



## Clinical trial results:

### Near infrared imaging and measurement of extremity lymphatic collector function using indocyanine green

#### Summary

|                          |                |
|--------------------------|----------------|
| EudraCT number           | 2013-004954-58 |
| Trial protocol           | DK             |
| Global end of trial date | 06 June 2017   |

#### Results information

|                                |               |
|--------------------------------|---------------|
| Result version number          | v1 (current)  |
| This version publication date  | 24 March 2020 |
| First version publication date | 24 March 2020 |

#### Trial information

##### Trial identification

|                       |          |
|-----------------------|----------|
| Sponsor protocol code | 2013-617 |
|-----------------------|----------|

##### Additional study identifiers

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

Notes:

#### Sponsors

|                              |  |
|------------------------------|--|
| Sponsor organisation name    | Bispebjerg & Frederiksberg Hospital, Capital Region of Denmark   |
| Sponsor organisation address | Ebba Lunds Vej 44, Copenhagen NW, Denmark, 2400  |
| Public contact               | Department of Clinical Physiology & Nuclear Medicine, Bispebjerg & Frederiksberg Hospital, 0045 3863 5530, mads.radmer.jensen@regionh.dk |
| Scientific contact           | Department of Clinical Physiology & Nuclear Medicine, Bispebjerg & Frederiksberg Hospital, 0045 3863 5530, mads.radmer.jensen@regionh.dk |

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:

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## Results analysis stage

|  |                 |
|--|-----------------|
| Analysis stage                                       | Final           |
| Date of interim/final analysis                       | 03 January 2020 |
| Is this the analysis of the primary completion data? | Yes             |
| Primary completion date                              | 06 June 2017    |
| Global end of trial reached?                         | Yes             |
| Global end of trial date                             | 06 June 2017    |
| Was the trial ended prematurely?                     | Yes             |

Notes:

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## General information about the trial

Main objective of the trial:

To assess effects of method variations and physical factors on visualisation of numbers of - and pumping activity in extremity lymphatic collectors in healthy adult subjects using indocyanine green (ICG) fluorescence lymphangiography.

Protection of trial subjects:

During the study visit:

- Discomfort/pain: To avoid precipitation in the injectate, ICG powder was dissolved in sterile water for injection purposes. However, this solution is hypotonic, and skin injection is therefore painful. This was alleviated by first dissolving ICG in 1 ml sterile water for injection purposes with subsequent dilution with sterile isotonic saline for injection purposes to the desired concentration.

- Infection: Although the risk of skin infection related to injection of the ICG solution is expectedly minor in healthy subjects, the following measures were taken to further reduce the risk: 1) ICG was reconstituted using sterile technique, 2) Depot injection sites were disinfected twice with alcohol swaps prior to injection, 3) Sterile disposable needles and - syringes were used.

- Acute adverse events and - reactions: Following depot injection and during the imaging procedure (minimum 180 min after 1. and 90 min after last injection) the subjects were monitored clinically for signs of acute local or systemic allergic reactions. None occurred, however if a reaction had occurred treatment and monitoring would have followed manufacturer (See SPC) and hospital guidelines. Necessary acute medication and utensils were always readily at hand in the study department.

After the study visit:

The subject was instructed to contact the primary investigator in case of suspected late adverse events and/or - reactions. One week after participation the subject participated in a short mandatory phone interview in order to identify any unrecognised adverse events or - reactions. None occurred; however, if an adverse reaction had been suspected, the subject would have been examined clinically and the event documented in detail in the CRF. Absence of adverse events or - reactions was specifically recorded in the CRF. Treatment, follow-up and/or referral – if necessary – would have been decided on a case-by-case basis

Background therapy:

None

Evidence for comparator:

Evidence has been published showing that skin injections of the fluorescent dye Indocyanine green (ICG) enable near infrared imaging of extremity lymphatic collectors. Through video analysis dynamics of peristaltic pumping activity in these collectors is possible. However, sparse evidence exists in the literature on the possible effects of study method variations, subject characteristics and physical factors on numbers of visualised lymphatic collectors and their pumping activity (lymph package frequency) in healthy adult subjects with normal Body Mass Index. Hence, this study is exploratory in nature and designed to elucidate possible large effects of below listed factors. The results may prove important to take into account in the design of future trials.

