Clinical Study Report

AN OPEN LABEL, LONGITUDINAL STUDY OF THE EFFECTS OF SUBCUTANEOUS ACUTE AND CHRONIC PASIREOTIDE (SOM230) THERAPY ON ADRENOCORTICOTROPHIC HORMONE AND TUMOUR VOLUME IN PATIENTS WITH NELSON'S SYNDROME

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Abbreviations

ACTH	adrenocotricotropin
ANOVA	analysis of variance
b.d.	twice daily
BMI	body mass index (calculated from weight[kg] divided by height $[m]^2$)
DVT	deep venous thrombosis
ECG	electrocardiogram
h	hour
Hba1c	glycated haemoglobin A1c
HC	hydrocortisone
i.m.	intramuscular
LAR	long-acting release
LFTs	liver function tests (total protein, albumin, bilirubin, ALP, ALT, GGT)
MRI	magnetic resonance imaging
o.d.	once daily
p.r.n.	as needed
QT	the time between the start of the Q-wave and the end of the subsequent T-wave in an ECG
QTcF	QT interval corrected for heart rate using Fridericia's formula
RR	the time between two consecutive R-waves in an ECG
s.c.	subcutaneous
SPSS	Statistical Package for the Social Sciences
t.d.s.	thrice daily
TIA	transient ischaemic attack
TSS	transsphenoidal pituitary surgery
U&Es	urea and electrolytes (sodium, potassium, urea and creatinine)
USS	ultrasound

Executive summary

Background: Nelson's syndrome is a very challenging condition that can develop following bilateral adrenalectomy for Cushing's disease. It is due to the development of a progressive tumour of the corticotroph cells in the pituitary that is often invasive and refractory to treatment, and patients can present with mass effects, headache, visual field defects, external ophthalmoplegia, and are characteristically pigmented with high ACTH levels. There is an unmet need for an effective medical therapy.

Hypothesis: Pasireotide lowers plasma ACTH levels in patients with Nelson's syndrome.

Objectives: 1) To assess the acute effects of pasireotide on circulating levels of plasma ACTH after a single 600µg s.c. injection, and whether this would allow prediction of individual longer-term response, 2) to assess the effects of four-weeks of pasireotide s.c. (300-600µg) twice daily on circulating plasma ACTH, 3) to assess the effects of pasireotide LAR (40-60mg every 28 days) for 6 months, on circulating plasma ACTH, and 4) to assess the effect of pasireotide s.c. and LAR in tumour volume.

Design: Open labelled non-randomised longitudinal trial in 3 steps; a placebo-controlled acute response arm, a short-term s.c. pasireotide twice-daily treatment, and a long-term pasireotide LAR monthly treatment.

Main outcome measures

Primary endpoint

Response criteria - change in plasma ACTH levels:

Complete success: Fall in basal plasma ACTH > 400ng/l; Partial success: Fall in basal plasma ACTH < 399ng/l >200ng/l; Not successful: Fall in basal plasma ACTH < 199ng/l

Secondary endpoints

Tumour volume assessed by MRI, safety and tolerability, fasting glucose, fasting insulin and HbA1c, changes in skin pigmentation.

Patients: Adult patients with Nelson's syndrome and no history of radiotherapy within one year of enrolment.

Results: Eight patients were recruited, all females; seven had s.c. treatment and 5 had LAR treatment. Recruitment was slow and the target sample size (17 patients) was not achieved. Overall, there was a significant reduction in baseline ACTH during treatment (mean baseline 1823+/-1286ng/l vs. 888.0+/- 812.8ng/l during the s.c. phase and vs. 829.0+/-1171ng/l during the LAR phase, p=0.001),which was not dependent on dose administered. When on s.c. pasireotide 5/8 patients had a

complete response, 2/8 had a partial response and 1 patient did not respond. At the end of 24-weeks of pasireotide LAR treatment or at the last visit, 3/5 patients had a complete response, 1/5 a partial response and 1/5 showed no response. An acute response to a test dose predicted outcome in the majority of patients. Overall, there was no significant change in tumour volumes between the pre-treatment and post-treatment scans (1.4+/-0.9 vs. 1.3+/-1.0, p=0.86). Four patients withdrew during the study. Hyperglycaemia was common and side effects frequently reported.

Conclusion: Pasireotide lowers ACTH levels in patients with Nelson's syndrome. A longer period of treatment may be needed to assess the effects of pasireotide on tumour volume. Active monitoring and management of glucose homeostasis is needed and patients counselled about this prior to therapy. In an individual patient, a positive effect is more likely if there has been a response to the test dose. If no clear response is noted after three months, a response is unlikely. The LAR preparation appears as effective as the s.c. preparation and is likely to be more acceptable to patients. It would seem reasonable to commence therapy at a lower dose and escalate if tolerated, as there appears to be no clear relationship between dose and effect.

Introduction

Nelson's syndrome is a very challenging condition that can develop following bilateral adrenalectomy for Cushing's disease (CD). It is due to the development of a progressive tumour of the corticotroph cells in the pituitary that is often invasive and refractory to treatment [1], and occurs in up to 30% of patients with CD undergoing bilateral adrenalectomy [2]. Corticotroph tumour progression as assessed on MRI is shown in up to 50% of cases [3]. The corticotroph tumour is often small but may also be locally invasive and patients can present with mass effects, headache, visual field defects, external ophthalmoplegia, and are characteristically pigmented with high ACTH levels [4, 5]. Pigmentation may be the source of considerable psychological disturbance to the patient. The levels of plasma ACTH reflect the activity of the tumour and may be used for monitoring [6].

Current treatment of Nelson's involves pituitary surgery and radiotherapy [7, 8]. Whilst these modalities can be effective in some patients the levels of ACTH can continue to rise, limiting treatment options for the tumour and are associated with higher levels of hypopituitarism.

There has been a long interest in medical therapy to attempt to control plasma ACTH levels and tumour growth but unfortunately, apart from isolated case reports, there is no medical therapy that has been shown to consistently achieve these goals. Disappointing or variable results have been seen with sodium valproate [9-14] and there are only occasional responses found with dopamine agonists, such as cabergoline [15-18]. Peroxisome proliferator-activated receptor (PPAR) gamma agonists have also been studied and one report showed that two out of three patients responded to rosiglitazone with lowering of plasma ACTH levels, but one of these subsequently escaped [19]. Higher than licensed doses of rosiglitazone (12mg/day) are not effective in reducing plasma ACTH levels, and by inference will not control tumour growth [4]. Temozolomide may be used for some aggressive pituitary tumours, but is associated with significant toxicity [20]. At the same time there are increasing data on the somewhat disappointing long-term outcome of transsphenoidal surgery for Cushing's disease, and laparoscopic bilateral adrenalectomy is increasingly used for failures of pituitary surgery or even as primary therapy [21] leading to some patients developing Nelson's syndrome. There is therefore a real need for an effective medical management for Nelson's syndrome.

Pasireotide (SOM230) is a somatostatin analogue with a broad somatostatin receptor binding profile that binds to subtypes 1, 2, 3, and with high affinity to

subtype 5. It has been shown to be effective in lowering cortisol levels in patients with active Cushing's disease [22-24] and it is licensed for the treatment of hypercortisolism associated with CD and acromegaly as a second line therapy. *In vitro* experiments have shown that pasireotide inhibits ACTH secretion in cultured adenoma cells from patients with Cushing's disease [25]. More recently, pasireotide LAR was used to treat a patient with an invasive corticotroph tumour resulting in clinical improvement, and reductions in tumour size and plasma ACTH levels [26].

