

CLINICAL TRIALS

Netazepide, a gastrin/cholecystokinin-2 receptor antagonist, can eradicate gastric neuroendocrine tumours in patients with autoimmune chronic atrophic gastritis

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AIMS

Netazepide, a gastrin/cholecystokinin 2 receptor antagonist, once daily for 12 weeks reduced the number of tumours and size of the largest one in 16 patients with autoimmune chronic atrophic gastritis (CAG), achlorhydria, hypergastrinaemia and multiple gastric neuroendocrine tumours (type 1 gastric NETs), and normalized circulating chromogranin A (CgA) produced by enterochromaffin-like cells, the source of the tumours. The aim was to assess whether longer-term netazepide treatment can eradicate type 1 gastric NETs.

METHODS

After a mean 14 months off netazepide, 13 of the 16 patients took it for another 52 weeks. Assessments were: gastroscopy; gene-transcript expression in corpus biopsies using quantitative polymerase chain reaction; blood CgA and gastrin concentrations; and safety assessments.

RESULTS

While off-treatment, the number of tumours, the size of the largest one, and CgA all increased again. Netazepide for 52 weeks: cleared all tumours in 5 patients; cleared all but one tumour in one patient; reduced the number of tumours and size of the largest one in the other patients; normalized CgA in all patients; and reduced mRNA abundances of CgA and histidine decarboxylase in biopsies. Gastrin did not increase further, confirming that the patients had achlorhydria. Netazepide was safe and well tolerated.

CONCLUSIONS

A gastrin/cholecystokinin 2 receptor antagonist is a potential medical and targeted treatment for type 1 gastric NETs, and an alternative to regular gastroscopy or surgery. Treatment should be continuous because the tumours will regrow if it is stopped. Progress can be monitored by CgA in blood or biomarkers in mucosal biopsies.

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Patients with autoimmune CAG develop multiple type 1 gastric NETs as result of hypergastrinaemia secondary to achlorhydria.
- Short-term treatment with netazepide, a gastrin/CCK₂ receptor antagonist, reduces the number of tumours, but does not eradicate them.

WHAT THIS STUDY ADDS

- The tumours regrow if netazepide treatment is stopped.
- Long-term, continuous netazepide treatment has the potential to eradicate them.

Table of Links

TARGETS	
G protein-coupled receptors [2]	SST ₂ receptor
CCK ₂ receptor	SST ₅ receptors

This Table lists key protein targets in this article that are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY [1], and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 [2].

Introduction

Gastric neuroendocrine tumours (NETs), previously called carcinoids [3, 4], arise from enterochromaffin-like (ECL) cells in the gastric mucosa, which express gastrin/cholecystokinin 2 (CCK₂) receptors [5]. Gastric NETs can be separated into three types [6, 7]. Type 1 occur in patients with autoimmune chronic atrophic gastritis (CAG), are usually multiple and <1–2 cm in diameter, and comprise about 80% of gastric NETs. An estimated 5% of type 1 gastric NETs metastasize [7]. The features of CAG are: chronic inflammation and atrophy of the gastric corpus; achlorhydria and secondary hypergastrinaemia; ECL-cell growth; and malabsorption of vitamin B12, which leads to pernicious anaemia and neurological signs in some patients [8, 9].

The management of type 1 gastric NETs is controversial. The European Neuroendocrine Tumour Society (ENETS) recommends that patients undergo yearly gastroscopy, during which the tumours are mapped and biopsied [10]. If there are concerns about the morphology and histology of the tumours, they can be treated surgically by endoscopic polypectomy or gastric antrectomy. Polypectomy does not remove the source of the hypergastrinaemia, and the tumours can recur [11]. Antrectomy leads to a reduction in circulating gastrin by removing the source of the hypergastrinaemia, but it is not effective in all patients, and it carries the risk of morbidity and mortality [12, 13]. Somatostatin (SST) analogues are sometimes used off-label to treat patients with type 1 gastric NETs; they reduce gastrin by negative feedback on the gastrin-secreting G cells in the gastric antrum, and by a direct effect on ECL cells [14, 15], which possess SST₂ and SST₅ receptors. Several studies have shown that SST analogues can cause regression of type 1 NETs, and are generally well-tolerated [14–19]. However, SST analogues inhibit release of various hormones, such as growth hormone, insulin, glucagon, thyroid stimulating hormone and cholecystokinin,

and neither ENETS nor the North American NETS recommends them for treating type 1 gastric NETs [10, 20].

