



## Clinical trial results:

**A randomised, double blind, double-dummy, placebo controlled trial of inhaled treatment to establish the mechanisms of prematurity-associated airway obstruction and inflammation.**

### Summary

|                          |                  |
|--------------------------|------------------|
| EudraCT number           | 2015-003712-20   |
| Trial protocol           | GB               |
| Global end of trial date | 30 November 2019 |

### Results information

|                                   |                                                                                                                                                                                                                                                |
|-----------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Result version number             | v1 (current)                                                                                                                                                                                                                                   |
| This version publication date     | 27 January 2022                                                                                                                                                                                                                                |
| First version publication date    | 27 January 2022                                                                                                                                                                                                                                |
| Summary attachment (see zip file) | Inhaled Corticosteroids Alone and in Combination With Long-Acting $\beta$ 2 Receptor Agonists to Treat Reduced Lung Function in Preterm-Born Children A Randomized Clinical Trial (jamapediatrics_goulden_2021_oi_210075_1637630272.60457.pdf) |

### Trial information

#### Trial identification

|                       |             |
|-----------------------|-------------|
| Sponsor protocol code | SPON1451-15 |
|-----------------------|-------------|

#### Additional study identifiers

|                                    |                |
|------------------------------------|----------------|
| ISRCTN number                      | ISRCTN14767962 |
| ClinicalTrials.gov id (NCT number) | -              |
| WHO universal trial number (UTN)   | -              |

Notes:

### Sponsors

|                              |                                                                                           |
|------------------------------|-------------------------------------------------------------------------------------------|
| Sponsor organisation name    | Cardiff University                                                                        |
| Sponsor organisation address | 30-36 Newport Road, Cardiff, United Kingdom, CF24 0DE                                     |
| Public contact               | Research Governance Office, Cardiff University, +44(0)29 20 879 131, resgov@cardiff.ac.uk |
| Scientific contact           | Research Governance Office, Cardiff University, +44(0)29 20 879 131, resgov@cardiff.ac.uk |

Notes:

### Paediatric regulatory details

|                                                                      |    |
|----------------------------------------------------------------------|----|
| Is trial part of an agreed paediatric investigation plan (PIP)       | No |
| Does article 45 of REGULATION (EC) No 1901/2006 apply to this trial? | No |
| Does article 46 of REGULATION (EC) No 1901/2006 apply to this trial? | No |

Notes:

---

## Results analysis stage

|                                                      |                  |
|------------------------------------------------------|------------------|
| Analysis stage                                       | Final            |
| Date of interim/final analysis                       | 30 November 2019 |
| Is this the analysis of the primary completion data? | Yes              |
| Primary completion date                              | 30 November 2019 |
| Global end of trial reached?                         | Yes              |
| Global end of trial date                             | 30 November 2019 |
| Was the trial ended prematurely?                     | No               |

Notes:

---

## General information about the trial

Main objective of the trial:

The overall aim of the research is to establish the reasons why children who were born prematurely have ongoing respiratory symptoms, potentially caused by narrowing of the airways (obstruction), inflammation and structural differences in the lung. The principle objective is to assess if 12 weeks of inhaler treatment can reduce this obstruction and inflammation.

To obtain updated information on the prevalence of respiratory symptoms (such as wheeze) in children who were born prematurely and investigate how these change over time.

To understand, in detail, the reasons (mechanisms) why inhaler treatment works in some participants, but not in others, by investigating the differences in laboratory markers of disease between responders and non-responders.

To understand more about the structure of the lungs and how this effects their function by comparing results from MRI scans.

To establish a cohort of children born prematurely in Wales for future studies.

---

Protection of trial subjects:

Trial has three parts

### PART 1

Research nurse contacts those expressing interest following return of the questionnaire.

Home visit- Full study explained ,consent obtained, medical history, current medications, repeat respiratory and neurological questionnaire (parent/care giver), blood pressure, heart rate, oxygen saturations, height, weight, body composition, assess pubertal status, collect saliva sample (optional); urine sample. exhaled nitric oxide. lung spirometry, administer salbutamol inhaler, reassess lung spirometry.