Hypothesis

It is hypothesised that treatment with the multi-ligand somatostatin analogue pasireotide (SOM230) would attenuate plasma ACTH levels and corticotroph tumour volume in patients with Nelson's syndrome.

Aims

The aim of this study was to investigate the effects of pasireotide on circulating plasma ACTH and tumour size in patients with Nelson's syndrome. In particular, the study was structured to assess: 1) the acute effects of pasireotide on circulating levels of plasma ACTH after a single 600µg s.c. injection, and whether this would allow prediction of individual longer-term response, 2) to assess the effects of four-weeks of pasireotide s.c. on circulating plasma ACTH, 3) to assess the effects of pasireotide LAR given monthly for 6 months, on circulating plasma ACTH, and 4) to assess the effect of pasireotide s.c. and LAR in tumour volume.

Methods

Study design

This was an open labelled non-randomised longitudinal trial over a 31-week period conducted in four tertiary centres in England (Sheffield Teaching Hospitals Trust, Sheffield, The Christie Hospital, Manchester, St Bartholomew's Hospital, London and Churchill Hospital, Oxford).

There were three parts in the study. Initially an acute response to pasireotide was assessed; this part was a placebo-controlled, randomised single-blinded crossover intervention. Patients were randomised to receive either an initial test dose of 600µg pasireotide s.c. or saline to establish if an acute response predicts drug efficacy. In the second part of the study patients underwent short-term (4-weeks) open label treatment with pasireotide twice-daily s.c. injection (600µg b.d. or 300µg b.d. if dose reduction due to tolerability was necessary). In the last part of the study patients had long-term open label treatment with pasireotide LAR 60mg (or 40mg if reduced for tolerability when on s.c. dosing) every 28 days for 24 weeks (Figure 1).

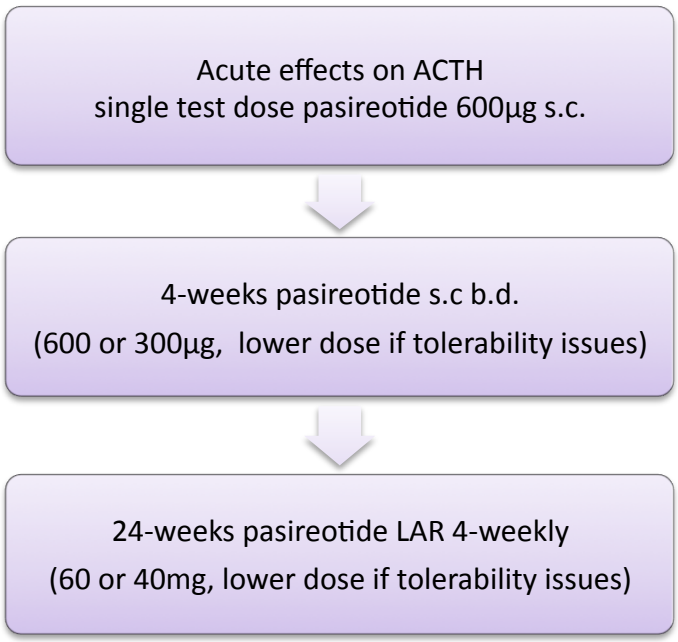


Figure 1: Pasireotide treatment in Nelson's syndrome: study design

Study endpoints

Primary endpoint

Early morning plasma ACTH sampled at 0, 1, 2, and 3 hours after morning hydrocortisone (HC) during 4 weeks of pasireotide s.c. 1200µg/day (or 600µg/day if reduced for tolerability issues) compared with levels at these respective time points found at baseline and after chronic depot pasireotide 60mg (or 40mg if reduced for tolerability issues) i.m. every 28 days. Response criteria were:

- Complete success: Fall in pre-HC plasma ACTH > 400ng/l, or 120 minutes after HC >200ng/l
- Partial success: Fall in pre-HC plasma ACTH < 399ng/l >200ng/l, or 120 minutes after HC <199ng/l >100ng/l
- Not successful: Fall in pre-HC plasma ACTH < 199ng/l, or 120 minutes after HC <99ng/l

For the baseline value of the pre-HC ACTH the mean was calculated from visits 1 to 4 (Table 1). Values from visit 1 only were the baseline of the post-HC ACTH. Baseline ACTH values were compared with ACTH levels at the end of treatment for the s.c. and the LAR phase (or the last visit if patient withdrew).

Table 1: Study visits and intervention

Visit 1	Screening
Visit 2	Test dose
Visit 3	Test dose
Visit 4	Start s.c. phase
Visit 5	S.c. phase
Visit 6	Start LAR phase
Visits 7-11	LAR phase
Visit 12	End of LAR phase and the study

Secondary endpoints:

1. Plasma ACTH at -60, -30, 0 minutes and 1, 2, 3, 4, 5, 6 hours after acute single dose of 600µg pasireotide or saline.
2. Tolerability of pasireotide and safety endpoints determined via adverse event monitoring, blood safety checks (U&Es/LFTs), and ECG at baseline and 4 weeks after s.c. pasireotide treatment.

3. Change in HbA1c, fasting insulin and glucose levels during pasireotide s.c. and LAR treatment.
4. Changes in skin pigmentation at the end of the study period compared with pre-treatment.
5. Changes in tumour volume at the end of the chronic pasireotide LAR compared with pre-treatment, determined by MRI.
6. *In vitro* analysis of available historical patient paraffin embedded samples to screen for somatostatin receptor expression.

Patients

Patients with Nelson's syndrome were eligible to take part in this study. The inclusion criteria were: male or female patients aged 18-80 years with signs, symptoms and biochemistry consistent with Nelson's syndrome and a negative pregnancy test (where applicable). The exclusion criteria were: (1) pituitary radiotherapy within the last 1 year prior to study entry, (2) recent significant deterioration in visual fields or other neurological signs related to tumour mass requiring surgery, (3) severe liver disease (i.e. cirrhosis, chronic active hepatitis or chronic persistent hepatitis, or persistent ALT, AST, alkaline phosphates 2X> upper limit of normal, or total bilirubin 1.5X> upper limit of normal), (4) symptomatic cholelithiasis, (5) clinically significant abnormal laboratory values, (6) a QTcF interval measured on the ECG >480ms, (7) pregnancy or lactation, (8) recent (last 6months) history of alcohol or drug abuse, (9) concurrent administration of investigational drug for another study, (10) history of non-compliance, or inability to complete the entire study for any reason.

Sample Size

A target number for recruitment of 17 patients was calculated taking into account the variability of ACTH levels and a 13% dropout rate which was recorded for a pasireotide phase 2 study [27]. Accounting for a within person variability of ACTH levels of approximately 400ng/l for the pre-HC dose and 250ng/l post-HC dose, 15 patients were needed to detect a clinically significant change of 200ng/l with a power of 80% with 5% significance [4]. Another two patients had to be recruited to cover possible dropout.

Electrocardiograms

ECGs were performed at screening (visit 1) and after 4-weeks of s.c. pasireotide treatment (visit 6). The local investigators reported all ECGs and the traces were retrieved for evaluation of the corrected QT interval. Corrected QT (QTcF) was calculated using the Fridericia's formula from the QT and RR intervals. QTcF values above 450msec were considered abnormal.

Imaging

Standard gadolinium-enhanced MRI of the pituitary was performed at the participating centres before and after treatment to assess the tumour volume. A single blinded radiologist assessed the scans and calculated a single maximum diameter as well as maximum diameters in 3 dimensions to assess tumour volume using standard volumetric techniques [28-30]. Abdominal USS was performed at screening and at the end of the study (week 28) to assess for the presence of cholelithiasis.

