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:

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:

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 i 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:

Population of trial subjects

Subjects enrolled per country

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

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)	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/m2
- 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 trail (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 subcutanously in the dorsum of the left foot (depot D).

Number of subjects in period 1	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:

Group A consisting of healthy adult volunteers recruited evenly from both genders (see Subject disposition, Period 1). The reporting groups comprise data collceted 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:

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).

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.

Primary: Mean number of packages in 30 min

End point title	Mean 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. 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.
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	Primary

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[1]	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

Attachments (see zip file)	GLMM mean package count/GLMM mean package count.pdf

Statistical analyses

Statistical analysis title	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[2]
P-value	= 0.02 [3]
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)		

Attachments (see zip file)	GLMM max package count/GLMM max package count.pdf

Statistical analyses

Statistical analysis title	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[4]
P-value	= 0.02 [5]
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 distributiondistribution (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)		

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

Statistical analyses

Statistical analysis title	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[6]
P-value	= 0.02 [7]
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.

End point values	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)			

Attachments (see zip file)	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 af depot A (see Subject disposition, Group A) was unintendedly 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	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 inititation 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. 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.	-

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