

**Clinical trial results:**

A 48-WEEK PHASE II, RANDOMISED, DOUBLE BLINDED PLACEBO CONTROLLED, PARALLEL-GROUP, MULTI-CENTRE TRIAL ON LIRAGLUTIDE'S SAFETY, EFFICACY AND ACTION ON LIVER HISTOLOGY AND METABOLISM IN OVERWEIGHT PATIENTS WITH NON-ALCOHOLIC STEATOHEPATITIS, WITH OR WITHOUT TYPE II DIABETES

Summary

EudraCT number	2009-016761-29
Trial protocol	GB
Global end of trial date	02 July 2014

Results information

Result version number	v1 (current)
This version publication date	26 September 2020
First version publication date	26 September 2020
Summary attachment (see zip file)	Lean Study Protocol V7.0 (Protocol version 7.0 (Cleaned).pdf)

Trial information**Trial identification**

Sponsor protocol code	RG_09-190
-----------------------	-----------

Additional study identifiers

ISRCTN number	ISRCTN85774727
ClinicalTrials.gov id (NCT number)	NCT01237119
WHO universal trial number (UTN)	-
Other trial identifiers	UK Competent authority (MHRA) Reference: 21761/0247/001-0001, NHS (UK) Ethics Ref number: 10/H0402/32

Notes:

Sponsors

Sponsor organisation name	The University of Birmingham
Sponsor organisation address	Research Support Group, Finance Office, Aston Webb building, Birmingham, United Kingdom, B15 2TT
Public contact	Dr Sean Jennings, The University of Birmingham, +44 (0)121 415 8011, s.jennings@bham.ac.uk
Scientific contact	Professor Philip Newsome, The University of Birmingham, +44 (0)121 414 5614, p.n.newsone@bham.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	20 August 2015
Is this the analysis of the primary completion data?	Yes
Primary completion date	02 July 2014
Global end of trial reached?	Yes
Global end of trial date	02 July 2014
Was the trial ended prematurely?	No

Notes:

General information about the trial

Main objective of the trial:

To investigate the safety and efficacy of 48 weeks treatment with once-daily injections of liraglutide in damaged liver tissue in overweight patients with non-alcoholic steatohepatitis [NASH] (i.e. the more severe, inflammatory form of fatty liver disease). To investigate whether the effect of liraglutide on NASH warrants further investigation.

Protection of trial subjects:

No specific measures were included within the study protocol specifically for the protection of trial subjects outside the normal safety monitoring that is included in a phase II (safety and efficacy) study. This monitoring included, but not limited to blood tests (Full blood counts and Biochemistry panel); physical exam and collection/evaluation of adverse event and serious adverse event data. These procedures were performed/repeated at multiple visits throughout the treatment period (monthly - 3 monthly visits) and the results of these procedures were recorded in the Case Report Form (CRF)

In addition a facility for 24/7 unblinding of study medication was available for medical physicians if required. This could be accessed by any physician who was treating a patient irrespective of whether they were directly involved in the clinical study. Trial subjects were provided with a participant study card which included details of the clinical trial and 24/7 emergency number. Limited medical advice was also available from this resource.

Background therapy:

The study did not include any Non-Investigational Medicinal Products (NIMPS). A full record of any medications that a trial subject was taking during the study period was recorded in the individual subjects Case Report Form (Concomitant medication page).

Evidence for comparator:

This clinical trial was a randomised, double blind phase II safety and efficacy study. The comparator was a liraglutide-matched Placebo provided by Novo-Nordisk Ltd (UK). The placebo included everything except the active ingredient.

Actual start date of recruitment	19 October 2010
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: 52
Worldwide total number of subjects	52
EEA total number of subjects	52

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)	49
From 65 to 84 years	3
85 years and over	0

Subject disposition

Recruitment

Recruitment details:

All trial participants were recruited / randomised into the clinical study between 19th October 2010 and 3rd May 2013. The trial participants were all recruited in the united kingdom from 4 participating centres; Queen Elizabeth Hospital: Birmingham, Queens Medical Centre: Nottingham, Hull royal infirmary: Hull, St James University Hospital: Leeds

Pre-assignment

Screening details:

Patients were screened using study inclusion and exclusion criteria detailed in the study protocol.

Key screening inclusion criteria were:

1. Age ≥ 18
2. BMI ≥ 25
3. Non-alcoholic steatohepatitis (NASH): Kleiner classification on liver biopsy within ≤ 6 months prior to randomisation.

Presence of any other liver aetiologies were excluded

Period 1

Period 1 title	Randomisation
Is this the baseline period?	Yes
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Carer, Assessor

Blinding implementation details:

After screening and completion of the inclusion / exclusion criterion patients were randomly assigned to either treatment or placebo on a 1:1 basis using a computer generated randomisation. This randomisation was stratified to insure equal numbers in each treatment group. Stratification variables were;

1. Type II diabetes (vs. non-diabetics)
2. Trial/ participating centre

All randomised patients were allocated a unique trial identification number.