Participants with FEV1  $\leq$  85% invited to part 2 provided with information - contacted at later date to book a visit if agreeable. Preterm born participant identified and willing to participate, noted to be taking inhaled corticosteroids, referred to consultant respiratory paediatrician to assess if washout from steroids appropriate before booking part 2

### PART 2

Families invited to clinic visit- consent and assent taken, medical history, current medications, respiratory and quality of life questionnaire ( parent/care giver), blood pressure, heart rate, oxygen saturation, height, weight, body composition, collect saliva sample (optional,) urine sample, perform skin-prick allergy test, exhaled nitric oxide test, lung spirometry, carbon monoxide transfer test, plethysmography, collect exhaled breath condensate,, exercise challenge test, reversibility test after exercise challenge, administer salbutamol inhaler, reassessing lung spirometry to test reversibility airway obstruction.

### PART 3

60 Preterm born children with decreases in lung function, 20 Preterm born controls and 20 term born controls (both normal lung function) invited to participate at the Royal Hallamshire Hospital to undertake a hyperpolarised helium MRI scan. Asked to inhale small amount of specially produced helium prior to the scan, Informed consent and assent taken, checklist completed to ensure the participant meets the criteria to undergo the scan. The scan is radiation free

---

Background therapy:

On completion of the Part 1 visit 1 schedule of assessments, eligibility will be confirmed by the attending study clinician and recorded on the Case Report Form (CRF); the participant will be randomised to receive either monotherapy (fluticasone), combination therapy (fluticasone/salmeterol) or placebo. Inhaler technique will be demonstrated by the attending members of the clinical and nursing team. These children will be invited to attend 2 clinic visits for extensive lung function testing; the two visits will be separated by a 12- week treatment trial of inhaled medicines commonly used to treat asthma. We compared the results of tests before and after the treatment trial to establish if the medicines work

(Part 2). In order to generalise our results we will invite 50 children born at term (37 weeks gestation and over) with normal lung function, and 50 children born prematurely with normal lung function, to participate in each part of the trial as comparison groups .

#### Evidence for comparator:

The trial was a randomised, double blind, double-dummy placebo-controlled. Eligible participants were randomised to receive blinded study medication, stratified by inhaled steroid status: not currently taking inhaled steroids (non-ICS) or weaned from inhaled steroids. Non-ICS participants, expected to comprise approximately 87% of the eligible children, were randomised to:

- a) Combination therapy- Inhaled long acting  $\beta_2$  agonist (25 $\mu$ g salmeterol xinafoate, total daily dose of 100 $\mu$ g) and corticosteroid (50 $\mu$ g fluticasone propionate, total daily dose of 200 $\mu$ g);
- b) Monotherapy- Inhaled steroid only (50 $\mu$ g fluticasone propionate, total daily dose of 200 $\mu$ g);
- c) Placebo.

Participants who were successfully weaned off steroids prior to visit 1 of part 2, expected to comprise approximately 15% of children, were randomised to:

- a) Combination therapy- Inhaled long acting  $\beta_2$  agonist (25 $\mu$ g salmeterol xinafoate, total daily dose of 100 $\mu$ g) and corticosteroid (50 $\mu$ g fluticasone propionate, total daily dose of 200 $\mu$ g);
- b) Monotherapy- Inhaled steroid only (50 $\mu$ g fluticasone propionate, total daily dose of 200 $\mu$ g)

The children were monitored for any adverse events during the 12-week treatment period and were reassessed after this period undergoing repeat spirometry and exercise testing. The trial was overseen by an independent trial and safety monitoring committee.

|                                                           |                  |
|-----------------------------------------------------------|------------------|
| Actual start date of recruitment                          | 21 November 2016 |
| Long term follow-up planned                               | No               |
| Independent data monitoring committee (IDMC) involvement? | Yes              |

Notes:

### Population of trial subjects

#### Subjects enrolled per country

|                                      |                    |
|--------------------------------------|--------------------|
| Country: Number of subjects enrolled | United Kingdom: 53 |
| Worldwide total number of subjects   | 53                 |
| EEA total number of subjects         | 0                  |

Notes:

#### Subjects enrolled per age group

|                                           |    |
|-------------------------------------------|----|
| In utero                                  | 0  |
| Preterm newborn - gestational age < 37 wk | 0  |
| Newborns (0-27 days)                      | 0  |
| Infants and toddlers (28 days-23 months)  | 0  |
| Children (2-11 years)                     | 43 |
| Adolescents (12-17 years)                 | 10 |
| Adults (18-64 years)                      | 0  |
| From 65 to 84 years                       | 0  |
| 85 years and over                         | 0  |

## Subject disposition

### Recruitment

Recruitment details:

We used our research patient database to identify potentially eligible participants, which contained linked-anonymised participant data only. We requested updated contact details from the NHS Wales Informatics Service for participants who gave consent to be contacted regarding further research. We initially sent a postal questionnaire .