Method variations (Group A):

- Time delay from depot injection to imaging: 0 min, 30 min, 120 min and 150 min.
- Injection method: Intradermal vs subcutaneous.
- Injected volume: 0,1 ml vs 0,3 ml.
- Injected concentration: 0,83 mg/ml, 1 mg/ml and 2,5 mg/ml.
- Extremity: Depot injection interdigitally on the dorsum of the hand vs the foot.

Physical factors (Group B\*):

- External skin heating: Heating lamp adjusted to 39 degrees centigrade skin temperature.
- Exercise: Treadmill walking at 4 km/h in 10 min.
- Tonicity of injectate: Sterile water dilution of the injectate vs dilution with isotonic saline.
- Venous stasis: Depot dependency 30 cm below heart level.

Other factors:

- Age
- Gender

\*Note: Data collection in group A was completed. It was our intend - according to protocol - to perform an interim analysis of the data acquired in group A. Based on these results we planned to choose the method to be applied in group B. However, due to lack of personnel resources the study was interrupted in the interim analysis period. Group B was never initiated.

|   |                  |
|---|------------------|
| Actual start date of recruitment                          | 03 February 2014 |
| Long term follow-up planned                               | No               |
| Independent data monitoring committee (IDMC) involvement? | No               |

Notes:

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### Population of trial subjects

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#### Subjects enrolled per country

|                                      |             |
|--------------------------------------|-------------|
| Country: Number of subjects enrolled | Denmark: 19 |
| Worldwide total number of subjects   | 19          |
| EEA total number of subjects         | 19          |

Notes:

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#### 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)                     | 0  |
| Adolescents (12-17 years)                 | 0  |
| Adults (18-64 years)                      | 19 |
| From 65 to 84 years                       | 0  |
| 85 years and over                         | 0  |

## Subject disposition

### Recruitment

#### Recruitment details:

Volunteers were recruited from the Capital Region of Denmark by approved advertisement on the website <http://www.forsøgsperson.dk>. Interested subjects contacted the subinvestigator by e-mail or phone. Written informed consent was obtained prior to participation. The first subject was recruited on 30.12.2014 and the last on 06.06.2017

### Pre-assignment

#### Screening details:

##### Inclusion:

- Healthy
- Capable
- Age 18-65 years
- BMI 18-25 kg/m<sup>2</sup>
- A negative urine-HCG test for fertile women

##### Exclusion:

- Any known disease or chronic medication
- Pregnancy or lactation
- Contraindication to ICG-PULSION® use (see SPC).
- Smoking/other nicotine use.
- Oedema, venous disease, local inflammation.

### Period 1

|                              |                                |
|------------------------------|--------------------------------|
| Period 1 title               | Overall trial (overall period) |
| Is this the baseline period? | Yes                            |
| Allocation method            | Non-randomised - controlled    |
| Blinding used                | Not blinded                    |

#### Blinding implementation details:

None

### Arms

|           |         |
|-----------|---------|
| Arm title | Group A |
|-----------|---------|

#### Arm description:

Effect of method variations on numbers of visualised lymphatic collectors and their pumping activity.

#### Assessed factors are:

- Time delay from depot injection to imaging: 0 min, 30 min, 120 min and 150 min.
- Injectate concentration: 0,1 mg/ml, 0,83 mg/ml and 2,5 mg/ml.
- Injected volume: 0,1 ml and 0,3 ml.
- Skin injection method: Intradermal and subcutaneous.

#### Other explanatory factors:

Gender, age and extremity (arm and leg).

|  |  |
|--|--|
| Arm type                               | Experimental   |
| Investigational medicinal product name | ICG-PULSION(R)   |
| Investigational medicinal product code |  |
| Other name                             | Indocyanine Green  |
| Pharmaceutical forms                   | Powder for concentrate for solution for injection/infusion |
| Routes of administration               | Intradermal use, Subcutaneous use                          |

#### Dosage and administration details:

Sterile utensils and techniques were used. One vial of ICG-PULSION® was used for each subject. A base solution was prepared by reconstituting 25 mg ICG powder with 1 ml sterile water for injection purposes in the vial. From this base solution 1 ml syringes with solutions for injection (A, B, C & D) were prepared by dilution with isotonic saline for injection purposes (details are described in the protocol).