Arms

Are arms mutually exclusive?	Yes
Arm title	Group 1

Arm description:

Control group: Treatment with once daily subcutaneous injection of inactive treatment (liraglutide placebo) - supplied by Novo Nordisk Ltd, UK.

Arm type	Placebo
Investigational medicinal product name	liraglutide placebo - supplied by Novo Nordisk Ltd, UK.
Investigational medicinal product code	PLACEBO
Other name	
Pharmaceutical forms	Injection
Routes of administration	Subcutaneous use

Dosage and administration details:

1.8mg daily after a 14 day dose escalation period

Arm title	Group 2
------------------	---------

Arm description:

Group 2: Experimental group. Treatment with once daily subcutaneous injections 1.8 mg active liraglutide (Victoza) supplied by Novo Nordisk Ltd, UK

Arm type	Experimental
----------	--------------

Investigational medicinal product name	Liraglutide
Investigational medicinal product code	Active
Other name	Victoza
Pharmaceutical forms	Injection
Routes of administration	Subcutaneous use

Dosage and administration details:

1.8mg Subcutaneous daily injection after a 2 week dose escalation period for a period of 48 weeks.

Number of subjects in period 1	Group 1	Group 2
Started	26	26
Completed	26	26

Period 2

Period 2 title	On Study Treatment and Follow-up
Is this the baseline period?	No
Allocation method	Randomised - controlled
Blinding used	Double blind
Roles blinded	Subject, Investigator, Monitor, Carer, Assessor

Blinding implementation details:

After screening and completion of the inclusion / exclusion criterion patients were randomly assigned to either treatment or placebo on a 1:1 basis using a computer generated randomisation. This randomisation was stratified to insure equal numbers in each treatment group. Stratification variables were;

1. Type II diabetes (vs. non-diabetics)
2. Trial/ participating centre

All randomised patients were allocated a unique trial identification number which was used to identify the patient.

Arms

Are arms mutually exclusive?	Yes
Arm title	Group 1 - Placebo

Arm description:

Placebo

Arm type	Placebo
Investigational medicinal product name	liraglutide placebo - supplied by Novo Nordisk Ltd, UK.
Investigational medicinal product code	PLACEBO
Other name	
Pharmaceutical forms	Injection
Routes of administration	Subcutaneous use

Dosage and administration details:

1.8mg daily after a 14 day dose escalation period

Arm title	Group 2 - Liraglutide - Active Treatment Group
------------------	--

Arm description:

Active- experimental

Arm type	Experimental
Investigational medicinal product name	Liraglutide
Investigational medicinal product code	Active
Other name	Victoza
Pharmaceutical forms	Injection
Routes of administration	Subcutaneous use

Dosage and administration details:

1.8mg Subcutaneous daily injection after a 2 week dose escalation period for a period of 48 weeks.

Number of subjects in period 2	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group
Started	26	26
Completed	22	23
Not completed	4	3
end Trt / No end of study biopsy- Patient choice	3	-
patient choice- end Trt/ No biospy @ 48 week	-	3
No Treatment received / ineligible after entry	1	-

Baseline characteristics

Reporting groups

Reporting group title	Group 1
Reporting group description:	
Control group: Treatment with once daily subcutaneous injection of inactive treatment (liraglutide placebo) - supplied by Novo Nordisk Ltd, UK.	
Reporting group title	Group 2
Reporting group description:	
Group 2: Experimental group. Treatment with once daily subcutaneous injections 1.8 mg active liraglutide (Victoza) supplied by Novo Nordisk Ltd, UK	

Reporting group values	Group 1	Group 2	Total
Number of subjects	26	26	52
Age categorical			
Units: Subjects			
Adults (18-64 years)	24	25	49
From 65-84 years	2	1	3
85 years and over	0	0	0
Age continuous			
Age			
Units: years			
arithmetic mean	52	50	
standard deviation	± 12	± 11	-
Gender categorical			
Units: Subjects			
Female	13	8	21
Male	13	18	31
Race			
Units: Subjects			
Asian (south Asian or oriental)	1	1	2
Black (African or caribbean)	0	1	1
Other (Including Mixed)	2	1	3
White	23	23	46
Smoking History			
Units: Subjects			
current smoker	2	2	4
ex-smoker	13	8	21
never smoked	11	16	27
Sex			
Units: Subjects			
Male	18	13	31
Female	8	13	21
Definite NASH			
Definite NASH			
Units: Subjects			
Positive	26	26	52
Kleiner fibrosis stages			
Kleiner fibrosis stages F0-F2 and F3-F4			