### Pre-assignment

Screening details:

Potential participants were approached if they agreed to future contact when completing the original study. Study information was mailed inviting them to take part in completing the questionnaire and inviting them to a screening visit.

A research nurse made telephone or email contact (as preferred by the family) to establish baseline eligibility.

### Period 1

|                              |                                                               |
|------------------------------|---------------------------------------------------------------|
| Period 1 title               | Overall Trial (overall period)                                |
| Is this the baseline period? | Yes                                                           |
| Allocation method            | Randomised - controlled                                       |
| Blinding used                | Double blind                                                  |
| Roles blinded                | Subject, Investigator, Monitor, Data analyst, Carer, Assessor |

Blinding implementation details:

Serevent® (25µg salmeterol xinafoate), and Flixotide® (50µg fluticasone propionate) metered-dose inhalers used for the active arms of the trial. Matching placebo canister, containing no active substances, was sourced.

For blinding purposes, all canisters removed from their original actuators and placed in plain-coloured actuators provided to the IMP manufacturer who provided evidence of the equivalency of Serevent and Flixotide actuators. The active inhalers and placebo were identical .

### Arms

|                              |             |
|------------------------------|-------------|
| Are arms mutually exclusive? | Yes         |
| Arm title                    | Monotherapy |

Arm description:

Preterm born children with decreases in lung function (FEV1 <=85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

Monotherapy treatment consisted of the following:-

- i) Fluticasone, two sucks to be taken twice a day (100 micrograms total per dose) - also Placebo added
- ii) Placebo (dummy) inhaler containing no active drug, two sucks to be taken twice a day

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Arm type                               | Active comparator                  |
| Investigational medicinal product name | Fluticasone Propionate             |
| Investigational medicinal product code | R03BA05                            |
| Other name                             | Flixotide                          |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

Dosage and administration details:

200µg microgram(s per day

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Investigational medicinal product name | Placebo                            |
| Investigational medicinal product code |                                    |
| Other name                             |                                    |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

Dosage and administration details:

Placebo (dummy) inhaler containing no active drug, two sucks to be taken twice a day

|                  |                     |
|------------------|---------------------|
| <b>Arm title</b> | Combination Therapy |
|------------------|---------------------|

**Arm description:**

Preterm born children with decreases in lung function (FEV1 ≤85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

The combination treatment consisted of :-

ii) Fluticasone and salmeterol together, two sucks to be taken twice a day (100 micrograms fluticasone, 50 micrograms salmeterol per dose)

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Arm type                               | Active comparator                  |
| Investigational medicinal product name | Salmeterol Xinfoate                |
| Investigational medicinal product code | R03AC12                            |
| Other name                             | Serevent                           |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

**Dosage and administration details:**

100µg microgram(s)

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Investigational medicinal product name | Fluticasone Propionate             |
| Investigational medicinal product code | R03BA05                            |
| Other name                             | Flixotide                          |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

**Dosage and administration details:**

200µg micrograms

|                  |         |
|------------------|---------|
| <b>Arm title</b> | Placebo |
|------------------|---------|

**Arm description:**

Pretermborn children with decreases in lung function (FEV1 ≤85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

The placebo treatment consisted of :-

iii) Placebo (dummy) inhaler containing no active drug, two sucks to be taken twice a day

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Arm type                               | Placebo                            |
| Investigational medicinal product name | Placebo                            |
| Investigational medicinal product code |                                    |
| Other name                             |                                    |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

**Dosage and administration details:**

2 sucks to be taken twice per day

For blinding purposes, all canisters removed from their original actuators and placed in plain-coloured actuators incorporating an atomising mouthpiece and fitted with dustcaps. One pressurised container delivers 120 actuations. The matching placebo canister, contains no active substances.. The remaining excipients (hydrofluoroalkane as propellant) are identical between active and placebo canisters.