- Solution A: 0,1 ml 2,5 mg/ml ICG injected intradermally in the dorsum of the right hand (depot A).
- Solution B: 0,1 ml 1,0 mg/ml ICG injected intradermally in the dorsum of the left hand (depot B).
- Solution C: 0,3 ml 0,83 mg/ml ICG injected intradermally in the dorsum of the right foot (depot C).
- Solution D: 0,1 ml 2,5 mg/ml ICG injected subcutaneously in the dorsum of the left foot (depot D).

| <b>Number of subjects in period 1</b> | Group A |
|---------------------------------------|---------|
| Started                               | 19      |
| Completed                             | 18      |
| Not completed                         | 1       |
| Adverse event, non-fatal              | 1       |

## Baseline characteristics

### Reporting groups

|   |               |
|---|---------------|
| Reporting group title   | Overall trail |
| Reporting group description:<br>Group A consisting of healthy adult volunteers recruited evenly from both genders (see Subject disposition, Period 1). The reporting groups comprise data collected from the same subjects (group A, 1 excluded, see non-serious adverse events). |               |

| Reporting group values   | Overall trail | Total |  |
|--|---------------|-------|--|
| Number of subjects   | 19            | 19    |  |
| Age categorical  |               |       |  |
| Being adult (age 18 - 64 years) was an inclusion criterion; however effect of age was not a factor under investigation as such. During recruitment, an even age dispersion in the adult age range was sought for both genders, but not treated as a recruitment criterion per se. Volunteering subjects were primarily young (< 30 years) in both genders as reflected in the final study population age median. |               |       |  |
| Units: Subjects  |               |       |  |
| In utero   | 0             | 0     |  |
| Preterm newborn infants (gestational age < 37 wks)   | 0             | 0     |  |
| Newborns (0-27 days)   | 0             | 0     |  |
| Infants and toddlers (28 days-23 months)   | 0             | 0     |  |
| Children (2-11 years)  | 0             | 0     |  |
| Adolescents (12-17 years)  | 0             | 0     |  |
| Adults (18-64 years)   | 19            | 19    |  |
| From 65-84 years   | 0             | 0     |  |
| 85 years and over  | 0             | 0     |  |
| Age continuous   |               |       |  |
| Being adult (age 18 - 64 years) was an inclusion criterion; however effect of age was not a factor under investigation as such. During recruitment, an even age dispersion in the adult age range was sought for both genders, but not treated as a recruitment criterion per se. Volunteering subjects were primarily young (< 30 years) in both genders as reflected in the final study population age median. |               |       |  |
| Units: years   |               |       |  |
| median   | 24            |       |  |
| full range (min-max)   | 18 to 59      | -     |  |
| Gender categorical   |               |       |  |
| An even gender distribution was sought during recruitment. One female subject was excluded due to unintended intravenous injection rendering the video unusable for later analysis (See non-serious adverse events).   |               |       |  |
| Units: Subjects  |               |       |  |
| Female   | 11            | 11    |  |
| Male   | 8             | 8     |  |

### Subject analysis sets

|                            |                        |
|----------------------------|------------------------|
| Subject analysis set title | Video analysis results |
| Subject analysis set type  | Full analysis          |

Subject analysis set description:

Results of visual and semi-automatic analysis of near infrared fluorescence videos recorded in group A except for one excluded subject (see non-serious adverse events). Results from each video are: (1) Mean package count (explained in "end points"), (2) maximum package count and (3) numbers of visualised lymphatic collectors.

|   |                    |
|---|--------------------|
| Subject analysis set title  | Age by gender      |
| Subject analysis set type   | Sub-group analysis |
| Subject analysis set description:   |                    |
| Age distribution in male and female subjects, who completed the protocol. |                    |