Units: Subjects			
F0-F2	11	14	25
F3-F4	15	12	27
Comorbidity - Type II Diabetes			
Units: Subjects			
Yes	8	9	17
No	18	17	35
Comorbidity - Hyperlipidaemia			
Units: Subjects			
Yes	7	9	16
No	19	17	36
Comorbidity - Hypertension			
Units: Subjects			
Yes	14	13	27
No	12	13	25
Comorbidity - Cardiovascular disease			
Units: Subjects			
Yes	3	0	3
No	23	26	49
Comorbidity - Thyroid Disease			
Units: Subjects			
Yes	4	3	7
No	22	23	45
Concomitant Drug Use - Metformin			
Units: Subjects			
Yes	8	9	17
No	18	17	35
Concomitant Drug Use - Sulfonylurea			
Units: Subjects			
Yes	1	1	2
No	25	25	50
Concomitant Drug Use - Anti-lipidaemic			
Units: Subjects			
Yes	7	10	17
No	19	16	35
Concomitant Drug Use - Anti-hypertensive			
Units: Subjects			
Yes	12	13	25
No	14	13	27
Concomitant Drug Use - Anti-platelet			
Units: Subjects			
Yes	5	5	10
no	21	21	42
Glucose			
Glucose (mmol/L)			
Units: mmol/L			
arithmetic mean	6.1	6.0	
standard deviation	± 1.5	± 1.7	-
Insulin			
Insulin (pmol/L)			

Units: pmol/L			
arithmetic mean	257	166	
standard deviation	± 289	± 80	-
HOMA-IR			
HOMA-IR (mmol.IU.L) (glucose [mmol/L] × insulin[mmol × U/L])			
Units: mmol.IU.L			
arithmetic mean	9.6	6.7	
standard deviation	± 9.8	± 4.7	-
HbA1c			
HbA1c mmol/mol			
Units: mmol/mol			
arithmetic mean	42.4	6.0	
standard deviation	± 9.3	± 0.9	-
HbA1c (%)			
HbA1c (%)			
Units: Percent %			
arithmetic mean	6.0	5.9	
standard deviation	± 0.9	± 0.7	-
NEFA			
NEFA			
Units: Micro mol / L			
arithmetic mean	836	967	
standard deviation	± 368	± 535	-
ADIPO-IR			
ADIPO-IR (mmol.IU.L)			
Units: mmol.IU.L			
arithmetic mean	30.5	22.2	
standard deviation	± 42.7	± 12.7	-
Weight (kg)			
Weight (kg)			
Units: kg			
arithmetic mean	108	101	
standard deviation	± 18	± 18	-
Body mass index			
Body mass index (Kg/m2)			
Units: Kg/m2			
arithmetic mean	37.7	34.2	
standard deviation	± 6.2	± 4.7	-
Waist circumference			
Waist circumference (cm)			
Units: cm			
arithmetic mean	120	110	
standard deviation	± 15	± 11	-
Systolic blood pressure			
Systolic blood pressure (mmHg)			
Units: mmHg			
arithmetic mean	133	130	
standard deviation	± 12	± 13	-
Diastolic blood pressure			
Diastolic blood pressure (mmHg)			
Units: mmHg			

arithmetic mean	78	79	
standard deviation	± 9	± 11	-
Total cholesterol			
Total cholesterol (mmol/L)			
Units: mmol/L			
arithmetic mean	5.0	4.5	
standard deviation	± 1.2	± 1.1	-
High density lipoprotein			
High density lipoprotein (mmol/L)			
Units: mmol/L			
arithmetic mean	1.3	1.1	
standard deviation	± 0.2	± 0.4	-
Low density lipoprotein			
Low density lipoprotein (mmol/L) Low density lipoprotein (LDL) calculated using the Friedwald formula. LDL= Total Cholesterol -HDL- (Triglycerides / 2.1929).			
Units: mmol/L			
arithmetic mean	2.9	2.6	
standard deviation	± 1.0	± 0.8	-
Triglycerides			
Triglycerides (mmol/L)			
Units: mmol/L			
arithmetic mean	1.8	1.9	
standard deviation	± 0.8	± 1.1	-
Creatinine			
Creatinine (µmol/L)			
Units: micro mol/L			
arithmetic mean	71	83	
standard deviation	± 15	± 20	-
Alanine aminotransferase			
Alanine aminotransferase (IU/L)			
Units: IU/L			
arithmetic mean	66	77	
standard deviation	± 42	± 34	-
Aspartate aminotransferase			
Aspartate aminotransferase (IU/L)			
Units: IU/L			
arithmetic mean	51	51	
standard deviation	± 27	± 22	-
Gamma-glutamyl transferase			
Gamma-glutamyl transferase (IU/L)			
Units: IU/L			
arithmetic mean	115	91	
standard deviation	± 174	± 69	-
Alkaline phosphatase			
Alkaline phosphatase (IU/L)			
Units: IU/L			
arithmetic mean	87	76	
standard deviation	± 41	± 25	-
Total bilirubin			
Total bilirubin (µmol/L)			
Units: µmol/L			