|                                        |                                    |
|----------------------------------------|------------------------------------|
| Investigational medicinal product name | Placebo                            |
| Investigational medicinal product code |                                    |
| Other name                             |                                    |
| Pharmaceutical forms                   | Pressurised inhalation, suspension |
| Routes of administration               | Inhalation use                     |

**Dosage and administration details:**

2 sucks to be taken twice per day

For blinding purposes, all canisters removed from their original actuators and placed in plain-coloured actuators incorporating an atomising mouthpiece and fitted with dustcaps. One pressurised container delivers 120 actuations. The matching placebo canister, contains no active substances.. The remaining excipients (hydrofluoroalkane as propellant) are identical between active and placebo canisters.

| <b>Number of subjects in period 1</b> | Monotherapy | Combination Therapy | Placebo |
|---------------------------------------|-------------|---------------------|---------|
| Started                               | 20          | 19                  | 14      |
| Completed                             | 18          | 17                  | 13      |
| Not completed                         | 2           | 2                   | 1       |
| Consent withdrawn by subject          | -           | 1                   | 1       |
| Adverse event, non-fatal              | 1           | -                   | -       |
| child worried about treatment         | 1           | -                   | -       |
| Poor Compliance                       | -           | 1                   | -       |

## Baseline characteristics

### Reporting groups

| Reporting group title                                                                                   | Overall Trial |
|---------------------------------------------------------------------------------------------------------|---------------|
| Reporting group description:                                                                            |               |
| 1) Children aged 7-12 at the time of screening                                                          |               |
| 2) Born at a gestational age $\leq 34$ weeks                                                            |               |
| 3) Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up |               |
| 4) Fully informed proxy consent from parents/guardians and assent from child where possible             |               |
| 5) Preterm-born children found during screening to have FEV1 $\leq 85\%$ predicted                      |               |

| Reporting group values                                                                                  | Overall Trial | Total |  |
|---------------------------------------------------------------------------------------------------------|---------------|-------|--|
| Number of subjects                                                                                      | 53            | 53    |  |
| Age categorical                                                                                         |               |       |  |
| 1) Children aged 7-12 at the time of screening                                                          |               |       |  |
| 2) Born at a gestational age $\leq 34$ weeks                                                            |               |       |  |
| 3) Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up |               |       |  |
| 4) Fully informed proxy consent from parents/guardians and assent from child where possible             |               |       |  |
| Units: Subjects                                                                                         |               |       |  |
| 7-12 years                                                                                              | 53            | 53    |  |
| Age continuous                                                                                          |               |       |  |
| - Children aged 7-12 at the time of screening                                                           |               |       |  |
| - Born at a gestational age $\leq 34$ weeks                                                             |               |       |  |
| - Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up  |               |       |  |
| - Fully informed proxy consent from parents/guardians and assent from child where possible              |               |       |  |
| Units: years                                                                                            |               |       |  |
| arithmetic mean                                                                                         | 10.8          |       |  |
| standard deviation                                                                                      | $\pm 1.2$     | -     |  |
| Gender categorical                                                                                      |               |       |  |
| 1) Children aged 7-12 at the time of screening                                                          |               |       |  |
| 2) Born at a gestational age $\leq 34$ weeks                                                            |               |       |  |
| 3) Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up |               |       |  |
| 4) Fully informed proxy consent from parents/guardians and assent from child where possible             |               |       |  |
| Units: Subjects                                                                                         |               |       |  |
| Female                                                                                                  | 29            | 29    |  |
| Male                                                                                                    | 24            | 24    |  |

### Subject analysis sets

| Subject analysis set title                                                                                                                                                                                                                                                                      | Overall Trial |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|
| Subject analysis set type                                                                                                                                                                                                                                                                       | Full analysis |
| Subject analysis set description:                                                                                                                                                                                                                                                               |               |
| To test why children who were born prematurely have ongoing respiratory symptoms, potentially caused by narrowing of the airways (obstruction), inflammation and structural differences in the lung and to assess if 12 weeks of inhaler treatment can reduce this obstruction and inflammation |               |