| Reporting group values   | Video analysis results | Age by gender |  |
|--|------------------------|---------------|--|
| Number of subjects   | 18                     | 18            |  |
| Age categorical  |                        |               |  |
| Being adult (age 18 - 64 years) was an inclusion criterion; however effect of age was not a factor under investigation as such. During recruitment, an even age dispersion in the adult age range was sought for both genders, but not treated as a recruitment criterion per se. Volunteering subjects were primarily young (< 30 years) in both genders as reflected in the final study population age median. |                        |               |  |
| Units: Subjects  |                        |               |  |
| In utero   | 0                      | 0             |  |
| Preterm newborn infants (gestational age < 37 wks)   | 0                      | 0             |  |
| Newborns (0-27 days)   | 0                      | 0             |  |
| Infants and toddlers (28 days-23 months)   | 0                      | 0             |  |
| Children (2-11 years)  | 0                      | 0             |  |
| Adolescents (12-17 years)  | 0                      | 0             |  |
| Adults (18-64 years)   | 18                     | 18            |  |
| From 65-84 years   | 0                      | 0             |  |
| 85 years and over  | 0                      | 0             |  |
| Age continuous   |                        |               |  |
| Being adult (age 18 - 64 years) was an inclusion criterion; however effect of age was not a factor under investigation as such. During recruitment, an even age dispersion in the adult age range was sought for both genders, but not treated as a recruitment criterion per se. Volunteering subjects were primarily young (< 30 years) in both genders as reflected in the final study population age median. |                        |               |  |
| Units: years   |                        |               |  |
| median   | 24                     | 24            |  |
| full range (min-max)   | 18 to 59               | 18 to 59      |  |
| Gender categorical   |                        |               |  |
| An even gender distribution was sought during recruitment. One female subject was excluded due to unintended intravenous injection rendering the video unusable for later analysis (See non-serious adverse events).   |                        |               |  |
| Units: Subjects  |                        |               |  |
| Female   | 10                     | 10            |  |
| Male   | 8                      | 8             |  |

## End points

### End points reporting groups

|   |                        |
|---|------------------------|
| Reporting group title   | Group A                |
| Reporting group description:<br>Effect of method variations on numbers of visualised lymphatic collectors and their pumping activity.<br>Assessed factors are: <ul style="list-style-type: none"><li>- Time delay from depot injection to imaging: 0 min, 30 min, 120 min and 150 min.</li><li>- Injectate concentration: 0,1 mg/ml, 0,83 mg/ml and 2,5 mg/ml.</li><li>- Injected volume: 0,1 ml and 0,3 ml.</li><li>- Skin injection method: Intradermal and subcutaneous.</li></ul> |                        |
| Other explanatory factors:<br>Gender, age and extremity (arm and leg).  |                        |
| Subject analysis set title  | Video analysis results |
| Subject analysis set type   | Full analysis          |
| Subject analysis set description:<br>Results of visual and semi-automatic analysis of near infrared fluorescence videos recorded in group A except for one excluded subject (see non-serious adverse events). Results from each video are: (1) Mean package count (explained in "end points"), (2) maximum package count and (3) numbers of visualised lymphatic collectors.  |                        |
| Subject analysis set title  | Age by gender          |
| Subject analysis set type   | Sub-group analysis     |
| Subject analysis set description:<br>Age distribution in male and female subjects, who completed the protocol.  |                        |

### Primary: Mean number of packages in 30 min

|  |                                   |
|--|-----------------------------------|
| End point title  | Mean number of packages in 30 min |
| End point description:<br>In each subject 8 videos were recorded of pumping activity in lymphatic collectors draining 4 depots injected dorsally and interdigitally in both hands and - feet with methodological variations and time delay as specified below. Pumping activity was quantified as spike-like fluorescence signal intensity increases recorded in 3 regions of interest (ROI) placed over each visualised collector enabling semi-automatic counting of package numbers in a 30 min recording. Mean package number is the average count from all ROIs in a video. As the number of visualised collectors varies, the number of ROIs also varies.<br>Depot A: 0,1 ml 2,5 mg/ml ICG-PULSION(R), i.d., right hand.<br>Depot B: 0,1 ml 1,0 mg/ml ICG-PULSION(R), i.d., left hand.<br>Depot C: 0,3 ml 0,83 mg/ml ICG-PULSION(R), i.d., right foot.<br>Depot D: 0,1 ml 2,5 mg/ml ICG-PULSION(R), s.c., left foot.<br>Time delays from depot injection to recording were 0, 30 & 150 min for depot A, 30 min for depot B & 30 & 120 min for depot C and D. |                                   |
| End point type   | Primary                           |
| End point timeframe:<br>Analysis results of videos recorded during study visits from 30.12.2014 to 30.05.2017.   |                                   |