arithmetic mean	13	13	
standard deviation	± 7	± 5	-
Albumin			
Albumin (g/L)			
Units: g/L			
arithmetic mean	43	45	
standard deviation	± 5	± 6	-
Cytokeratin-18 M30			
Cytokeratin-18 M30 (IU/L)			
Units: IU/L			
arithmetic mean	352	394	
standard deviation	± 370	± 304	-
Enhanced Liver Fibrosis (ELF) test			
Enhanced Liver Fibrosis (ELF) test			
Units: Enhanced Liver Fibrosis (ELF) test			
arithmetic mean	9.4	9.3	
standard deviation	± 1.3	± 0.9	-
SF-36, physical component			
SF-36, physical component Quality of Life			
Units: Score			
arithmetic mean	40	45	
standard deviation	± 13	± 11	-
SF-36, mental component			
SF-36, mental component Quality of Life			
Units: Score			
arithmetic mean	45	51	
standard deviation	± 14	± 10	-
Dietary consumption Total calories			
Dietary consumption (Block FFQ) Total calories (Kcal)			
Units: Kcal			
arithmetic mean	1926	1885	
standard deviation	± 677	± 700	-
Dietary consumption Total protein			
Dietary consumption (Block FFQ) Total protein (g)			
Units: grams			
arithmetic mean	70	72	
standard deviation	± 25	± 34	-
Dietary consumption Total fat			
Dietary consumption (Block FFQ) Total fat (g)			
Units: grams			
arithmetic mean	74	71	
standard deviation	± 35	± 30	-
Dietary consumption Total carbohydrate			
Dietary consumption (Block FFQ) Total carbohydrate (g)			
Units: grams			
arithmetic mean	248	240	
standard deviation	± 89	± 87	-
Dietary consumption Caffeine			

Dietary consumption (Block FFQ) Caffeine (mg)			
Units: mg			
arithmetic mean	26	21	
standard deviation	± 45	± 30	-
Dietary consumption Alcohol			
Dietary consumption (Block FFQ) Alcohol (g)			
Units: grams			
arithmetic mean	4.8	6.3	
standard deviation	± 8.4	± 8.3	-
NAFLD activity score			
Liver histology Total NAFLD activity score (0-8)			
Units: Score			
arithmetic mean	4.8	4.9	
standard deviation	± 0.9	± 0.9	-
Hepatocyte ballooning score			
Liver histology Hepatocyte ballooning score (0-2)			
Units: Score			
arithmetic mean	1.5	1.5	
standard deviation	± 0.4	± 0.5	-
Steatosis score			
Liver histology Steatosis score (0-3)			
Units: Score			
arithmetic mean	1.9	2.1	
standard deviation	± 0.7	± 0.7	-
Lobular inflammation score			
Liver histology Lobular inflammation score (0-3)			
Units: Score			
arithmetic mean	1.4	1.4	
standard deviation	± 0.4	± 0.3	-
Kleiner fibrosis stage			
Liver histology Kleiner fibrosis stage (0-4)			
Units: Score			
arithmetic mean	2.3	2.3	
standard deviation	± 1.3	± 0.9	-
Liver biopsy length			
Liver biopsy length			
Units: mm			
arithmetic mean	19.7	21.0	
standard deviation	± 5.7	± 7.6	-
Number of portal tracts			
Liver Histology Number of portal tracts			
Units: Count			
arithmetic mean	16.2	18.5	
standard deviation	± 5.3	± 7.1	-

End points

End points reporting groups

Reporting group title	Group 1
Reporting group description: Control group: Treatment with once daily subcutaneous injection of inactive treatment (liraglutide placebo) - supplied by Novo Nordisk Ltd, UK.	
Reporting group title	Group 2
Reporting group description: Group 2: Experimental group. Treatment with once daily subcutaneous injections 1.8 mg active liraglutide (Victoza) supplied by Novo Nordisk Ltd, UK	
Reporting group title	Group 1 - Placebo
Reporting group description: Placebo	
Reporting group title	Group 2 - Liraglutide - Active Treatment Group
Reporting group description: Active- experimental	
Subject analysis set title	Evaluable Patient Set
Subject analysis set type	Modified intention-to-treat
Subject analysis set description: All randomised patients that were evaluable with biopsy at the 48 weeks endpoint irrespective of treatment received.	
Subject analysis set title	Safety patient cohort (ITT)
Subject analysis set type	Safety analysis
Subject analysis set description: All randomised patients as per intention to treat.	

Primary: Histology: clearance of NASH and no worsening of fibrosis

End point title	Histology: clearance of NASH and no worsening of fibrosis
End point description: Action number is the proportion of patients with an improvement in liver histology between biopsy at baseline and biopsy after 48 weeks of treatment: The definition of an improvement in liver histology requires both of the following; - Disappearance of NASH (defined as a disappearance of hepatocyte ballooning) AND No worsening of fibrosis. Liver biopsy results were reviewed by two independent pathologists that were blinded to the study group/ treatment.	
End point type	Primary
End point timeframe: Baseline (Screening within 6 months of randomisation) - End of treatment (within 14 days). A protocol defined treatment (completion of) is 48 weeks	

End point values	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	22	23		
Units: number	2	9		