|                                                                                                                                                                                                                                                                                                          |               |  |  |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------|--|--|
| <b>Reporting group values</b>                                                                                                                                                                                                                                                                            | Overall Trial |  |  |
| Number of subjects                                                                                                                                                                                                                                                                                       | 53            |  |  |
| Age categorical                                                                                                                                                                                                                                                                                          |               |  |  |
| 1) Children aged 7-12 at the time of screening<br>2) Born at a gestational age $\leq 34$ weeks<br>3) Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up<br>4) Fully informed proxy consent from parents/guardians and assent from child where possible |               |  |  |
| Units: Subjects                                                                                                                                                                                                                                                                                          |               |  |  |
| 7-12 years                                                                                                                                                                                                                                                                                               | 53            |  |  |
| Age continuous                                                                                                                                                                                                                                                                                           |               |  |  |
| - Children aged 7-12 at the time of screening<br>- Born at a gestational age $\leq 34$ weeks<br>- Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up<br>- Fully informed proxy consent from parents/guardians and assent from child where possible     |               |  |  |
| Units: years                                                                                                                                                                                                                                                                                             |               |  |  |
| arithmetic mean                                                                                                                                                                                                                                                                                          | 10.8          |  |  |
| standard deviation                                                                                                                                                                                                                                                                                       | $\pm 1.2$     |  |  |
| Gender categorical                                                                                                                                                                                                                                                                                       |               |  |  |
| 1) Children aged 7-12 at the time of screening<br>2) Born at a gestational age $\leq 34$ weeks<br>3) Resident in the south Wales area whom, in the opinion of the Investigator, are possible to follow up<br>4) Fully informed proxy consent from parents/guardians and assent from child where possible |               |  |  |
| Units: Subjects                                                                                                                                                                                                                                                                                          |               |  |  |
| Female                                                                                                                                                                                                                                                                                                   | 29            |  |  |
| Male                                                                                                                                                                                                                                                                                                     | 24            |  |  |

## End points

### End points reporting groups

|                       |             |
|-----------------------|-------------|
| Reporting group title | Monotherapy |
|-----------------------|-------------|

Reporting group description:

Preterm born children with decreases in lung function (FEV1  $\leq$ 85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

Monotherapy treatment consisted of the following:-

- i) Fluticasone, two sucks to be taken twice a day (100 micrograms total per dose) - also Placebo added
- ii) Placebo (dummy) inhaler containing no active drug, two sucks to be taken twice a day

|                       |                     |
|-----------------------|---------------------|
| Reporting group title | Combination Therapy |
|-----------------------|---------------------|

Reporting group description:

Preterm born children with decreases in lung function (FEV1  $\leq$ 85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

The combination treatment consisted of :-

- ii) Fluticasone and salmeterol together, two sucks to be taken twice a day (100 micrograms fluticasone, 50 micrograms salmeterol per dose)

|                       |         |
|-----------------------|---------|
| Reporting group title | Placebo |
|-----------------------|---------|

Reporting group description:

Preterm born children with decreases in lung function (FEV1  $\leq$ 85%) randomised to receive 1 of 3 different inhalers to be taken for 12 weeks:

The placebo treatment consisted of :-

- iii) Placebo (dummy) inhaler containing no active drug, two sucks to be taken twice a day

|                            |               |
|----------------------------|---------------|
| Subject analysis set title | Overall Trial |
|----------------------------|---------------|

|                           |               |
|---------------------------|---------------|
| Subject analysis set type | Full analysis |
|---------------------------|---------------|

Subject analysis set description:

To test why children who were born prematurely have ongoing respiratory symptoms, potentially caused by narrowing of the airways (obstruction), inflammation and structural differences in the lung and to assess if 12 weeks of inhaler treatment can reduce this obstruction and inflammation

### Primary: Analysis of Covariance of percentage predicted FEV1

|                 |                                                     |
|-----------------|-----------------------------------------------------|
| End point title | Analysis of Covariance of percentage predicted FEV1 |
|-----------------|-----------------------------------------------------|

End point description:

Analysis of Covariance was performed on outcomes, the post-treatment value was adjusted for sex, gestation, bronchopulmonary dysplasia, intrauterine growth restriction, pre-treatment corticosteroid status, group and pre-treatment value. Adjusted post-treatment values were compared between groups, with the null hypothesis of no difference, and using the Games Howell adjustment for multiple comparisons. The sample size required was 53, multiple imputation was employed to impute missing data

|                |         |
|----------------|---------|
| End point type | Primary |
|----------------|---------|

End point timeframe:

12 weeks

| End point values                     | Monotherapy       | Combination Therapy | Placebo           | Overall Trial        |
|--------------------------------------|-------------------|---------------------|-------------------|----------------------|
| Subject group type                   | Reporting group   | Reporting group     | Reporting group   | Subject analysis set |
| Number of subjects analysed          | 20 <sup>[1]</sup> | 19 <sup>[2]</sup>   | 14 <sup>[3]</sup> | 53 <sup>[4]</sup>    |
| Units: % predicted FEV1              |                   |                     |                   |                      |
| arithmetic mean (standard deviation) | 81.6 (± 12.0)     | 88.0 (± 7.1)        | 73.9 (± 11.5)     | 80.2 (± 11.9)        |

Notes:

[1] - 18 completed and results imputed for 2 participants

[2] - data for 17 and imputed data for 2 participants

[3] - data for 13 and imputed data for 1 participant

[4] - 48 completed analysis and 5 participants data imputed, total 53 participants.

## Statistical analyses

| Statistical analysis title | Analysis of Covariance of percentage predicted FEV |
|----------------------------|----------------------------------------------------|
|----------------------------|----------------------------------------------------|

Statistical analysis description:

Analysis of Covariance was performed on outcomes, the post-treatment value was adjusted for sex, gestation, bronchopulmonary dysplasia, intrauterine growth restriction, pre-treatment corticosteroid status, group and pre-treatment value. Adjusted post-treatment values were compared between groups, with the null hypothesis of no difference, and using the Games Howell adjustment for multiple comparisons. The sample size required was 53, multiple imputation was employed to impute missing data.

|                                         |                                |
|-----------------------------------------|--------------------------------|
| Comparison groups                       | Placebo v Monotherapy          |
| Number of subjects included in analysis | 34                             |
| Analysis specification                  | Pre-specified                  |
| Analysis type                           | superiority <sup>[5]</sup>     |
| P-value                                 | < 0.05 <sup>[6]</sup>          |
| Method                                  | ANCOVA                         |
| Parameter estimate                      | Mean difference (final values) |
| Point estimate                          | 7.7                            |
| Confidence interval                     |                                |
| level                                   | 95 %                           |
| sides                                   | 2-sided                        |
| lower limit                             | -0.3                           |
| upper limit                             | 15.7                           |
| Variability estimate                    | Standard error of the mean     |
| Dispersion value                        | 4.1                            |

Notes:

[5] - This was the analysis as defined in the statistical analysis plan

[6] - P-values for group comparisons were adjusted for multiple comparisons using the Games Howell method.

| Statistical analysis title | Analysis of Covariance of percentage pr... |
|----------------------------|--------------------------------------------|
|----------------------------|--------------------------------------------|

Statistical analysis description:

Analysis of Covariance was performed on outcomes, the post-treatment value was adjusted for sex, gestation, bronchopulmonary dysplasia, intrauterine growth restriction, pre-treatment corticosteroid status, group and pre-treatment value. Adjusted post-treatment values were compared between groups, with the null hypothesis of no difference, and using the Games Howell adjustment for multiple comparisons. The sample size required was 53, multiple imputation was employed to impute missing data.

|                   |                               |
|-------------------|-------------------------------|
| Comparison groups | Combination Therapy v Placebo |
|-------------------|-------------------------------|

|                                         |                                |
|-----------------------------------------|--------------------------------|
| Number of subjects included in analysis | 33                             |
| Analysis specification                  | Pre-specified                  |
| Analysis type                           | superiority <sup>[7]</sup>     |
| P-value                                 | < 0.05 <sup>[8]</sup>          |
| Method                                  | ANCOVA                         |
| Parameter estimate                      | Mean difference (final values) |
| Point estimate                          | 14.1                           |
| Confidence interval                     |                                |
| level                                   | 95 %                           |
| sides                                   | 2-sided                        |
| lower limit                             | 7.3                            |
| upper limit                             | 21                             |
| Variability estimate                    | Standard error of the mean     |
| Dispersion value                        | 3.5                            |

Notes:

[7] - This was the analysis as defined in the statistical analysis plan

[8] - P-values for group comparisons were adjusted for multiple comparisons using the Games Howell method.

|                                   |                                    |
|-----------------------------------|------------------------------------|
| <b>Statistical analysis title</b> | Analysis of Covariance of perce... |
|-----------------------------------|------------------------------------|