Skin pigmentation

Medical photographs of participants were performed at screening, at the start (study week 4) and at the end (study week 28) of pasireotide LAR treatment. The photographs of all participants were collected and analysed centrally. Two blinded researchers reviewed the photographs and graded the skin pigmentation as 'improved', 'stable', or 'worsened'. Medical documentation from the physical examination during the last visit (visit 12) was also reviewed.

Biochemistry

Fasting insulin and ACTH samples were collected and analysed at the central Clinical Chemistry laboratory (Sheffield Teaching Hospitals NHS Trust). Insulin was measured with the Roche electrochemiluminescence immunoassay on a cobas e602 module. ACTH was measured by a chemiluminescent immunometric assay on the Siemens Immulite 2000 analyser. Baseline biochemistry was analysed at the local laboratories of the participating hospitals (glucose, U&Es, Hba1c, and LFTs). The latest available samples were used for analysis when study visits were repeated.

Statistical Analysis

Statistical analysis was performed using GraphPad and SPSS v22. The main aim of the analysis was to establish whether ACTH levels change over time after pasireotide therapy. ACTH levels at 0h (before morning hydrocortisone dose) were compared before treatment (i.e. 'baseline' or visits 1-3) and during treatment with s.c. pasireotide (visits 4-5) and LAR (visits 6-12) using one-way ANOVA. QTcF at screening and after 4-weeks of s.c. pasireotide treatment were compared using a non-parametric t-test (Wilcoxon matched pairs signed rank test).Tumour volumes before and after treatment were compared by paired t-test, assuming a normal distribution. A p value <0.05 was considered statistically significant.

Results

Patients

Eight patients were recruited, all females; recruitment was slow and the target number of 17 patients was not reached. Of the eight patients who were recruited, four withdrew during the s.c. phase (101, 401) and the LAR phase of treatment (301, 303) and 4 patients (102, 201, 202, 302) completed all of the study visits (Tables 1 and 2). In all patients any radiotherapy had been administered >5 years prior to study entry.

Table 2: Summary of patients and response to pasireotide treatment

	s.c. phase			LAR phase		
Patient ID	**Daily dose (µg)**	**Time treated**	**Response***	**Monthly dose (mg)**	**Time treated**	**Response***
101	1200	11 days	C	-	-	-
102	1200	4 weeks	C	60	24 weeks	C
201	600	4 weeks	C	40	24 weeks	No
202	600	4 weeks	C	40	24 weeks	C
301	1200	4 weeks	C	-	-	-
302	1200	4 weeks	P	60	24 weeks	C
303	1200	4 weeks	P	60	12 weeks	P
401	1200	0.5 day	No	-	-	-
Protocol		**4-weeks**			**24-weeks**	

*C: complete success, P: partial success, No: no success (see Methods)

Patient 101 was 46 years old at the screening visit with Cushing's disease diagnosed in 1992 that was treated by transsphenoidal hypophysectomy (in 1992 and 1998) and bilateral adrenalectomy (2003). The past medical history also included DVT (2002), cholecystectomy (1995), migraines, and sinusitis. At the time of screening she was treated with hydrocortisone 10mg b.d., fludrocortisone 50 µg

o.d., omeprazole 20mg o.d., atorvastatin 20mg o.d., levothyroxine 125 µg o.d., and pseudoephedrine spray. An abdominal USS showed evidence of previous cholecystectomy. On examination the BMI was 28, blood pressure was normal (119/82), there was evidence of skin hyperpigmentation in the shins, knuckles, chin, tongue, and face and a laparoscopy scar in the abdomen from the previous cholecystectomy. At screening, fasting blood sugar was normal (4.5mmol/l) and Hba1c was elevated (58mmol/mol), U&Es and LFTs were normal, and ACTH prior to the morning dose of hydrocortisone was elevated (1520ng/l). The patient attended for visits 2 and 3 (Table 2) but did not have the pasireotide/ placebo test dose. The patient completed visit 4 but withdrew during visit 5 following the baseline blood tests for this visit and having completed 11 days of pasireotide 600ug s.c. b.d. treatment. Her ACTH results were therefore included in the ACTH efficacy analysis for 0h and 2h post HC for the s.c. but not the LAR phase.

Patient 102 was 48 years old at the screening visit with Cushing's disease treated with TSS (1995), pituitary radiotherapy (1995), and bilateral adrenalectomy. There was also a medical history of psoriatic arthropathy (2008), hypertension, palpitations and irregular heart beat (2004), and partial 3rd nerve palsy (06/2011). At the time of the screening visit, she was treated with prednisolone (5mg and 2.5mg), fludrocortisone 100µg, atenolol 25mg, amitriptyline 50mg, levothyroxine 100µg, adcalD3 1 b.d., methotrexate 15mg weekly, folic acid 5mg, simvastatin 40mg, ranitidine 150mg, mirapexin 125mg, growth hormone 0.5mg, progesterone only pill, and Furosemide 20mg. A pregnancy test was negative and an abdominal USS was normal. BMI was 22.2 and clinical examination revealed skin hyperpigmentation and a normal blood pressure (127/ 80). Baseline biochemical investigations at screening showed a normal fasting blood sugar (4.8mmol/l) and an elevated HbA1c (50mmol/mol), normal U&Es and LFTs. Plasma ACTH prior to the morning dose of hydrocortisone was elevated (>1250ng/l). The patient completed all visits.

Patient 201 was 43 years old at the screening visit with a past medical history of Cushing's disease (1993) treated with TSS (1995) and bilateral adrenalectomy (04/1999), and previous cholecystectomy (1999). At the time of the screening visit she was treated with hydrocortisone (10/5/5mg), fludrocortisone 100µg bid, cyclizine 50mg t.d.s. p.r.n., loperamide 2mg p.r.n., hyoscine 10-20mg p.r.n., folic acid 5mg, ferrous sulphate 200mg o.d. On examination the BMI was 25.1, blood pressure was low (88/55), there was a laparotomy scar as well as scars over the right knee and foot. Blood results at screening were; normal fasting blood sugar (4.5mmol/l), HbA1c (40mmol/mol), U&Es and LFTs, and an elevated ACTH prior to the morning dose of hydrocortisone (4872.0ng/l). Following visit 4 the dose of pasireotide s.c. was changed from 1200 to 600µg daily due to poor tolerance and

visit 4 was repeated. The patient received the reduced dose (40mg) during the LAR phase and completed all study visits.

Patient 202 was 47 years old at the screening visit and had a past medical history of Cushing's disease treated with TSS (1994), pituitary radiotherapy (1994), bilateral adrenalectomy (1998), and stereotactic radiotherapy (2001), migraines (2001), and hysterectomy (07/2011). She was treated with hydrocortisone (10/5/5mg), fludrocortisone 100µg, growth hormone 0.2/0.1mg on alternative days o.d., and sumitriptan 50mg p.r.n. An abdominal USS was normal. On examination the BMI was 31.5, blood pressure was normal (126/61) and there were two abdominal scars. Blood tests at screening were: normal fasting blood sugar (4.1mmol/l), HbA1c (32mmol/mol), normal U&Es and LFTs, and elevated ACTH prior to the morning dose of hydrocortisone (1854ng/l). During visit 5 the dose of s.c. pasireotide was reduced to 300µg daily due to adverse events; following protocol amendment the dose was changed to 600µg daily and visit 4 was repeated. The patient was treated with the reduced dose during the LAR phase (40mg) and completed all study visits.