Statistical analyses

Statistical analysis title	Histological
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.019
Method	Chi-squared
Parameter estimate	Risk ratio (RR)
Point estimate	4.3
Confidence interval	
level	95 %
sides	2-sided
lower limit	1
upper limit	17.7
Variability estimate	Standard error of the mean

Secondary: Effect of treatment on individual histological features of Non-alcoholic steatohepatitis (NASH)

End point title	Effect of treatment on individual histological features of Non-alcoholic steatohepatitis (NASH)
End point description: The liver biopsy's (baseline and end of treatment) were reviewed by two independent pathologists and the individual features of NASH including; 1. Steatosis 2. Hepatocyte inflammation and injury 3. Fibrosis Histological measures (change between baseline and end of treatment biopsy for individual patients) included; 1. Calculation of the mean changes in NAFLD activity score (NAS) between the two treatment groups. 2. Changes in the independent features of NAS; Steatosis, lobular inflammation and hepatocyte ballooning between the two treatment groups. 3. Changes in fibrosis stage according to Kleiner Non-Alcoholic Fatty Liver Disease (NAFLD) fibrosis score (F0-F4) between the two treatment groups.	
End point type	Secondary
End point timeframe: Baseline (within 6 months of randomisation date) and within 7 days of end of study treatment (maximum protocol defined treatment duration 48 weeks)	

End point values	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	22	23		
Units: Patient numbers / percentage	22	23		

Attachments (see zip file)	Histology Data/1 EudraCT Histology.pdf
-----------------------------------	--

Statistical analyses

Statistical analysis title	Changes from baseline for NAS.
Statistical analysis description: Changes from baseline in histopathological parameters - Total NAFLD activity score	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.24 ^[1]
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-0.5
Confidence interval	
level	95 %
sides	2-sided
lower limit	-1.3
upper limit	0.3

Notes:

[1] - From linear regression (equivalent to ANCOVA).

Statistical analysis title	Patients with improvement in NAS
Statistical analysis description: Changes from baseline in histopathological parameters - Patients with improvement in NAS	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.46
Method	Chi-squared
Parameter estimate	Risk ratio (RR)
Point estimate	1.2
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.8
upper limit	1.7

Statistical analysis title	Hepatocyte ballooning score - mean change
Statistical analysis description:	
Changes from baseline in histopathological parameters	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.15
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-0.3
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.7
upper limit	0.1

Statistical analysis title	Patients with improved Hepatocyte ballooning
Statistical analysis description:	
Changes from baseline in histopathological parameters	
Hepatocyte ballooning score - N improved	
Patients with improvement	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.05
Method	Chi-squared
Parameter estimate	Risk ratio (RR)
Point estimate	1.9
Confidence interval	
level	95 %
sides	2-sided
lower limit	1
upper limit	3.8

Statistical analysis title	Steatosis - Change in Score
Statistical analysis description:	
Changes from baseline in histopathological parameters	
Steatosis	
Change in score	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group

Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.32
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-0.2
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.6
upper limit	0.2

Statistical analysis title	Patients with improvement in Steatosis
-----------------------------------	--

Statistical analysis description:

Changes from baseline in histopathological parameters

Steatosis

Patients with improvement

Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.009
Method	Chi-squared
Parameter estimate	Risk ratio (RR)
Point estimate	1.8
Confidence interval	
level	95 %
sides	2-sided
lower limit	1.1
upper limit	3

Statistical analysis title	Lobular inflammation score - mean change
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.97
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-0.01
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.3
upper limit	0.3

Statistical analysis title	Patients with improvement in Lobular Inflammation
Statistical analysis description: Changes from baseline in histopathological parameters Lobular inflammation - Patients with improvement	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.65
Method	Chi-squared
Parameter estimate	Risk ratio (RR)
Point estimate	0.9
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.5
upper limit	1.6

Statistical analysis title	Kleiner Fibrosis score - mean change
Statistical analysis description: Changes from baseline in histopathological parameters	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.11
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-0.4
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.8
upper limit	0.1

Statistical analysis title	Patients with improvement in Kleiner Fibrosis
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group

Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.46
Method	Fisher exact
Parameter estimate	Risk ratio (RR)
Point estimate	1.9
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.5
upper limit	6.7

Statistical analysis title	Patients with worsening in Kleiner Fibrosis
-----------------------------------	---

Statistical analysis description:

Changes from baseline in histopathological parameters

Patients with worsening LIRAGLUTIDE - 2 (9%) PLACEBO - 8 (36%)

Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.04
Method	Fisher exact
Parameter estimate	Risk ratio (RR)
Point estimate	0.2
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.1
upper limit	1

Secondary: Change to weight, BMI and waist: hip Circumference

End point title	Change to weight, BMI and waist: hip Circumference
-----------------	--

End point description:

Each participants weight (kg), height (cm), waist and hip circumferences were measured at visits 1-8 (except visit 2) in order to calculate each individual patients body mass index (BMI) (Kg/m²) and waist: hip circumference.