Statistical analysis description:

Analysis of Covariance was performed on outcomes, the post-treatment value was adjusted for sex, gestation, bronchopulmonary dysplasia, intrauterine growth restriction, pre-treatment corticosteroid status, group and pre-treatment value. Adjusted post-treatment values were compared between groups, with the null hypothesis of no difference, and using the Games Howell adjustment for multiple comparisons. The sample size required was 53, multiple imputation was employed to impute missing data.

|                                         |                                   |
|-----------------------------------------|-----------------------------------|
| Comparison groups                       | Monotherapy v Combination Therapy |
| Number of subjects included in analysis | 39                                |
| Analysis specification                  | Pre-specified                     |
| Analysis type                           | superiority <sup>[9]</sup>        |
| P-value                                 | < 0.05 <sup>[10]</sup>            |
| Method                                  | ANCOVA                            |
| Parameter estimate                      | Mean difference (final values)    |
| Point estimate                          | 6.4                               |
| Confidence interval                     |                                   |
| level                                   | 95 %                              |
| sides                                   | 2-sided                           |
| lower limit                             | 0.25                              |
| upper limit                             | 12.6                              |
| Variability estimate                    | Standard error of the mean        |
| Dispersion value                        | 3.2                               |

Notes:

[9] - This was the analysis as defined in the statistical analysis plan

[10] - P-values for group comparisons were adjusted for multiple comparisons using the Games Howell method.

## Adverse events

### Adverse events information

Timeframe for reporting adverse events:

All adverse events, including non-serious adverse events, were recorded in the participant's medical records and on their case report form. All adverse events were reported from consent of participant up to the final study visit

Adverse event reporting additional description:

13 adverse events in total throughout the trial - 7 adverse events seen in 4 children randomised to placebo, 2 adverse events seen in 2 children randomised to combination therapy and 4 adverse event seen in 4 children randomised to monotherapy.

|                 |                |
|-----------------|----------------|
| Assessment type | Non-systematic |
|-----------------|----------------|

### Dictionary used

|                 |        |
|-----------------|--------|
| Dictionary name | MedDRA |
|-----------------|--------|

|                    |    |
|--------------------|----|
| Dictionary version | 14 |
|--------------------|----|

### Reporting groups

|                       |               |
|-----------------------|---------------|
| Reporting group title | Overall Trial |
|-----------------------|---------------|

Reporting group description: -

| Serious adverse events                            | Overall Trial  |  |  |
|---------------------------------------------------|----------------|--|--|
| Total subjects affected by serious adverse events |                |  |  |
| subjects affected / exposed                       | 0 / 53 (0.00%) |  |  |
| number of deaths (all causes)                     | 0              |  |  |
| number of deaths resulting from adverse events    | 0              |  |  |

Frequency threshold for reporting non-serious adverse events: 2.65 %

| Non-serious adverse events                            | Overall Trial    |  |  |
|-------------------------------------------------------|------------------|--|--|
| Total subjects affected by non-serious adverse events |                  |  |  |
| subjects affected / exposed                           | 13 / 53 (24.53%) |  |  |
| General disorders and administration site conditions  |                  |  |  |
| General symptom                                       |                  |  |  |
| subjects affected / exposed                           | 13 / 53 (24.53%) |  |  |
| occurrences (all)                                     | 13               |  |  |