A gastrin/CCK₂ receptor antagonist is a more logical treatment of type 1 gastric NETs, because they are gastrin driven. Many have been described, but to date none has been developed into a medicine [21]. In nonclinical studies, netazepide (YF476) is a potent, highly-selective, competitive gastrin/CCK₂ receptor antagonist with good oral bioavailability [22]. Netazepide prevented hypergastrinaemia-induced increases in ECL-cell activity, density and oxyntic mucosal thickness in rats [23], and reduced substantially the incidence of ECL-cell carcinomas in a strain of female cotton rats that develop such tumours spontaneously as a result of hypergastrinaemia secondary to gastric hypoacidity [24]. Furthermore, netazepide not only prevented formation of gastric NETs accelerated by hypergastrinaemia induced by loxidine, an insurmountable histamine H₂-receptor antagonist, in *Mastomys* rodents – which have a genetic predisposition to gastric NETs – but also caused shrinkage of formed lesions [25].

Netazepide is also an orally-active gastrin/CCK₂ receptor antagonist in healthy subjects. It caused dose-dependent, persistent inhibition of pentagastrin-induced gastric acid secretion [26] and prevented the increase in plasma CgA – a biomarker of ECL-cell hyperactivity – resulting from proton pump inhibitor-induced hypergastrinaemia. Furthermore, netazepide reduced baseline plasma CgA – a sign of ECL-cell hypoactivity [27].

Thus, there is evidence from animal models and healthy subjects to justify developing a gastrin/CCK₂ receptor antagonist as a medical treatment for patients with gastric NETs type 1, which are gastrin-driven and comprise the majority of all gastric NETs [6, 7]. Indeed, in two separate studies in Trondheim, Norway, and Liverpool, UK, in patients with type 1 gastric NETs, netazepide 50 mg once daily for 12 weeks, reduced the number of tumours and the size of

the largest one [28, 29]. Current toxicology studies of netazepide allow treatment for only 13 weeks. However, because of those favourable results, the UK Medicines and Healthcare products Regulatory Agency and the Norwegian Regulatory Agency (NoMA) allowed netazepide treatment for longer without the extended toxicology studies normally required [30].

Aim

Our aim was to treat patients with type 1 gastric NETs from the aforementioned 12-week studies with netazepide for another 52 weeks, and pool the results: (1) to assess if netazepide for longer can eradicate the tumours; (2) to identify tumour biomarkers; and (3) to continue to assess the safety and tolerability of netazepide.

Methods

Study design

The two centres in Trondheim and Liverpool first did an open study in which *Helicobacter pylori* negative patients with CAG, hypergastrinaemia, multiple type 1 gastric NETs and raised circulating CgA were treated with netazepide 50 mg once daily for 12 weeks, with 12-week follow-up. The same protocol was used for both studies. There were seven outpatient visits. Visits 1 and 2 were to assess eligibility and to obtain consent. Visits 3, 4, 5 and 6 were at 3, 6, 9 and 12 weeks after starting treatment, respectively. Visit 7 was at 12 weeks after stopping treatment. Gastroscopy was done at visits 2, 4, 6 and 7, during which the number of tumours was counted, the diameter of the largest tumour was measured against the open biopsy forceps, and the tumours and flat corpus mucosa were biopsied. Blood was collected at visits 2, 3, 4, 5, 6 and 7 for assay of fasting serum gastrin, plasma or serum CgA, and plasma netazepide, as described previously [28, 29]. Safety and tolerability were assessed by: medical examination; echocardiography; blood and urine tests; and adverse events, which were recorded by the patient on a diary card and transferred to the patient's case report form by the investigator. Trio Medicines (London, UK) supplied netazepide 25 mg capsules.

The Medicines and Healthcare products Regulatory Agency approved a protocol amendment for the Liverpool patients to receive netazepide 50 mg once daily for another 52 weeks. The frequency of clinic visits and gastroscopy was reduced to 3- and 6-monthly, respectively, to make the extension to the study less demanding. NoMA would not agree to a similar protocol amendment for the Trondheim study. However, they did agree to long-term treatment with netazepide 25 mg once daily under the Norwegian 'named patient' scheme. Cambridgeshire 1 Research Ethics Committee (REC) and East of England – Cambridge East approved the Liverpool 12- and 52-week studies, respectively (reference 10/H0304/51). The Regional REC approved the Trondheim studies (reference 2010/1617). Subjects gave written, informed consent. The 12-week studies were completed during January 2011 to July 2012, and the 52-week studies during October 2012 to April 2014. There was a mean interval of 14

(range 8–19) months between the end of dosing patients in the 12-week studies and the start of dosing them in the 52-week studies. The interval off-treatment was required to obtain regulatory and REC approvals. The studies complied with the ICH Guideline for Good Clinical Practice and the EU Clinical Trial Directive. The Liverpool study was registered as ClinicalTrials.gov NCT01339169, and the Trondheim study as ClinicalTrials.gov NCT01444014.