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

End point timeframe:

Data recorded during the clinical trial at multiple time points between baseline (visit 1) and 3 months post end of treatment visit.

End point values	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	22	23		
Units: Numbers and percentage	22	23		

Attachments (see zip file)	Fig 3a Abs Weight.pdf Fig 2a Weight.pdf Physical Data/1 EudraCT Physical.pdf
-----------------------------------	--

Statistical analyses

Statistical analysis title	Changes from baseline for Weight
Statistical analysis description: Absolute weight (kg) Lira change -5.3 (4.7) Placebo change -0.6 (4.4)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.003 ^[2]
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-4.39
Confidence interval	
level	95 %
sides	2-sided
lower limit	-7.19
upper limit	-1.59

Notes:

[2] - *p values and adjusted treatment changes determined by linear regression analysis regressing change on the baseline characteristic score and treatment

Statistical analysis title	Changes from baseline for BMI
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.005 ^[3]
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-1.59

Confidence interval	
level	95 %
sides	2-sided
lower limit	-2.66
upper limit	-0.51

Notes:

[3] - *p values and adjusted treatment changes determined by linear regression analysis regressing change on the baseline characteristic score and treatment

Statistical analysis title	Changes from baseline for Waist circumference
Statistical analysis description: Waist circumference (cm)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.29 ^[4]
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-2.54
Confidence interval	
level	95 %
sides	2-sided
lower limit	-7.32
upper limit	2.25

Notes:

[4] - *p values and adjusted treatment changes determined by linear regression analysis regressing change on the baseline characteristic score and treatment.

Secondary: Quality of Life (SF-36) / AUDIT

End point title	Quality of Life (SF-36) / AUDIT
End point description: The SF36 (version 2.0) was used /requested at three protocol defined time points (Visit 1, 7 and 8).	
End point type	Secondary
End point timeframe: Data recorded during the clinical trial at multiple time points between baseline (visit 1) and 3 months post end of treatment visit.	

End point values	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	22	23		
Units: SF-36 score				
arithmetic mean (standard deviation)	-0.5 (± 8.0)	1.9 (± 5.1)		

Attachments (see zip file)	Questionnaires/1 EudraCT Questionnaires.pdf
-----------------------------------	---

Statistical analyses

Statistical analysis title	Quality of Life Physical Component
Statistical analysis description: Quality of life (SF-36v2) Mean (SD) change from baseline to 48 weeks Physical component	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.04
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	4.05
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.2
upper limit	7.9

Statistical analysis title	Quality of life Mental component
Statistical analysis description: Quality of life (SF-36v2) Mean (SD) change from baseline to 48 weeks Physical component	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.62
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	1.5
Confidence interval	
level	95 %
sides	2-sided
lower limit	-4.64
upper limit	7.65

Secondary: A reduction in non-invasive inflammatory and fibrosis markers

End point title	A reduction in non-invasive inflammatory and fibrosis markers
-----------------	---

End point description:

Changes in Liver function measured by the use of non invasive biomarker (Fibroscan) and multiple Blood Tests including; Liver function Tests (LFTs), and Cytokeratin-18. Glycaemic Control measurements relating to A reduction in global (hepatic, adipose and muscle) insulin resistance and A reduction in hepatic de-novo lipogenesis (DNL)

Reporting details the Mean (SD) from baseline to 48weeks

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

End point timeframe:

Baseline to end of Treatment / Study follow up

End point values	Group 1 - Placebo	Group 2 - Liraglutide - Active Treatment Group		
Subject group type	Reporting group	Reporting group		
Number of subjects analysed	22	23		
Units: Numerical				
arithmetic mean (standard deviation)				
Bilirubin	-1.1 (± 3.1)	-1.7 (± 3.1)		
Alkaline Phosphatase	-1.2 (± 19.1)	-5.1 (± 11.7)		
Alanine Transferase	-10.2 (± 35.8)	-26.6 (± 34.4)		
Aspartate Transferase	-8.6 (± 28.3)	-15.8 (± 21.8)		
CytoKeratin-18 (CK)	-92 (± 327)	-185 (± 295)		
HbA1c	0.0 (± 8.7)	-5.7 (± 6.9)		
Glucose	0.72 (± 2.3)	-1.0 (± 1.5)		
HOMA-IR	0.70 (± 9.49)	-1.8 (± 3.7)		
Triglycerides	0.18 (± 1.29)	-0.02 (± 0.64)		
Total Cholesterol	-0.13 (± 0.91)	0.01 (± 0.60)		
HDL	-0.04 (± 0.13)	0.07 (± 0.19)		
LDL	-0.1 (± 0.9)	-0.1 (± 0.7)		

Attachments (see zip file)	Clinical Data/1 EudraCT Clinical Data.pdf
----------------------------	---

Statistical analyses

Statistical analysis title	Glucose
----------------------------	---------

Statistical analysis description:

Mean (95% CI) changes from baseline

Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
-------------------	--

Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.005
Method	Regression, Linear
Parameter estimate	Mean difference (final values)
Point estimate	-1.67
Confidence interval	
level	95 %
sides	2-sided
lower limit	-2.81
upper limit	-0.53