| End point values               | Group A           | Video analysis results |  |  |
|--------------------------------|-------------------|------------------------|--|--|
| Subject group type             | Reporting group   | Subject analysis set   |  |  |
| Number of subjects analysed    | 18 <sup>[1]</sup> | 18                     |  |  |
| Units: Package count in 30 min |                   |                        |  |  |
| median (full range (min-max))  |                   |                        |  |  |
| Depot A 0 min                  | 11 (3 to 42)      | 11 (0 to 42)           |  |  |
| Depot A 30 min                 | 14 (1 to 34)      | 14 (1 to 34)           |  |  |



|                 |              |              |  |  |
|-----------------|--------------|--------------|--|--|
| Depot A 150 min | 11 (2 to 38) | 11 (2 to 38) |  |  |
| Depot B 30 min  | 11 (5 to 37) | 11 (5 to 37) |  |  |
| Depot C 30 min  | 8 (1 to 24)  | 8 (1 to 24)  |  |  |
| Depot C 120 min | 12 (2 to 30) | 12 (2 to 30) |  |  |
| Depot D 30 min  | 9 (0 to 35)  | 9 (0 to 35)  |  |  |
| Depot D 120 min | 12 (4 to 29) | 12 (4 to 29) |  |  |

Notes:

[1] - xx

|                                   |   |
|-----------------------------------|---|
| <b>Attachments (see zip file)</b> | GLMM mean package count/GLMM mean package count.pdf |
|-----------------------------------|---|

## Statistical analyses

|                                   |                           |
|-----------------------------------|---------------------------|
| <b>Statistical analysis title</b> | GLMM mean "package" count |
|-----------------------------------|---------------------------|

Statistical analysis description:

No power calculation was performed, since insufficient human data existed in the literature. The study was exploratory in nature aiming at identifying large systematic differences; the null hypothesis being no difference in lymphatic collector pumping activity between described method variations (time delay, injection technique, ICG solution concentration, injected volume) or dependence on extremity (hands vs. feet), gender or age.

|   |                                  |
|---|----------------------------------|
| Comparison groups                       | Group A v Video analysis results |
| Number of subjects included in analysis | 36                               |
| Analysis specification                  | Post-hoc                         |
| Analysis type                           | equivalence <sup>[2]</sup>       |
| P-value                                 | = 0.02 <sup>[3]</sup>            |
| Method                                  | Mixed models analysis            |
| Parameter estimate                      | Estimated marginal means         |
| Point estimate                          | 12.4                             |
| Confidence interval                     |                                  |
| level                                   | 95 %                             |
| sides                                   | 2-sided                          |
| lower limit                             | 10.1                             |
| upper limit                             | 15.1                             |
| Variability estimate                    | Standard error of the mean       |
| Dispersion value                        | 1.2                              |

Notes:

[2] - Data was best fitted to a gamma probability distributiondistribution (Log link function).

Information criterion: Akaike corrected 302,179

The model adjusted for multiple comparisons.

The target was "mean package count".

Fixed effects were: Gender, extremity, injection (subcutaneous vs intradermal), concentration, time delay and age.

Subject ID was treated as a random effect.

[3] - Intercept:  $p < 0,00$

Gender:  $p = 0,02$ , Table 1 & Figure 1

No significant effect of extremity ( $p = 0,21$ ), injection ( $p = 0,69$ ), concentration ( $p = 0,65$ ), time delay ( $p = 0,39$ ) or age ( $p = 0,29$ )

Residual effect: Estimate 0,34,  $p < 0,00$ .

Random effect:  $p = 0,09$

## Secondary: Maximum number of packages in 30 min

|                 |                                      |
|-----------------|--------------------------------------|
| End point title | Maximum number of packages in 30 min |
|-----------------|--------------------------------------|

End point description:

In each subject 8 videos were recorded of pumping activity in lymphatic collectors draining 4 depots injected dorsally and interdigitally in both hands and - feet with methodological variations and time delay as specified below. Pumping activity was quantified as spike-like fluorescence signal intensity

increases recorded in 3 regions of interest (ROI) placed over each visualised collector enabling semi-automatic counting of package numbers in a 30 min recording. Maximum package number is the highest count found in any ROI in a video. As the number of visualised collectors varies, the number of ROIs also varies.

Depot A: 0,1 ml 2,5 mg/ml ICG-PULSION(R), i.d., right hand.

Depot B: 0,1 ml 1,0 mg/ml ICG-PULSION(R), i.d., left hand.

Depot C: 0,3 ml 0,83 mg/ml ICG-PULSION(R), i.d., right foot.

