Study Title

A Phase I/II randomised, placebo-controlled, double blind trial to assess the safety, tolerability, pharmacodynamics and exploratory efficacy of Heparin 25 mg inhalation powder in patients with Cystic Fibrosis (CF)

Study Centre

Twenty-five (25) sites were initiated of which 15 sites enrolled patients: 10 sites in the UK and Ireland, 3 sites in Poland, 1 site in Italy and 1 site in Australia.

Study Period	**Phase of Development**
10 November 2008 to 25 October 2010	1/2

Objectives

Primary Objective

To investigate the safety and tolerability of Heparin inhalation powder in patients with CF.

Secondary Objectives

- To investigate the pharmacodynamic (PD) effects of Heparin inhalation powder by means of:
  - Expectorated mucus properties (i.e. rheological properties including viscoelasticity / physicochemical measurements);
  - The effect on inflammatory markers in induced sputum (i.e. neutrophil elastase [NE], interleukins [ILs] [IL-6 and IL-8]) and cell counts (i.e. total cell count [TCC], % neutrophil count, % macrophage count);
  - The effect on inflammatory markers in blood plasma (i.e. NE, NE/alpha-1 antitrypsin [AAT] complex, IL-6, IL-8, neutrophil count and C-reactive protein [CRP] level);
  - The effect on blood coagulation (i.e. activated partial thromboplastin time [aPTT] and platelet count) (although planned as a PD objective, coagulation was measured as part of safety in this study).
- To evaluate efficacy by:
  - Visual analogue scales (VAS) based on change in symptoms, including: cough resolution; expectorated sputum clearability, thickness, volume, colour, viscoelasticity (stickiness); breathlessness; general well-being (including feeling, energy, physical activity, appetite and sleep);
  - Microbiological analysis of expectorated sputum for effects on bacterial growth, density, antibiotic sensitivity of the following organisms are included: *Pseudomonas aeruginosa* [mucoid and other types], *Burkholderia cepacia*, *Haemophilus influenzae* and *Staphylococcus aureus*;
  - Volume and weight of 24-hour cumulative expectorated sputum sample;
  - Pulmonary function measured by forced expiratory volume in one second (FEV_1), forced vital capacity (FVC), forced mid-expiratory flow (FEF_{25-75}) and arterial oxygen saturation (SaO_2);
  - Response to the Cystic Fibrosis Questionnaire-Revised (CFQ-R).

Methodology

Phase 1/2, multi-centre, randomised, double blind, placebo-controlled trial with 4 parallel groups with a 1:1:1:1 ratio (nominal doses of VR496 11400 IU [62 mg], VR496 22800 IU [124 mg], VR496 45600 IU [248 mg] or placebo) administered twice daily for 4 consecutive weeks in at least 64 evaluable patients with CF.

Number of Subjects (Planned and Analysed)

Planned: at least 64 evaluable patients

Analysed: 77 patients in both the Safety Population and Intention To Treat (ITT) Population; 65 patients in the Per Protocol (PP) Population

Main Criteria for Inclusion

Male and female non-smoking patients, ≥ 16 years with confirmed diagnosis of CF lung disease and who provided written informed consent were eligible for study entry. Patients who were clinically stable with acceptable FEV_1 values (i.e. predicted FEV_1 at Screening and Baseline between 40 and 90%, FEV_1 at Baseline within ± 15% of Screening FEV_1), regular mucus production due to CF, ease of sputum expectoration (i.e. clearability) VAS score of ≤ 80 mm, acceptable induced sputum NE and/or IL-8 levels and adequate contraceptive measures, with the ability to comply with all the requirements of the protocol and to use the study inhaler satisfactorily.

Test Product, Dose and Mode of Administration

Heparin 25 mg inhalation powder, hard capsules (hereafter referred to as VR496) for inhalation via the Monohaler® (single-unit DPI device) at daily nominal doses of VR496 11400 IU (62 mg), VR496 22800 IU (124 mg) and VR496 45600 IU (248 mg).

Duration of Treatment

Treatment administered twice daily for 4 consecutive weeks.

Reference Product, Dose and Mode of Administration

Placebo by inhalation

Criteria for Evaluation

Safety and Tolerability Parameters:

Monitoring and assessment of:

- Adverse events (AEs) / serious adverse events (SAEs);
- Sitting vital sign parameters (blood pressure, heart rate, respiratory rate, temperature), weight (weight at post dose was not recorded as planned as a safety assessment) and physical examination;
- Clinical laboratory parameters, including haematology, clinical chemistry and urinalysis;
- Platelet counts;
- Patients were contacted by the Investigative Site staff every 3-4 days whilst on the study to ensure no bleeding or thrombosis had been experienced.

Pharmacodynamic (PD) Parameters:

Assessment of:

- Expectorated sputum measurement parameters (i.e. rheological viscoelasticity/ physicochemical parameters);
- Induced sputum inflammatory markers (i.e. NE, IL-6 and IL-8 and cell counts [i.e. TCC, % neutrophil count, % macrophage count]);
- Blood plasma inflammatory markers (i.e. NE, NE/AAT complex, neutrophil count, IL-6, IL-8 and CRP levels);
- Blood coagulation parameters (i.e. aPTT and platelet count) (although planned as a PD objective, coagulation was measured as part of the safety assessment in this study).