Patient 301 was 53 years old at the screening visit with Cushing's disease treated with transsphenoidal hypophysectomy (1992) and bilateral adrenalectomy (1983). The past medical history also included hysterectomy for a uterine malignancy (1987), cholecystectomy (1986), right nephrectomy (1988), thyroidectomy for goitre (1990), hypoparathyroidism, tachycardia (1995), shortness of breath, TIAs (1996), and depression. At the time of the screening visit her medications were hydrocortisone (5/5mg), fludrocortisone 50µg o.d., fluconazole inhaler 250µg, levothyroxine 100µg o.d., omeprazole 20mg o.d., agomelatine 50mg o.d., alphacalciferol 75µg o.d., atorvastatin 10mg o.d., and dipyridamole/aspirin 200/25mg o.d. On examination the BMI was 33.2, blood pressure was normal (96/73) and there was evidence of skin hyperpigmentation consistent with Nelson's syndrome. At screening, fasting blood sugar (5.8mmol/l), HbA1c (5.9%), and LFTs were normal. Urea (13.7mmol/l), creatinine (140µmol/l), and ACTH taken prior to the morning dose of hydrocortisone (4080.0ng/l) were elevated. The patient missed 3 evening doses between visit 4 and visit 5 and withdrew after completing the s.c. phase (attended for visit 6 but did not start LAR) therefore she was not included in the ACTH efficacy analysis for the LAR phase.

Patient 302 was 62 years old at the screening visit with a past medical history of Cushing's disease (1994) treated with transsphenoidal hypophysectomy (1995), g-knife radiosurgery (2007) and bilateral adrenalectomy (05/2013). She also had a fractured pubic ramus (2013), osteoporosis, and headaches. Her treatment at the time of the screening visit was hydrocortisone (10/5/5mg), fludrocortisone 150µg

o.d., alendronic acid 70mg weekly, adcal-D3 b.d., levothyroxine 50µg, growth hormone 0.5mg o.d., and ibuprofen 200mg p.r.n.. At screening she had a negative pregnancy test and an abdominal USS was normal. On examination the BMI was 21.2, blood pressure was normal (99/68), there was symmetrical muscle weakness and evidence of widespread skin hyperpigmentation. Fasting blood sugar (3.9mmol/l), HbA1c (37mmol/mol), LFTs were normal, electrolytes and creatinine (114µmol/l) were normal. Urea was mildly raised (10mmol/l). Plasma ACTH levels prior to the morning dose of hydrocortisone was elevated (142.0ng/l). The patient completed all visits.

Patient 303 was 62 years old at the screening visit and had Cushing's disease (2007) treated with transsphenoidal hypophysectomy (2007, 2010) and bilateral adrenalectomy (2011). The past medical history also included hysterectomy (1996), hypertension, and cholecystectomy (2003). At screening she was treated with hydrocortisone (10/10/5mg), fludrocortisone 50/100µg on alternative days, losartan 25mg, levothyroxine 100µg, pravastatin 10mg, and fluoxetine 100mg. On examination the BMI was 34.0 and blood pressure was normal (125/83). Fasting blood sugar (3.4mmol/l), HbA1c (45mmol/mol), U&Es and LFTs were normal. ACTH prior to the morning dose of hydrocortisone was elevated (381.0ng/l). The patient withdrew following visit 9 due to significant hyperglycaemia.

Patient 401 was 59 years old at the screening visit and had a past medical history of Cushing's disease treated with radiotherapy and bilateral adrenalectomy. At the time of the screening visit she was treated with hydrocortisone (20/10mg) and fludrocortisone 100µg. At screening she had a negative pregnancy test and an abdominal USS showed cholelithiasis. On examination the BMI was 23.0 and blood pressure was normal (119/73). Fasting blood glucose (4.6mmol/l), HbA1c (6%), U&Es and LFTs were normal, and ACTH prior to the morning dose of hydrocortisone was significantly elevated (>7500ng/l). The patient withdrew following the first s.c. pasireotide injection at visit 4 and was therefore excluded from most analyses due to the lack of values (only included in the efficacy analysis for the s.c. phase for the 2h post-HC ACTH levels) (Table 1 and 2).

Withdrawals

Patient 101 withdrew after 11 days of s.c. pasireotide 1200 µg b.d., due to abdominal cramps. The symptoms resolved after stopping pasireotide. Patient 301 withdrew after completing the s.c. phase (following visit 5). Patient 303 withdrew during the LAR phase (after visit 9) due to significant hyperglycaemia that persisted after stopping pasireotide. Patient 401 withdrew during the first visit of the s.c.

phase (visit 4) due to adverse events (felt unwell, drowsy, had a headache and was hypotensive during the visit).

Study Endpoints

Primary Endpoint

Plasma ACTH levels improved during pasireotide treatment

ACTH levels at 0h prior to the morning hydrocortisone dose at baseline (ACTH 0h pre-HC, visits 1 to 4) were compared with ACTH 0h pre-HC levels during the s.c. phase (visits 5 and 6) and ACTH 0h pre-HC levels during the LAR phase (visits 7 to 12) (Figure 2a). Overall, there was a significant reduction in baseline ACTH during treatment (mean baseline 1823+/-1286ng/l vs. 888.0 +/- 812.8ng/l during the s.c. phase and vs. 829.0+/-1171ng/l during the LAR phase, p=0.001) (Figure 2b).

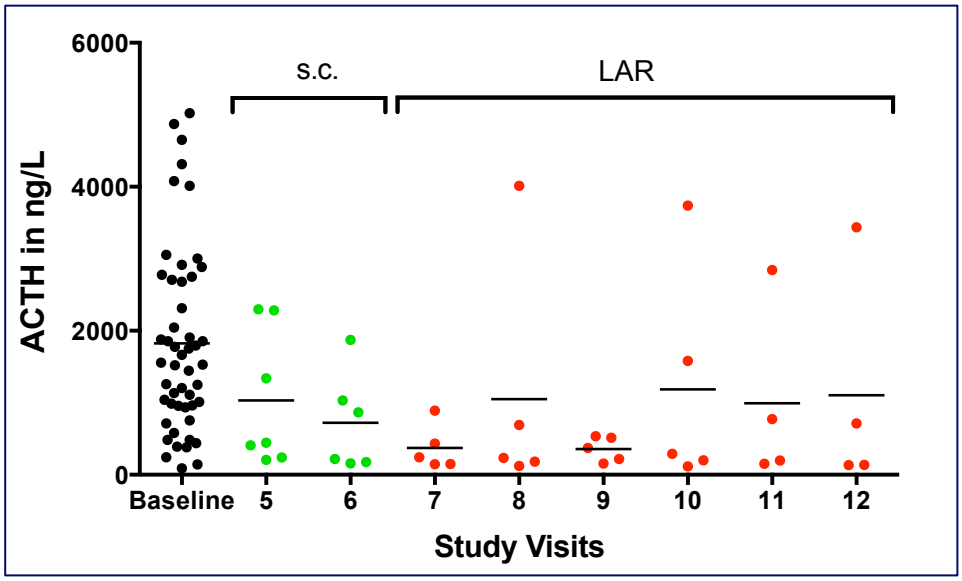


Figure 2a: Plasma ACTH at 0hours prior to the morning dose of hydrocortisone at baseline (mean of visits 1-4) and during s.c. (visits 5 and 6) and LAR (visits 7 to 12) pasireotide treatment (7 patients included, patient 401 had no values during treatment)

Based on the ACTH criteria for response shown above, at the end of 4-weeks of s.c. pasireotide (or at the last visit if patient withdrew prior to the end of this phase) 5/8 patients had a complete response, 2/8 had a partial response and 1 patient did not respond (Table 3). The patient who did not respond withdrew very early from the study after one s.c. dose of pasireotide (patient 401). At the end of 24-weeks of pasireotide LAR treatment or at the last visit, 3/5 patients had a complete response, 1/5 a partial response and 1/5 showed no response (Table 4, patients

101, 401, and 301 had no samples during this treatment period therefore they were not included in the analysis). Overall, 6 patients had complete or partial responses at their last biochemical assessment (either at the end of the study or last visit before withdrawal (Figure 3). There was no clear relationship between dose administered and effect.