Depot D: 0,1 ml 2,5 mg/ml ICG-PULSION(R), s.c., left foot.

Time delays from depot injection to recording were 0, 30 & 150 min for depot A, 30 min for depot B & 30 & 120 min for depot C and D

|                |           |
|----------------|-----------|
| End point type | Secondary |
|----------------|-----------|

End point timeframe:

Analysis results of videos recorded during study visits from 30.12.2014 to 30.05.2017.

| End point values                        | Group A         | Video analysis results |  |  |
|---|-----------------|------------------------|--|--|
| Subject group type                      | Reporting group | Subject analysis set   |  |  |
| Number of subjects analysed             | 18              | 18                     |  |  |
| Units: Frequency (package count/30 min) |                 |                        |  |  |
| median (full range (min-max))           |                 |                        |  |  |
| A 0 min                                 | 16 (3 to 54)    | 16 (3 to 54)           |  |  |
| A 30 min                                | 23 (1 to 77)    | 23 (1 to 77)           |  |  |
| A 150 min                               | 14 (3 to 56)    | 14 (3 to 56)           |  |  |
| B 30 min                                | 15 (6 to 68)    | 15 (6 to 68)           |  |  |
| C 30 min                                | 10 (2 to 47)    | 10 (2 to 47)           |  |  |
| C 120 min                               | 17 (4 to 59)    | 17 (4 to 59)           |  |  |
| D 30 min                                | 14 (0 to 56)    | 14 (0 to 56)           |  |  |
| D 120 min                               | 20 (6 to 40)    | 20 (6 to 40)           |  |  |

|                                   |   |
|-----------------------------------|---|
| <b>Attachments (see zip file)</b> | GLMM max package count/GLMM max package count.pdf |
|-----------------------------------|---|

## Statistical analyses

|                                   |                              |
|-----------------------------------|------------------------------|
| <b>Statistical analysis title</b> | GLMM maximum "package" count |
|-----------------------------------|------------------------------|

Statistical analysis description:

No power calculation was performed, since insufficient human data existed in the literature. The study was exploratory in nature aiming at identifying large systematic differences; the null hypothesis being no difference in lymphatic collector pumping activity between described method variations (time delay, injection technique, ICG solution concentration, injected volume) or dependence on extremity (hands vs. feet), gender or age.

|   |                                  |
|---|----------------------------------|
| Comparison groups                       | Group A v Video analysis results |
| Number of subjects included in analysis | 36                               |
| Analysis specification                  | Post-hoc                         |
| Analysis type                           | equivalence <sup>[4]</sup>       |
| P-value                                 | = 0.02 <sup>[5]</sup>            |
| Method                                  | Mixed models analysis            |
| Parameter estimate                      | Grand Mean Estimate              |
| Point estimate                          | 18.2                             |

|                      |                            |
|----------------------|----------------------------|
| Confidence interval  |                            |
| level                | 95 %                       |
| sides                | 2-sided                    |
| lower limit          | 14.5                       |
| upper limit          | 22.9                       |
| Variability estimate | Standard error of the mean |
| Dispersion value     | 2.06                       |

Notes:

[4] - Data was best fitted to a gamma probability distribution (Log link function).

Information criterion: Akaike corrected 332,890

The model adjusted for multiple comparisons.

The target was "maximum package count".

Fixed effects were: Gender, extremity, injection (subcutaneous vs intradermal), concentration, time delay and age.

Subject ID was treated as a random effect.

[5] - Intercept ( $p < 0,00$ )

Gender:  $p = 0,02$ , Table 2 & Figure 2

No significant effect of extremity ( $p=0,42$ ), injection ( $p=0,80$ ), concentration ( $p=0,48$ ), time delay ( $p=0,47$ ) & age ( $p=0,31$ )

Residual effect: Estimate 0.42,  $p < 0,00$ .

Random effect:  $p = 0,07$

## Secondary: Number of lymphatic collectors by depot type and delay

|                 |  |
|-----------------|--|
| End point title | Number of lymphatic collectors by depot type and delay |
|-----------------|--|

End point description:

In each subject 8 videos were recorded of pumping activity in lymphatic collectors draining 4 depots injected dorsally and interdigitally in both hands and - feet with methodological variations and time delay as specified below. The number of lymphatic collectors visualised in the beginning of each video was counted by visual assessment.