Efficacy Parameters:

Assessment of:

- VAS parameters, (i.e. expectorated sputum clearability, thickness, volume, colour, viscoelasticity (stickiness); cough resolution; breathlessness; general well-being including feeling, energy, physical activity, appetite and sleep);
- Volume and weight of 24-hour cumulative expectorated sputum sample;
- Microbiology assays of expectorated sputum (i.e. analysis of bacterial growth, density, antibiotic sensitivity of the following organisms are included: *Pseudomonas aeruginosa* [mucoid and other types], *Burkholderia cepacia*, *Haemophilus influenzae* and *Staphylococcus aureus*);
- Pulmonary function parameters FEV_1, FVC and FEF_{25-75} assessed by spirometry and SaO_2 assessed by finger oximetry;
- Response to the CFQ-R.

Statistical Methods

In general, categorical data are presented using counts and percentages, whilst continuous variables are presented using the mean, standard deviation (SD), median, minimum, maximum and number of patients. All the statistical analyses were performed at 2-sided 5% significance level.

The ITT Population was used for the analysis of efficacy and PD, unless otherwise specified. Primary analyses are also presented using the PP Population.

Efficacy and PD results are presented in summary tables and analysis tables. The summary table shows the absolute values of the scores/results, the changes from Baseline and the % changes from Baseline at each assessment. Change from Baseline to Week 2 and Week 4 was analysed using an analysis of covariance (ANCOVA) model with Baseline level as covariate and site as a fixed effect. Estimates of the difference between treatments are presented with the 95% confidence intervals (CIs) for the comparison of each active dose versus placebo, as well as pooled active dose versus placebo.

The Safety Population was used for the analysis of safety.

This proof of concept study was not formally powered for statistical comparisons. At least 16 patients per treatment arm had been selected as a feasible number of patients to treat, to provide at least 64 evaluable patients with CF to be included in the analysis.

Summary of Results:

Safety:

Active study treatment (VR496) was well tolerated as evidenced by the following;

1. High patient study completion rate complemented by high dosing compliance in active and placebo groups.
2. No clinically relevant difference in AE incidence or severity between active and placebo groups. No evidence of a pro-inflammatory effect was noted which is consistent with the reported safety outcomes.
3. No clinically relevant difference in exacerbation and haemoptysis incidence between active and placebo group with the number of reported events being consistent with the background rate in the CF population.
4. No evidence of thrombosis with active treatment and no clinically relevant change in blood coagulation parameters (i.e. aPTT and platelet count) compared to placebo.
5. Good local tolerability of active treatment with low incidence of AEs such as cough, throat irritation, dyspnoea and oropharyngeal pain.

The safety outcomes are consistent with reported nebulised heparin experience.

Blood coagulation parameters

No clinically relevant changes in coagulation parameters were reported.

Efficacy:

The reported outcomes confirm the multi-modal pharmacology of VR496 as evidenced by clinically relevant improvements in mucolytic and induced sputum inflammatory marker endpoints.

Mucolytic-related outcomes

A number of VAS outcomes confirm the mucolytic properties of VR496 with relevant improvements reported for sputum clearability, thickness, colour and viscoelasticity ("stickiness"). Furthermore, this activity was maintained during the "no treatment" period from Week 4 to Week 6. Further support for effective mucolytic activity is illustrated by these improvements being associated with increased cumulative sputum volume over the 24-hour period prior to each clinic visit.

The rheological analysis of expectorated sputum showed a 0.2 log reduction in mucus viscoelasticity (log G*) with the VR496 high dose.

Pharmacodynamics (PDs)

Induced sputum inflammatory markers

VR496 resulted in reductions from Baseline to Week 4 of key induced sputum inflammatory markers (i.e. NE, TCC and IL-6) compared to placebo.

Plasma inflammatory markers

No clinically relevant change (Baseline to Week 4) in any plasma inflammatory marker tested was observed in any treatment group effect. Importantly, there was no evidence of any pro-inflammatory effect with VR496, consistent with previously described safety observations (e.g. AE incidence, withdrawal rate, exacerbation rate) with nebulised heparin in patients with CF and other patients with airway inflammatory diseases. Similar rates of AEs were reported compared to placebo. The lack of any systemic activity closely correlates with the lack of effect on blood coagulation outcomes (e.g. aPTT and platelet count).

Other VAS outcomes

A small non-significant increase in VAS breathlessness was reported with VR496. However, no clear dose relationship was observed and this observation did not correlate with relevant safety measures such as AEs where incidents of treatment-related dyspnea were low and comparable to placebo.

Lung function

Study outcomes were consistent with the *a priori* criteria of no significant changes in lung function (FEV_1 and FVC) being reported from Baseline to Week 4. The small changes in FEV_1 relative to placebo were not significant and remained within the American Thoracic Society (ATS) range of acceptable variability (successive values within ± 150 ml) and therefore not considered to be of clinical relevance.

Microbiology

The microbiological evaluation of expectorated sputum samples did not show any relevant change over the study duration (Baseline to Week 4).

Cystic Fibrosis Questionnaire-Revised (CFQ-R)

No significant difference in change of CFQ-R parameters was observed between any active treatment or pooled active treatment group and placebo.

Summary - Conclusions

The VR496 middle and high doses improved key sputum inflammatory and mucolytic markers.

VR496 was well tolerated in CF patients with a wide range of lung disease severity. The safety outcomes are consistent with reported nebulised heparin experience.