Four patients completed the study (102, 201, 202, 302); 3/4 had a complete response at the end of the study and 1/4 did not respond (Table 2).

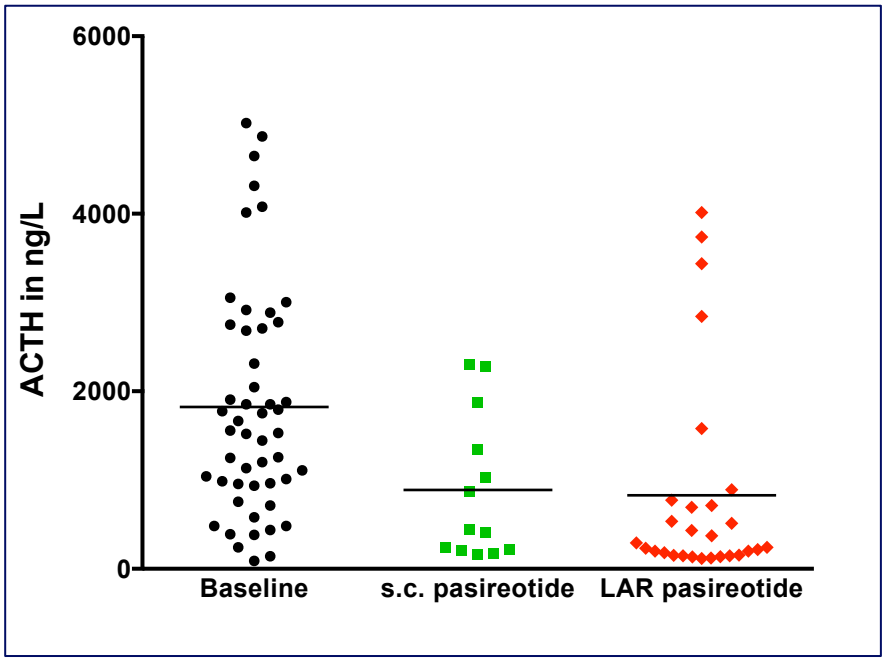


Figure 2b: Mean plasma ACTH at 0hours prior to the morning dose of hydrocortisone improved during pasireotide treatment (mean baseline 1823+/-1286ng/l vs. 888.0+/-812.8ng/l during the s.c. phase and vs. 829.0+/-1171ng/l during the LAR phase, p=0.001)

Table 3: Response of ACTH during the s.c. phase based on ACTH response criteria

ACTH response	0h pre-HC*	2h post-HC	Combined
Complete success	5/7 (101, 102^{+}, 201^{+}, 202^{+}, 301)	3/8 (101, 202^{+}, 301)	5/8 (101, 102^{+}, 201^{+}, 202^{+}, 301)
Partial success	2/7 (302^{+}, 303)	1/8 (102^{+})	2/8 (302^{+}, 303)
No success		4/8 (201^{+}, 302^{+}, 303, 401)	1/8 (401)

( ) Patient ID, + Completed all study visits , * Subject 401 excluded as there were no ACTH 0h values on s.c. treatment

Table 4: Response of ACTH during the LAR phase based on ACTH fall criteria

ACTH response	0h pre-HC	2h post-HC	Combined
Complete success	3/5 (102^{+}, 202^{+}, 302^{+})	3/5 (102^{+}, 202^{+}, 302^{+})	3/5 (102^{+}, 202^{+}, 302^{+})
Partial success	1/5 (201^{+})	1/5 (303)	1/5 (303)
No success	1/5 (303)	1/5 (201^{+})	1/5 (201^{+})

( ) Patient ID, + Completed all study visits

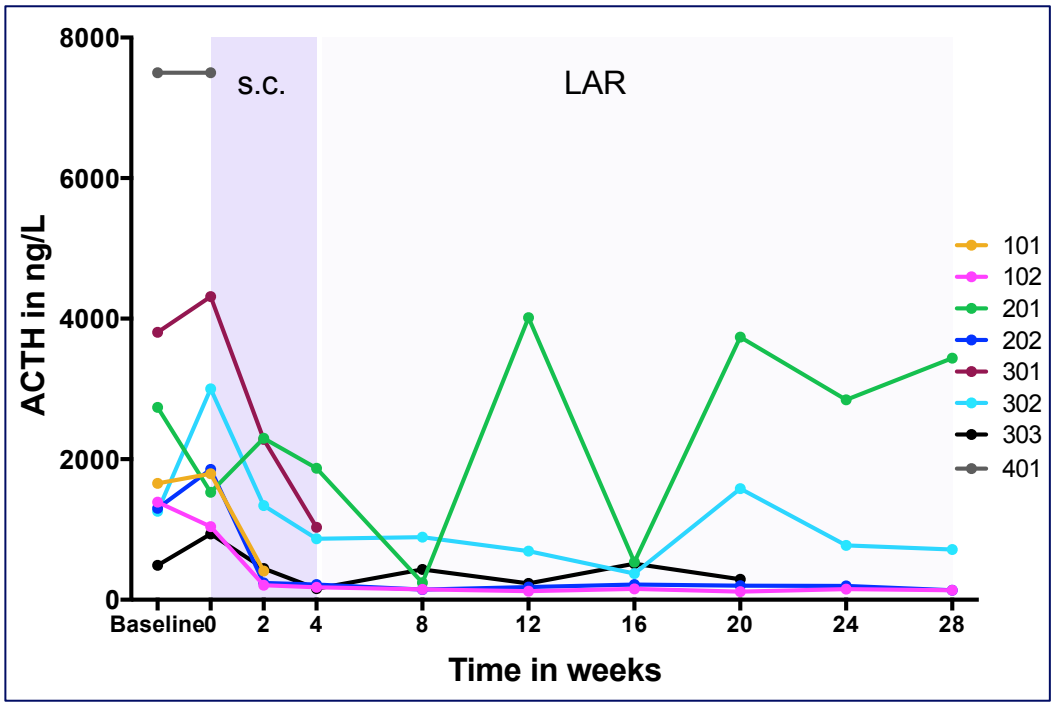


Figure 3: Individual plasma ACTH changes during the study in eight patients (ACTH levels before the morning dose of hydrocortisone)

Secondary Endpoints

1. Acute response to pasireotide test dose

Seven patients received the pasireotide/placebo test dose (Figure 4); 5/7 patients showed a consistent reduction in plasma ACTH levels and 2/7 (302, 401) did not respond. Patient 401 showed no response to the test; ACTH levels remained above

the reference range for the assay throughout. Plasma ACTH levels from patient 302 showed significant variability.