Statistical analysis title	HOMA-IR
Statistical analysis description: Mean (95% CI) changes from baseline	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.23
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-2.74
Confidence interval	
level	95 %
sides	2-sided
lower limit	-7.24
upper limit	1.76

Statistical analysis title	Glycated haemoglobin A1c
Statistical analysis description: Mean (95% CI) changes from baseline (liraglutide vs placebo)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.03
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-5.18

Confidence interval	
level	95 %
sides	2-sided
lower limit	-9.91
upper limit	-0.44

Statistical analysis title	Total Cholesterol
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.79
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	0.066
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.427
upper limit	0.56

Statistical analysis title	HDL
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.01
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	0.134
Confidence interval	
level	95 %
sides	2-sided
lower limit	0.029
upper limit	0.238

Statistical analysis title	LDL
-----------------------------------	-----

Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - LDL (mmol/L)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.61
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-0.126
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.622
upper limit	0.371

Statistical analysis title	Triglycerides
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Triglycerides (mmol/L)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.53
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-0.197
Confidence interval	
level	95 %
sides	2-sided
lower limit	-0.834
upper limit	0.439

Statistical analysis title	Alanine aminotransferase
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Alanine aminotransferase (U/L) - ALT	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.16
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-10.7

Confidence interval	
level	95 %
sides	2-sided
lower limit	-25.9
upper limit	4.5

Statistical analysis title	Aspartate aminotransferase
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Aspartate aminotransferase (U/L) AST	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.29
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-6.7
Confidence interval	
level	95 %
sides	2-sided
lower limit	-19.3
upper limit	5.9

Statistical analysis title	γ-glutamyl transferase (U/L)
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Gamma-glutamyl transferase (U/L) - GGT	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.01
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-22.8
Confidence interval	
level	95 %
sides	2-sided
lower limit	-40.4
upper limit	-5.2

Statistical analysis title	Alkaline phosphatase
-----------------------------------	----------------------

Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Alkaline phosphatase (U/L)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.22
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-5.46
Confidence interval	
level	95 %
sides	2-sided
lower limit	-14.36
upper limit	3.43

Statistical analysis title	Bilirubin
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Total bilirubin (µmol/L)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.51
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-0.63
Confidence interval	
level	95 %
sides	2-sided
lower limit	-2.52
upper limit	1.26

Statistical analysis title	CK-18
Statistical analysis description:	
Mean (95% CI) changes from baseline (liraglutide vs placebo) - Caspase-cleaved cytokeratin-18 fragment M30 (U/L)	
Comparison groups	Group 1 - Placebo v Group 2 - Liraglutide - Active Treatment Group
Number of subjects included in analysis	45
Analysis specification	Pre-specified
Analysis type	superiority
P-value	= 0.1
Method	Regression, Linear
Parameter estimate	Mean difference (net)
Point estimate	-86

Confidence interval	
level	95 %
sides	2-sided
lower limit	-188
upper limit	16

Secondary: Non Serious Adverse Events

End point title	Non Serious Adverse Events
-----------------	----------------------------

End point description:

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

End point timeframe:

AEs reported over the course of the trial will be attached as a pdf to this end point analysis.

End point values	Safety patient cohort (ITT)			
Subject group type	Subject analysis set			
Number of subjects analysed	52			
Units: Patients	52			

Attachments (see zip file)	Adverse Events/AE listing for EUDRACT.pdf
-----------------------------------	---

Statistical analyses

No statistical analyses for this end point

Adverse events

Adverse events information^[1]

Timeframe for reporting adverse events:

Date of consent to end of study visit (3 months post end of treatment) for non-serious adverse events.
Date of consent to 30 days post end of study treatment (48 weeks) or 30 days post last administration of IMP for serious adverse events

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

Dictionary used

Dictionary name	CTCAE
Dictionary version	4

Reporting groups

Reporting group title	Group 1: Placebo
-----------------------	------------------

Reporting group description:

Control group. Treatment with once-daily subcutaneous injection of inactive treatment (liraglutide placebo) (Supplied by Novo Nordisk Ltd, UK)

Reporting group title	Group 2: Active
-----------------------	-----------------

Reporting group description:

Experimental group. Treatment with once-daily subcutaneous injections 1.8mg active liraglutide (Victoza ®) (Supplied by Novo Nordisk Ltd, UK)

Notes:

[1] - There are no non-serious adverse events recorded for these results. It is expected that there will be at least one non-serious adverse event reported.

Justification: Results are posted in outcome section as validation error prevented uploading despite correct information- had to set number of patients not affected by non serious AE as `0` to post result as unable to over-ride validation warning to post results- group 1 is 24 and group 2 is 23.