## More information

### Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

| Date              | Amendment                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 01 July 2016      | <ul style="list-style-type: none"> <li>Flow chart updated to include QoL and Health Economic questionnaires</li> <li>Information on accelerometry removed</li> <li>Information on completion of CHU-9D questionnaire added</li> <li>Detail on completion of health economic questionnaire and CHU-9D added</li> <li>Detail added regarding collection of peak flow data</li> <li>Amended to include detail on placebo formulation</li> <li>Table 1 scheduled of procedures updated</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| 21 September 2016 | <ul style="list-style-type: none"> <li>Version control modified to reflect NWOORTH procedures</li> <li>Addition of ISRCRN</li> <li>Description of intervention' updated with details of new IMP treatment groups</li> <li>Flowchart updated to include: Cardiovascular assessment, Helium dilution test and FOT test. Removal of CO2 transfer test</li> <li>Clarification of restrictions prior to part 1 home visit</li> <li>Reversibility test: Removal of the specific use of Ventolin brand salbutamol</li> <li>Clarification of restrictions prior to part 2 lab visit 1</li> <li>Clarification that the respiratory questionnaire will be repeated if part 1 visit 1 is &gt;3 months after the initial questionnaire is completed</li> <li>Addition of detail regarding cardiovascular assessment</li> <li>Removal of CO transfer test</li> <li>Addition of the FOT test</li> <li>Addition of the inert gas washout test</li> <li>Exercise challenge: Small clarifications to the procedure</li> <li>Exercise challenge: Removal of the specific use of Ventolin brand salbutamol</li> <li>Sputum induction: Addition of safety precaution- FEV1 to be within 90% of baseline before starting test</li> <li>Change of treatment allocation to double-dummy design. Inclusion of Serevent in the combination therapy group and removal of Seretide</li> <li>Clarification that participants must take each inhaler, twice daily (AM and PM)</li> <li>Clarification of temperature storage requirements</li> <li>Clarification that local pharmacy will dispose of returns</li> <li>Change of process from on-line system to envelopes for emergency unblinding procedure</li> <li>Removal of the specific use of Ventolin brand salbutamol</li> <li>Removal of the specific use of Ventolin brand salbutamol</li> <li>Derivation of sample size calculations have been described in more detail</li> <li>Table 1 in the protocol Updated to reflect addition/removal of lung function tests</li> </ul> |
| 06 December 2016  | <ul style="list-style-type: none"> <li>Clarification of source of placebo canister</li> <li>Addition of section "treatment withdrawal criteria".</li> <li>Discussion of potential inclusion of females of reproductive potential as requested by the MHRA</li> <li>Addition of section "Nature and frequency of safety assessments" as requested by the MHRA</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

|                  |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 14 March 2017    | <ul style="list-style-type: none"> <li>• Addition of multiple breath washout (lung clearance index) to part 3 of study (LCI aspect removed from part 2)</li> <li>• Clarification of source of reference safety information</li> <li>• New source of placebo canister- Pharmaserve North West Ltd.</li> <li>• Removal of detail regarding use of masking device.</li> <li>• Change of manufacturer from Catalent to St Marys Pharmaceutical Unit</li> </ul>                                                                                                                                                                                                                                                                                           |
| 27 July 2017     | <ul style="list-style-type: none"> <li>• Additional recruitment strategy added- contact of participants outside of current RHiNO database</li> <li>• Typographical error: change to <math>p &lt; 0.05</math></li> <li>• Clarification of data required for internal pilot of sample size assumptions</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| 07 December 2017 | <ul style="list-style-type: none"> <li>• Change to number of research sites</li> <li>• Addition of Statistician's signature</li> <li>• Additional research sites added</li> <li>• Clarification of IMP packaging and labelling procedure</li> <li>• Change of text in relation to nomenclature for consistency with IMP manufacture and randomisation</li> </ul>                                                                                                                                                                                                                                                                                                                                                                                     |
| 13 March 2018    | <ul style="list-style-type: none"> <li>• Additional recruitment of potential participants</li> <li>• Addition of Adverse event to schedule of procedures for Part 3 MRI</li> <li>• Reference to data management plan</li> <li>• Change to the inhaler procurement</li> <li>• Change to the number of inhalers provided to participants</li> <li>• Revision of temperature storage requirements</li> <li>• Change to the inhaler procurement</li> <li>• Revision of temperature storage requirements</li> <li>• Corrected spelling errors</li> </ul>                                                                                                                                                                                                  |
| 16 October 2018  | <ul style="list-style-type: none"> <li>• Typographical errors corrected</li> <li>• Clarification of number of weeks between visit 1 and 2, to ensure convenience for children and parents</li> <li>• Change to xenon as the hyperpolarised gas used in MRI</li> <li>• Addition of an MRI performed before and after a bronchodilator</li> <li>• Clarification of placebo canister supplier- GSK</li> <li>• Clarification that GSK placebo inhalers are CE marked</li> <li>• Clarification that a third batch of inhalers will be procured in late 2018</li> <li>• Clarification of FEV1 % required for pre-term born and term born control children.</li> <li>• Derivation of sample size calculations have been described in more detail</li> </ul> |

Notes:

## Interruptions (globally)

Were there any global interruptions to the trial? No

## Limitations and caveats

None reported