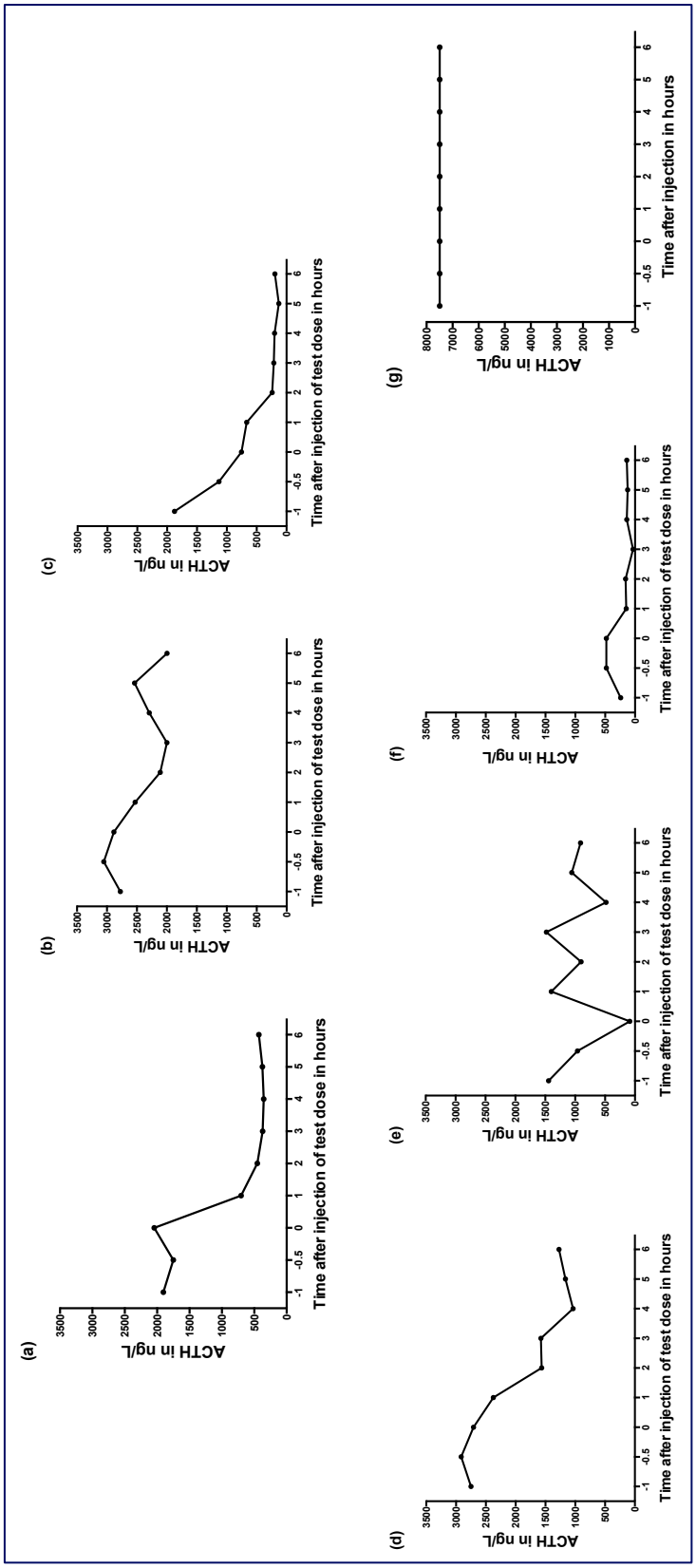
The relative decrease between baseline (=mean of ACTH levels at -1h, -0.5h and 0h before the pasireotide test dose) and 1, 2, 3, 4, 5, and 6h following the test dose was calculated for the six patients (Table 5). Patients who had a relative decrease of at least 42% of their baseline ACTH levels following a test dose showed some response (complete or partial) to pasireotide treatment. However, it is not possible to ascertain if an acute response to the pasireotide test dose can reliably predict which patients are more likely to respond to long-term treatment.

Table 5: Decrease in plasma ACTH levels from baseline following a single s.c. pasireotide test dose (600µg)

Patient	Maximum relative decrease	Range	Time of maximum decrease
102	81%	63-81%	4h
201	31%	12-31%	6h
202	90%	47-90%	5h
301	63%	15-63%	4h
302	42%	(-78)-42%	4h
303	91%	63-91%	3h
401*	0%	0%	-

*took 10mg hydrocortisone 2hours after the test dose injection

Figure 4: Acute response of plasma ACTH levels to a single dose of pasireotide 600µg s.c. in 7 patients [Patients (a) 102, (b) 201, (c) 202, (d) 301, (e) 302, (f) 303, (g) 401]



2. *Tumour volume*

Five patients had MRIs at screening and at the end of the study (102, 201, 202, 302, 303). Only 4 patients completed 28-weeks of the treatment protocol; patient 303 had the full s.c. treatment (4-weeks) but withdrew after 12 weeks of LAR treatment (Table 2). Table 6 shows the maximum adenoma diameters and tumour volumes (calculated using diameters in 3 dimensions) before and after treatment. The relative change in tumour volume was negative indicating reduction post treatment in three patients [(patient ID) relative change; (102) -1%, (201) -27%, (302) -4%] and positive (indicating increase in volume) in 2 patients; (202) 22%, (303) 2%. Overall, there was no significant change in tumour volumes between the pre-treatment and post-treatment scans (1.4+/-0.9 vs. 1.3+/-1.0, p=0.86) (Figure 5).

Table 6: Change in maximum adenoma diameters and tumour volumes before and after pasireotide treatment (s.c. and LAR) in five patients with Nelson's syndrome

Patient	**Maximum diameter in mm**		**Tumour volume in cm^3**	
ID	**Screening**	**End of LAR phase**	**Screening**	**End of LAR phase**
102	19.7	19.7	1.98	1.95
201	21.2	18.7	1.97	1.43
202	19.1	21	2.02	2.46
302	12.9	12.9	0.72	0.69
303	5.6	5.6	0.06	0.07

3. Skin pigmentation

A review of the medical documentation from the case report files shows that the attending physicians felt that 3 patients had an improvement in the skin pigmentation (201, 302, 303) and 1 patient had stable appearances (202). There was no relevant documentation in the remaining patients.

Six patients had medical photographs before treatment and at the end of LAR treatment. The two-blinded researchers felt that overall there was no change in the pigmentation in 5 patients (201, 202, 301, 302, and 303) and one researcher felt there was some reduction in the pigmentation of one patient (102).

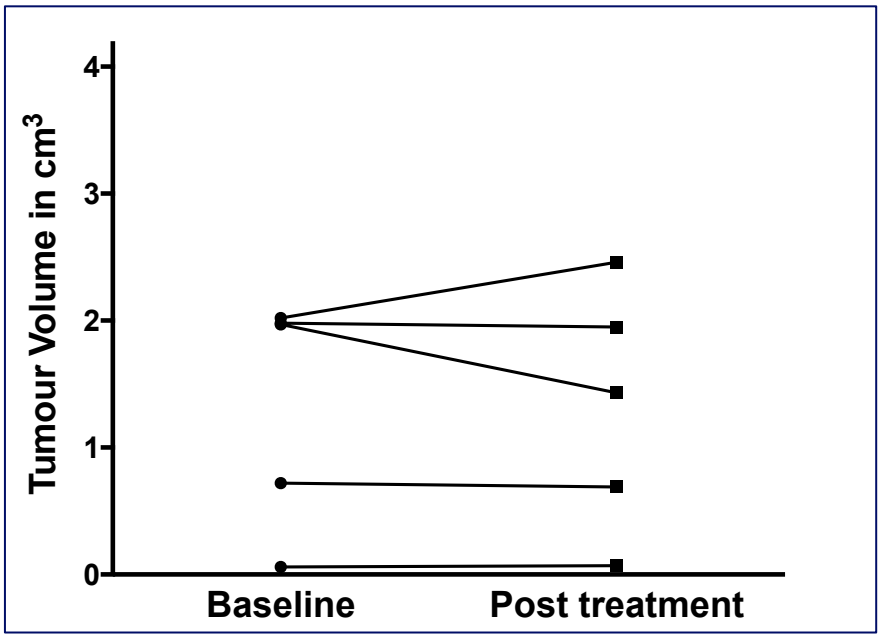


Figure 5: Tumour volumes calculated from gadolinium-enhanced pituitary MRI before and after pasireotide treatment showed no significant change (p=0.83)

Safety analysis

Baseline bloods tests and tolerability

There were no clinically significant events relating to baseline blood tests (U&Es, and LFTs). Adverse events were frequent (Table 7); during the study the majority of patients reported diarrhoea (7 patients), nausea and headaches (6 patients), dizziness (5), abdominal cramps (4), flu-like symptoms (4) and symptoms of hyperglycaemia (4).