Depot A: 0,1 ml 2,5 mg/ml ICG-PULSION(R), i.d., right hand.

Depot B: 0,1 ml 1,0 mg/ml ICG-PULSION(R), i.d., left hand.

Depot C: 0,3 ml 0,83 mg/ml ICG-PULSION(R), i.d., right foot.

Depot D: 0,1 ml 2,5 mg/ml ICG-PULSION(R), s.c., left foot.

Time delays from depot injection to recording were 0, 30 & 150 min for depot A, 30 min for depot B & 30 & 120 min for depot C and D.

|                |           |
|----------------|-----------|
| End point type | Secondary |
|----------------|-----------|

End point timeframe:

Analysis results of videos recorded during study visits from 30.12.2014 to 30.05.2017.

| End point values              | Group A         | Video analysis results |  |  |
|-------------------------------|-----------------|------------------------|--|--|
| Subject group type            | Reporting group | Subject analysis set   |  |  |
| Number of subjects analysed   | 18              | 18                     |  |  |
| Units: Count                  |                 |                        |  |  |
| median (full range (min-max)) |                 |                        |  |  |
| A 0 min                       | 2 (1 to 3)      | 2 (1 to 3)             |  |  |
| A 30 min                      | 3 (1 to 4)      | 3 (1 to 4)             |  |  |
| A 150 min                     | 4 (1 to 8)      | 4 (1 to 8)             |  |  |
| B 30 min                      | 2 (1 to 3)      | 2 (1 to 3)             |  |  |
| C 30 min                      | 3 (1 to 5)      | 3 (1 to 5)             |  |  |
| C 120 min                     | 3 (1 to 5)      | 3 (1 to 5)             |  |  |
| D 30 min                      | 2 (1 to 5)      | 2 (1 to 5)             |  |  |
| D 120 min                     | 2 (1 to 4)      | 2 (1 to 4)             |  |  |

|                                   |   |
|-----------------------------------|---|
| <b>Attachments (see zip file)</b> | Numbers of collectors by delay/collectors by delay - line plot. |
|-----------------------------------|---|

## Statistical analyses

|                                   |   |
|-----------------------------------|---|
| <b>Statistical analysis title</b> | Number of visualised lymphatic collectors |
|-----------------------------------|---|

Statistical analysis description:

No power calculation was performed, since insufficient human data existed in the literature. The study was exploratory in nature aiming at identifying large systematic differences; the null hypothesis being no difference in number of lymphatic collectors between depot ID (defining extremity, injection technique, ICG solution concentration and injected volume), time delay, gender and age.

|   |                                  |
|---|----------------------------------|
| Comparison groups                       | Group A v Video analysis results |
| Number of subjects included in analysis | 36                               |
| Analysis specification                  | Post-hoc                         |
| Analysis type                           | equivalence <sup>[6]</sup>       |
| P-value                                 | = 0.02 <sup>[7]</sup>            |
| Method                                  | Mixed models analysis            |
| Parameter estimate                      | Grand Mean Estimate              |
| Point estimate                          | 2.4                              |
| Confidence interval                     |                                  |
| level                                   | 95 %                             |
| sides                                   | 2-sided                          |
| lower limit                             | 2.1                              |
| upper limit                             | 2.8                              |
| Variability estimate                    | Standard error of the mean       |
| Dispersion value                        | 0.2                              |

Notes:

[6] - The target distribution was Loglinear.

Information criterion: Akaike corrected 227,398

The model adjusted for multiple comparisons.

The target was "number of lymphatic collectors".

Fixed effects were: Gender, age, depot ID (A, B, C & D) and time delay (min).

Subject ID was a random effect

[7] - Intercept: p=0.03

Delay: p=0.02 (coefficient 0.002 95% CI 0.00 - 0.004)

No significant effect of gender (p=0.47), age (p=0.50) or depot: p>0.05 (see box-plot)

Random effect: p=0.42

## Secondary: Age by gender

|                 |               |
|-----------------|---------------|
| End point title | Age by gender |
|-----------------|---------------|

End point description:

Age distribution by gender in group A.

|                |           |
|----------------|-----------|
| End point type | Secondary |
|----------------|-----------|

End point timeframe:

Analysis results from subjects recruited from 30.12.2014 to 30.05.2017.

