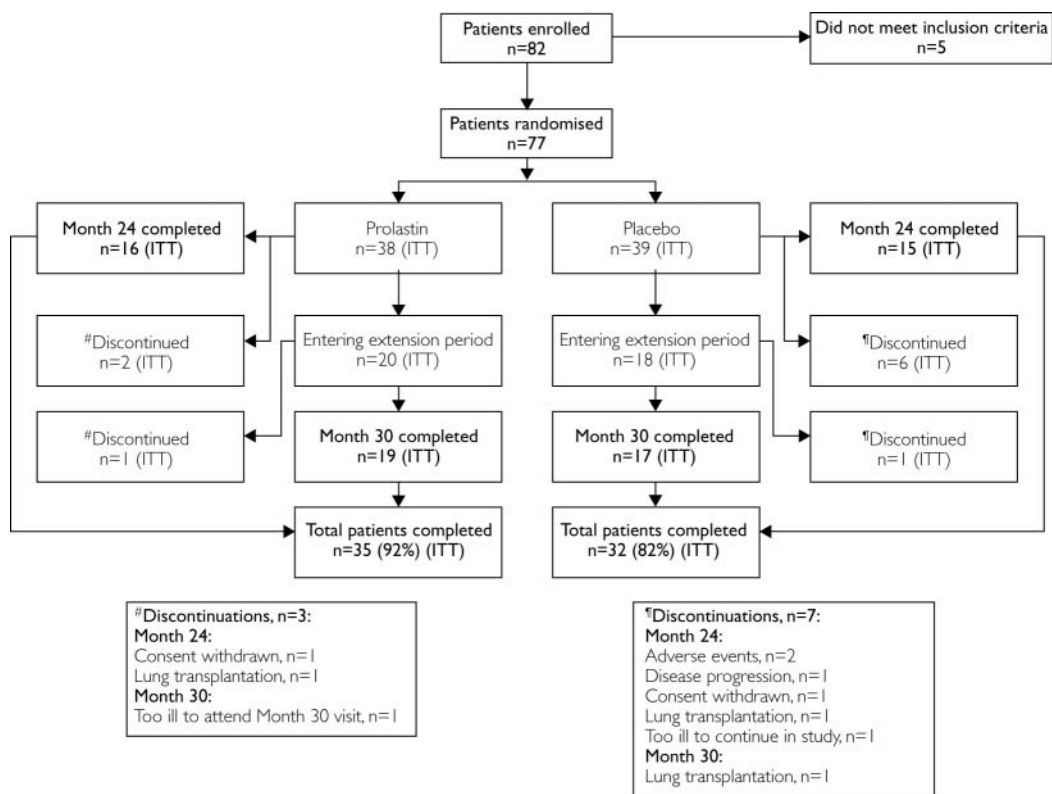
**FIGURE 4.** Plot and regression line of change from baseline to end-point in statistically adjusted 15th percentile lung density (PD15, g/L) *versus* lung volume (mL) (modified intent-to-treat population; Prolastin<sup>®</sup> [●—●] and placebo [○- -○]).

**FIGURE 5.** Correlation between the change in lung density (TLC-adjusted 15th percentile lung density [PD15; g/L]) *versus* change in forced expiratory volume in 1 s (FEV<sub>1</sub>) (L) (modified intent-to-treat population; both Prolastin<sup>®</sup> and placebo groups combined). Correlation coefficient (R) = 0.316; p=0.007.