ECGs were performed in all patients at screening and after the s.c. phase of pasireotide treatment (visits 1 and 6). There was no abnormal QT prolongation and no difference in QTcF before and after 4-weeks of s.c. pasireotide (p=0.63). The post treatment ECG from patient 302 showed (asymptomatic) sinus bradycardia; all other post-treatment ECGs showed no clinically significant changes.

Table 7: Frequency of adverse events in relation to number of patients reporting the symptom during the study

Adverse event	Number of patients
Diarrhoea	7
Nausea	6
Headache	6
Dizziness	5
Abdominal cramps	4
Flu-like symptoms	4
Diabetes/ hyperglycaemia	4
Injection site skin reaction or pain	3
Fatigue	2
Hot flushes	2
Muscle fatigue	2
Urine infection	2
Visual disturbance	2

Cholelithiasis

The presence of cholelithiasis was assessed by abdominal USS performed at screening and at the end of LAR phase (week 28 or visit 12). Patients 102 and 401 only had an USS before treatment; the result was a normal study for the first and uncomplicated cholelithiasis for the latter. Four patients 101, 201, 301, and 303 had a previous cholecystectomy and two patients (202 and 302) had normal USS before and after treatment. There were no recorded adverse events attributed to cholelithiasis.

Hyperglycaemia

Fasting blood glucose and Hba1c increased during therapy in 6/7 patients (fasting glucose: mean at baseline 4.6+/-0.6mmol/l vs. 6.9+/-1.6mmol/l during s.c. phase vs. 9.6+/-2.9mmol/l during LAR phase, $p<0.01$. Hab1c in mmol/mol: mean at baseline 42.9+/-7.8 vs. 45.6+/-8.5 vs. 60.0+/-13.6, $p<0.01$) (Figures 6 and 7). One patient (303) withdrew from the study due to significant hyperglycaemia after 16 weeks of treatment.

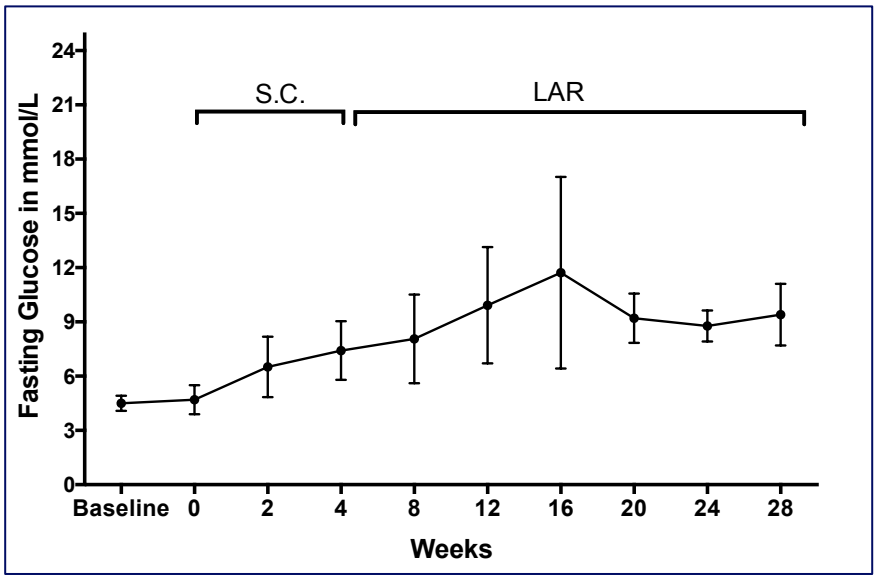


Figure 6a: Mean fasting glucose increased during pasireotide treatment (samples from 7 patients included in the baseline mean value and s.c. phase, 5 patients for the LAR phase)

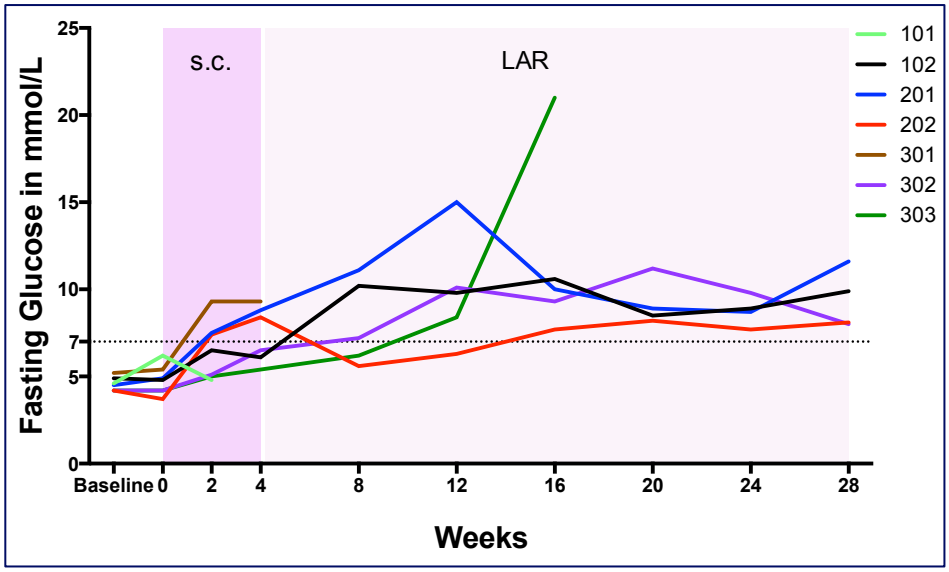


Figure 6b: Individual fasting glucose levels in 7 patients at baseline and during pasireotide treatment. Baseline is the mean of three visits prior to treatment (visits 1-3)

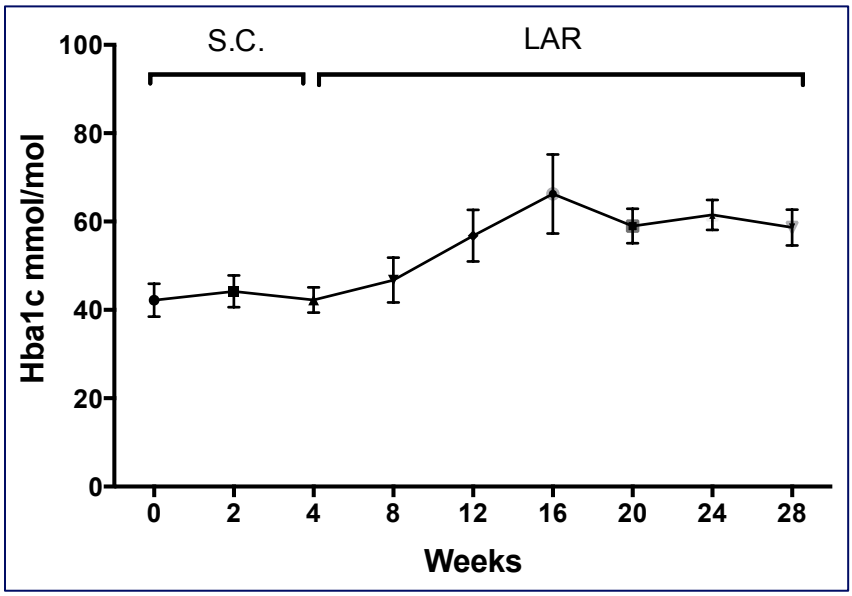


Figure 7a: Mean HbA1c levels increased during pasireotide treatment (levels from 7 patients during s.c. phase and 5 patients during LAR phase)