|                               |                 |  |  |  |
|-------------------------------|-----------------|--|--|--|
| <b>End point values</b>       | Group A         |  |  |  |
| Subject group type            | Reporting group |  |  |  |
| Number of subjects analysed   | 18              |  |  |  |
| Units: Years                  |                 |  |  |  |
| median (full range (min-max)) |                 |  |  |  |
| Males                         | 25 (18 to 59)   |  |  |  |
| Females                       | 24 (19 to 46)   |  |  |  |

|                                   |  |
|-----------------------------------|--|
| <b>Attachments (see zip file)</b> | Age by gender/Age by gender box plot.pdf |
|-----------------------------------|--|

### Statistical analyses

No statistical analyses for this end point

## Adverse events

### Adverse events information

Timeframe for reporting adverse events:

30-12-2017 to 06-06-2017

Adverse event reporting additional description:

Clinical observation of possible acute local or systemic SAR during the study visit from injection of the first depot to completion of video recordings.

Instruction to phone primary investigator in case of suspected delayed AE or AR.

Mandatory phone interview by subinvestigator 1 week after the study visit to confirm no occurrence of AE or AR

|                 |            |
|-----------------|------------|
| Assessment type | Systematic |
|-----------------|------------|

### Dictionary used

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

|                    |    |
|--------------------|----|
| Dictionary version | 16 |
|--------------------|----|

### Reporting groups

|                       |         |
|-----------------------|---------|
| Reporting group title | Group A |
|-----------------------|---------|

Reporting group description:

All healthy adult volunteers that were included in the study.

| Serious adverse events                            | Group A        |  |  |
|---|----------------|--|--|
| Total subjects affected by serious adverse events |                |  |  |
| subjects affected / exposed                       | 0 / 19 (0.00%) |  |  |
| number of deaths (all causes)                     | 0              |  |  |
| number of deaths resulting from adverse events    | 0              |  |  |

Frequency threshold for reporting non-serious adverse events: 5 %

| Non-serious adverse events                            | Group A   |  |  |
|---|---|--|--|
| Total subjects affected by non-serious adverse events |   |  |  |
| subjects affected / exposed                           | 1 / 19 (5.26%)  |  |  |
| Investigations  |   |  |  |
| Unintended intravenous injection                      | Additional description: In one female subject, injection of depot A (see Subject disposition, Group A) was unintentionally injected intravenously instead of the planned intradermal injection. This did not result in any adverse reactions (neither acute nor delayed). |  |  |
| subjects affected / exposed                           | 1 / 19 (5.26%)  |  |  |
| occurrences (all)                                     | 1   |  |  |

## More information

### Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

| Date         | Amendment   |
|--------------|---|
| 08 July 2016 | Increase in the allowed total number of subjects to be recruited to a maximum of 40 if deemed necessary. The amendment enabled replacement of subjects with an incomplete dataset or a dataset that could not be analysed due to reduced technical quality ensuring sufficient data for a comprehensible analysis of overall trial results. |

Notes:

### Interruptions (globally)

Were there any global interruptions to the trial? Yes

| Date         | Interruption  | Restart date |
|--------------|---|--------------|
| 06 June 2017 | <p>The last subject completed participation on 06-jun-2017. After, the interim analysis period started and until 04-03-2019 it was still our intend to complete the trial as described in the protocol. However, due to lack of personnel resources to complete the remaining study visits and following data analysis Sponsor and Investigator agreed to terminate the study before initiation of group B. This lack of personnel resources developed due to a combination of changes in employee composition and increasing clinical work load, and was not likely to improve in the foreseeable future.</p> <p>No adverse reactions occurred in the trial period; however, due to the small number of study subjects examined, rare adverse reactions are not likely to occur in this material. Overall the present data do not have a negative effect on the overall risk benefit assessment.</p> | -            |

Notes:

### Limitations and caveats

Limitations of the trial such as small numbers of subjects analysed or technical problems leading to unreliable data.

The study population is relatively small increasing the risk of type 2 errors i.e. not finding small but significant effects of applied method variations, time delay, extremity and age. Studies with larger populations are needed to elucidate this.

Notes:

### Online references

<http://www.ncbi.nlm.nih.gov/pubmed/22924087>

<http://www.ncbi.nlm.nih.gov/pubmed/26993717>

<http://www.ncbi.nlm.nih.gov/pubmed/22808440>