Serious adverse events	Group 1: Placebo	Group 2: Active	
Total subjects affected by serious adverse events			
subjects affected / exposed	2 / 26 (7.69%)	2 / 26 (7.69%)	
number of deaths (all causes)	0	0	
number of deaths resulting from adverse events	0	0	
Investigations			
Calcitonin stimulation test	Additional description: Calcitonin levels found to be abnormal		
subjects affected / exposed	1 / 26 (3.85%)	0 / 26 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Cardiac disorders			
Conduct disorder	Additional description: ECG showed completed Heart Block and patient subsequently fitted with ventricular pacemaker. Occurred prior to any treatment being given. Ref: HE2013/0010/01		
subjects affected / exposed	1 / 26 (3.85%)	0 / 26 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	
Nervous system disorders			
Headache	Additional description: Patient admitted with headache and associated nausea and dizziness. MRI and CT scan of head showed no abnormalities SAE ref: HE2013/0049/01		

subjects affected / exposed	0 / 26 (0.00%)	1 / 26 (3.85%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Hepatobiliary disorders			
Granulomatous liver disease	Additional description: End of trial biopsy showed incidental finding of multiple granulomas. Patient was found to have active tuberculosis and treated with 6 months of antibiotics ref: HE2013/0012/01		
subjects affected / exposed	0 / 26 (0.00%)	1 / 26 (3.85%)	
occurrences causally related to treatment / all	0 / 0	0 / 1	
deaths causally related to treatment / all	0 / 0	0 / 0	
Metabolism and nutrition disorders			
Hypoglycaemia	Additional description: Patient diagnosed with REACTIVE HYPOGLYCAEMIA. REF: HE2013/0010/02		
subjects affected / exposed	1 / 26 (3.85%)	0 / 26 (0.00%)	
occurrences causally related to treatment / all	0 / 1	0 / 0	
deaths causally related to treatment / all	0 / 0	0 / 0	

Frequency threshold for reporting non-serious adverse events: 0 %

Non-serious adverse events	Group 1: Placebo	Group 2: Active	
Total subjects affected by non-serious adverse events			
subjects affected / exposed	0 / 26 (0.00%)	0 / 26 (0.00%)	

More information

Substantial protocol amendments (globally)

Were there any global substantial amendments to the protocol? Yes

Date	Amendment
01 July 2010	<p>Amendment 2.0 The following statements were added to the exclusion criteria (p38 & p39):</p> <ul style="list-style-type: none"> • Patients with Multiple Endocrine Neoplasia Syndrome 2 (MEN2) • Family history of medullary thyroid cancer <p>Reason: These additional exclusion criteria are in keeping with the current recommended contraindications of Liraglutide (Victoza®) use in patients.</p> <p>Non-Substantial Amendments to the Protocol</p> <p>Throughout the protocol a number of non substantial amendments were made, specifically the contact details (p66) of Novo Nordisk Ltd offices have been updated. The central contact point between the sponsor (The University of Birmingham) and the Drug Manufacturer and Holder of the IMP MA (Novo Nordisk). In addition the current national ethical and regulatory approval status of study has been updated in the protocol sponsor section as well as some additional information in the finance section (p75) of the protocol concerning the supply of pen-injection needles for use on the study.</p> <ol style="list-style-type: none"> 1. Changes to IMP Labelling 2. Change to Patient Card: Inclusion of DUN Numbers (Prescribed study drug number)
20 November 2010	<p>Amendment 3.0: Change to exclusion criteria:</p> <ul style="list-style-type: none"> - No upper cut-off for BMI - removal of exclusion of patients that have had a significant change in dose of Angiotensin converting enzymes and (ACE) inhibitors and/or Angiotensin Receptor Blockers (ARBs) from exclusion criteria and inclusion of Multi-vitamins / vitamin E (containing > 200% recommended daily amount; >30mg/day). -Addition of a new participating site.
20 January 2011	<p>Amendment 4.0</p> <ul style="list-style-type: none"> - addition of new participating site - New wording added to protocol in relation to the requirement for sites to report pregnancies to study office. - New wording added to protocol in relation to the time period for reporting adverse events
28 April 2011	<p>Amendment 5.0 Change to clinical trial protocol</p> <ol style="list-style-type: none"> 1. Trial Site addition of Hull Royal Infirmary 2. Change to Target dates for trial completion. 3. Amendment to visit schedule - Amendment to visit 8, with regards to 12 weeks post visit 7 rather 24 weeks
01 September 2011	<p>Amendment 6.0: Addition of new participating centre and update to study protocol (protocol version 7.0) - site addition only.</p>

Notes:

Interruptions (globally)

Were there any global interruptions to the trial? No

Limitations and caveats

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

The information / data contained in this report is constrained /limited by the reporting structure of this report- for additional information and content please review the published articles and contact the corresponding author: Prof Philip Newsome.

Notes:

Online references

<http://www.ncbi.nlm.nih.gov/pubmed/24189085>

<http://www.ncbi.nlm.nih.gov/pubmed/26608256>

<http://www.ncbi.nlm.nih.gov/pubmed/26394161>

<http://www.ncbi.nlm.nih.gov/pubmed/23163663>