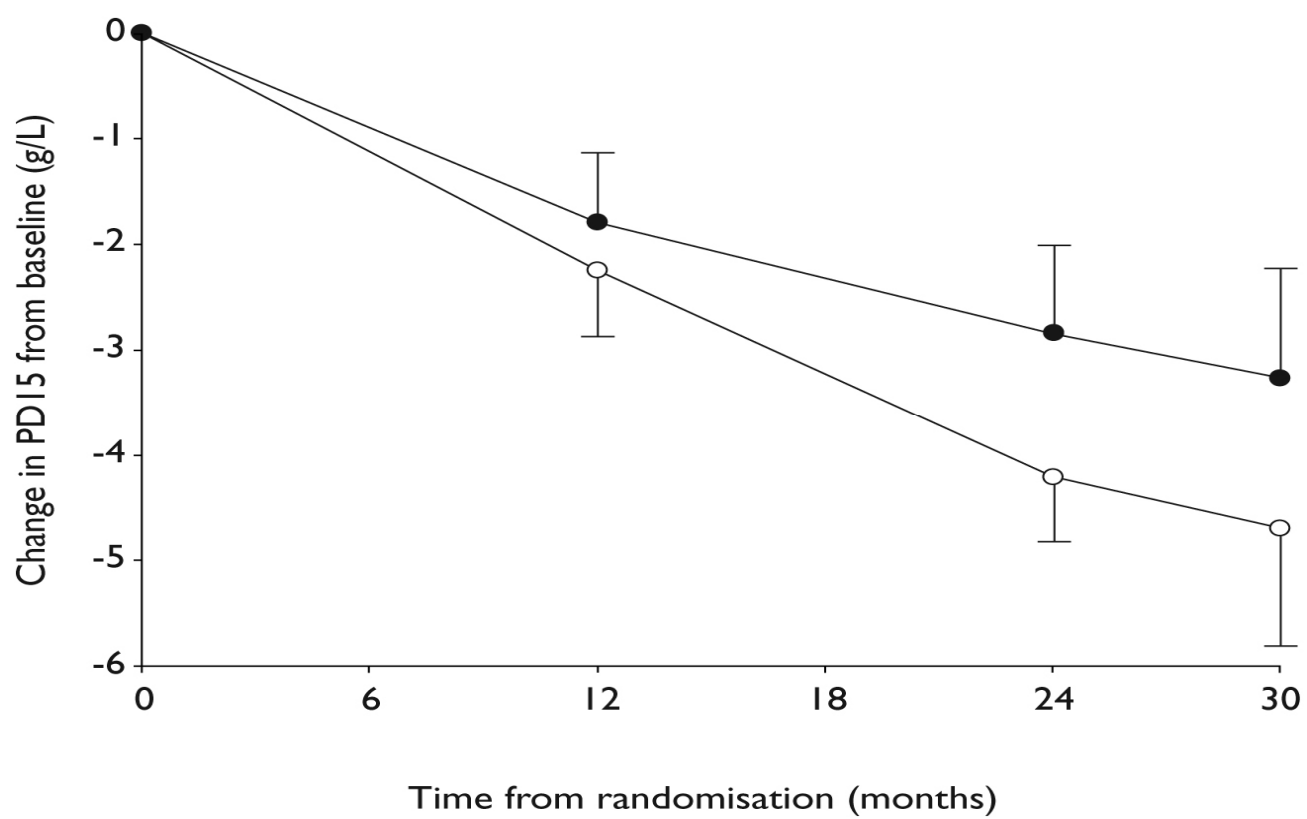
**Figure 1.**

		Statistical approach	
		Slope analysis	End-point analysis
Volume correction method	Physiological adjustment	Method 1	Method 3
	Statistical adjustment	Method 2	Method 4

**Figure 2.**



**Figure 3.**



**Figure 4.**

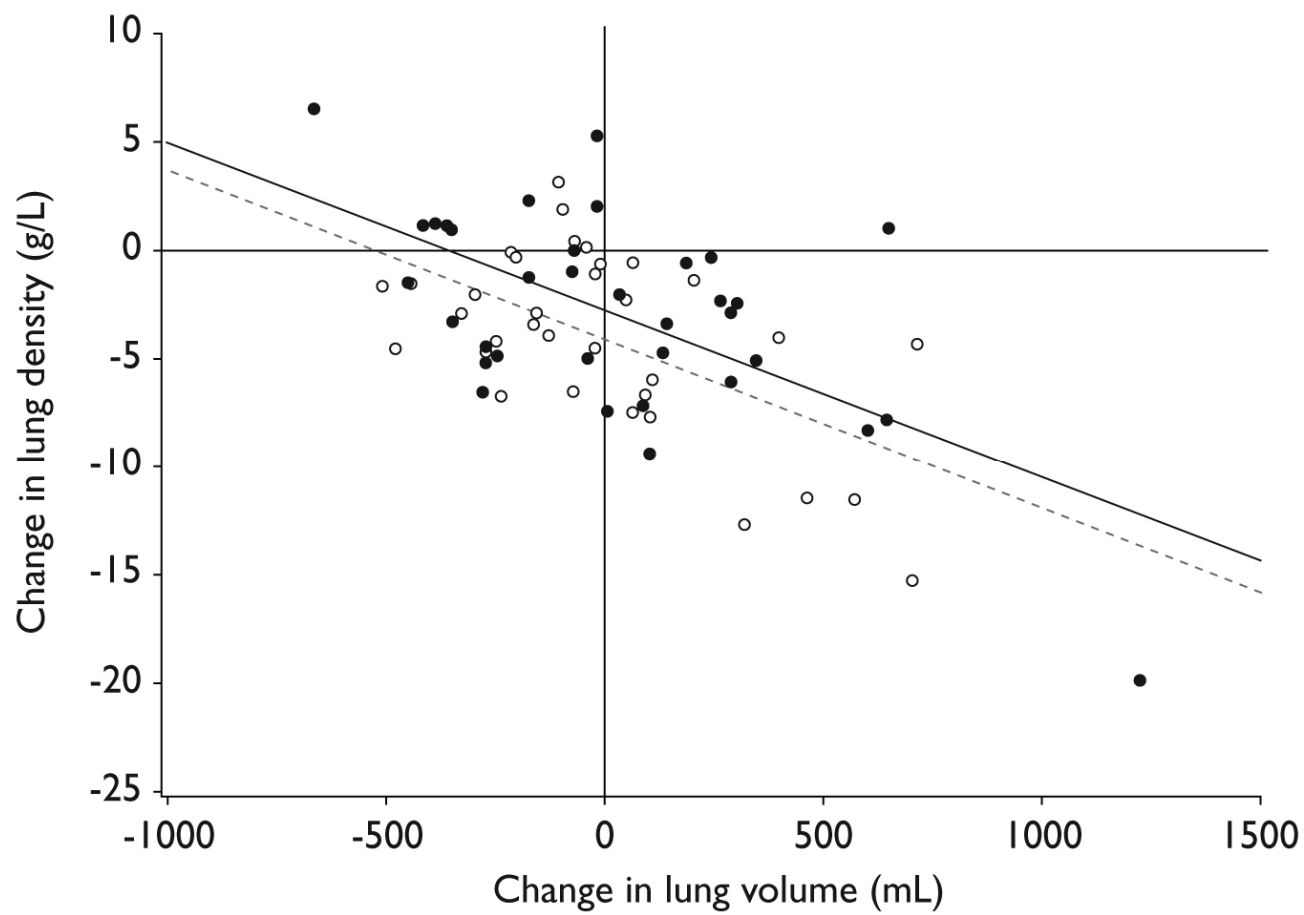




Figure 5.