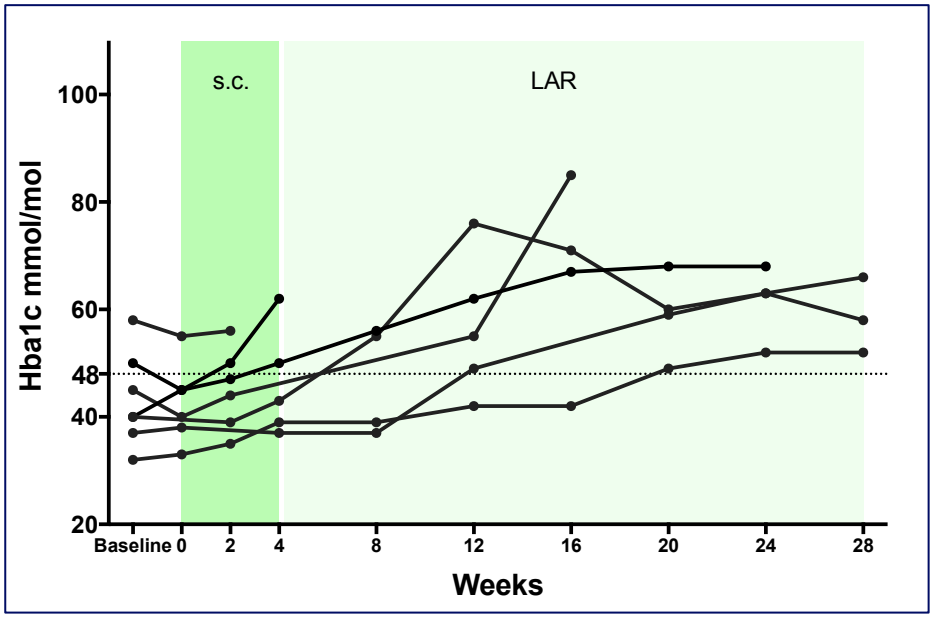


Figure 7b: Individual change in HbA1c levels during pasireotide treatment in 7 patients. Baseline is the mean of three visits prior to treatment

Fasting Insulin

Ten samples (from six patients) were significantly affected by haemolysis and were excluded from the analysis. Patient 401 was also excluded from the analysis due to early withdrawal and lack of samples during treatment. In the 7 remaining patients, fasting insulin levels reduced during s.c. and LAR pasireotide treatment (mean baseline 118.1+/-23.70 vs. mean during s.c. treatment 51.09+/-12.52 vs. 64.94+/111.90, p=0.04) (Figure 8).

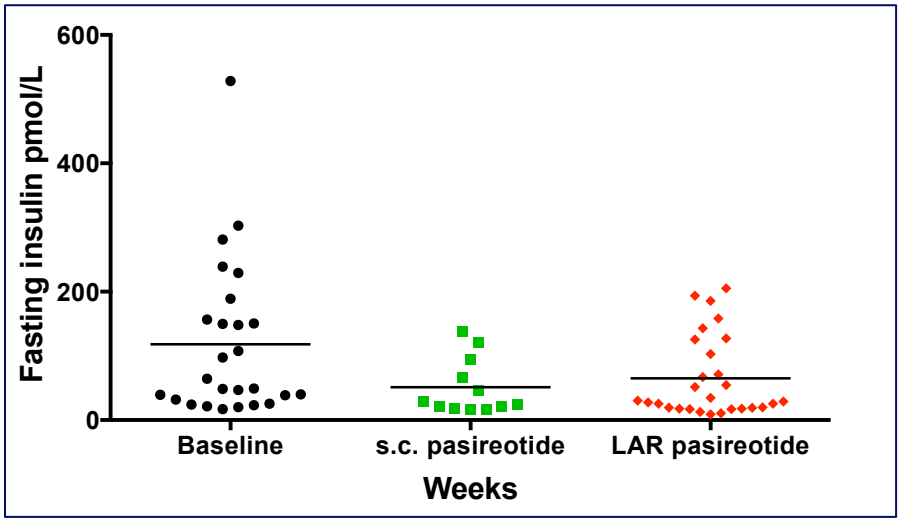


Figure 8: Fasting insulin levels reduced during pasireotide treatment (p=0.04) (baseline levels from visits 1-4, s.c. from visits 5-6, and LAR from visits 7-12)

Discussion

Nelson's syndrome remains a significant clinical challenge to manage. Overall, pasireotide reduces plasma ACTH levels in patients with Nelson's syndrome. However the present study includes a small number of patients and this precludes the complete generalisability of these data. Nevertheless pasireotide should be considered for the management of patients with Nelson's syndrome, on an individualised basis. The lowering of plasma ACTH would be anticipated to be associated with a reduction in tumour volume on longer-term treatment, at least in some patients, as is found in patients with acromegaly treated with first and second generation somatostatin analogues [31].

A positive acute response to pasireotide test dose (i.e. reduction in plasma ACTH levels following a single 600µg s.c. dose) may predict response to long-term treatment, but not in every patient. Four out of five patients who had a consistent reduction in plasma ACTH after a test dose had a response to pasireotide treatment. However due to small patient numbers and significant variability in ACTH levels it is not possible to have absolute confidence that a lack of an acute response to pasireotide test dose predicts lack of effect to long-term treatment.

The majority of patients who responded overall did so soon after initiation of pasireotide. Thus, it would be reasonable to consider that a complete lack of response after three months as a failure of response and a time when further therapy could be discontinued.

As a group, there were no significant changes in the corticotroph tumour volume following pasireotide. This could be due to small patient numbers or the duration of treatment. Nevertheless, there was at least one patient in whom there was a minimal improvement in tumour volume (Table 6, Figure 5). Whether this patient or others would have experienced changes in tumour volume on longer-term therapy is not established.

Side effects were frequent and hyperglycaemia in particular was significant. Six out of seven patients developed abnormal fasting glucose and either new or worsening diabetes. Fasting glucose and HbA1c continued to increase during treatment in spite of the clinicians' attempts to treat this medically. One patient withdrew due to hyperglycaemia. Similarly rates of hyperglycaemia are

reported in 73% of patients treated with s.c. pasireotide (1200 or 1800 µg daily) for CD [22]; in this study 6% of patients discontinued treatment due to a hyperglycaemia related adverse event and 46% had to start a new antidiabetic medication. The significant fall in insulin levels is consistent with suppression of insulin from beta cells of the pancreas, and is in line with the known action of pasireotide at the somatostatin subtype 5 receptors on these cells.

The place of pasireotide in patients with Nelson's syndrome needs to be balanced by its side effects, especially hyperglycaemia. Greater physician awareness of the pasireotide-associated hyperglycaemia and more aggressive management of glucose-related AEs is likely to make pasireotide more acceptable for managing this challenging condition, for which there is no established effective medical management.

Conclusions

Pasireotide treatment (s.c. and LAR) was effective in reducing ACTH levels and may be considered for treating patients with Nelson's syndrome. Active monitoring and management of glucose homeostasis is needed and patients counselled about this prior to therapy. In an individual patient, a positive effect is more likely if there has been a response to the test dose. If no clear response is noted after three months, a response is unlikely. The LAR preparation appears as effective as the s.c. preparation and is likely to be more acceptable to patients. It would seem reasonable to commence therapy at a lower dose and escalate if tolerated, as there appears to be no clear relationship between dose and effect.

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