Biopsies

The Liverpool centre took biopsies of the tumours and flat corpus mucosa at each endoscopy during both the 12- and 52-week studies and assessed them in detail, so only those procedures are described here. Results of biopsies taken during the 12-week study in Trondheim are published elsewhere [28].

Histology

The extent of ECL-cell proliferation in tumour biopsies was classified as linear ECL-cell hyperplasia, micronodular ECL-cell hyperplasia, ECL-cell dysplasia or NET, as described previously [29]. The histopathologist was blind to the clinical and endoscopic findings.

Biomarkers

RNA was extracted from corpus mucosal biopsies by Tri-Reagent and reverse transcribed before assessment of real-time polymerase chain reaction (qPCR) abundances of CgA, histidine decarboxylase (HDC) and matrix metalloproteinase-7 (MMP-7). qPCR used the primers, probes, master mix, standards, and 7500 real-time PCR machine (Applied Biosystems, Warrington, UK), as described previously [29]. Absolute abundances relative to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were calculated.

Statistics

Wilcoxon signed rank test was used to test for significant differences with respect to pooled data from the two centres for the number of tumours, size of the largest tumour, and circulating gastrin and CgA for the 12-week and 52-week studies, and for any changes during the period off-treatment. Data were illustrated by box-whisker plots for median, interquartile range and range of results. Because the two centres used different assays with different units for CgA [28, 29], we adjusted measurements to the upper limit of normal (Liverpool 22 U l⁻¹; Trondheim 6 nmol l⁻¹). Data for qPCR were analysed by SPSS v20 after testing for normality using the Kolmogorov-Smirnov test, and then analysed using a Wilcoxon signed rank test. Differences were deemed significant when $P < 0.05$.

Results

12 weeks' netazepide treatment

Sixteen patients, eight per centre, mean age 61 (range 50–76) years, entered and completed the study. Eleven were receiving vitamin B12 for treatment of pernicious anaemia.

At baseline, the median number of tumours was 10 (range 4–30) and the median size of the largest one was 6 (range 3–15) mm (Table S1; Figure 1). Netazepide 50 mg once daily for 12 weeks reduced significantly the number of tumours ($P < 0.001$) and the size of the largest tumour ($P < 0.001$), and reduced plasma or serum CgA in all patients to within normal limits ($P < 0.001$). The reduction in CgA was evident at the first assessment, at 3 weeks.

Netazepide did not affect ($P < 0.1$) the high serum gastrin observed at baseline (median 415 pmol l^{-1} ; normal ≤ 40 pmol l^{-1} for both centres). At 24 weeks, when patients had been off-treatment for 12 weeks, the number of tumours, the size of the largest tumour, and circulating CgA had all increased again, but the effect of netazepide was still significant, albeit less so.

Plasma netazepide concentrations before and 1 h after dosing ranged from 4.6 to 7.0 ng ml^{-1} and 87 to 220 ng ml^{-1} , respectively, at 3, 6, 9 and 12 weeks. There were no clinically relevant changes in safety tests, and 10 mild-to-moderate adverse events, none of which was deemed related to treatment by the investigator. No patient had their treatment stopped.

Interval off-treatment

All eight patients from the Liverpool study and six from the Trondheim study consented to a further 52 weeks' treatment with netazepide. One Trondheim patient withdrew soon after, for personal reasons. The mean interval between the end of 12 weeks' netazepide treatment and the start of 52 weeks' treatment in 13 patients was 14 (range 8–19) months. During the period off-treatment, the number of tumours ($P < 0.01$), the size of the largest tumour ($P < 0.05$), and plasma or serum CgA ($P < 0.001$) all increased. Serum gastrin was unaffected (Figure 2).

52 weeks' netazepide treatment

Thirteen patients from the two centres entered and completed another 52 weeks' treatment with netazepide 50 mg (Liverpool) or 25 mg (Trondheim) once daily for 52 weeks. Netazepide cleared all tumours in five of the 13 patients and reduced the number of tumours ($P < 0.01$) and the size of

the largest one ($P < 0.001$) in the other patients, and reduced plasma or serum CgA to within normal limits in all patients ($P < 0.001$; Table S2; Figure 3). One patient was left with only one tumour. Serum gastrin was unaffected. There were no clinically relevant changes in safety tests and 28 adverse events. One adverse event was considered severe and the others mild-to-moderate. None of them was deemed related to treatment by the investigator. No patient had their treatment stopped.

Biopsies

Histology. When Liverpool patients were screened for the study, biopsies of their tumours and flat mucosa showed that all eight patients had NETs and ECL-cell hyperplasia, respectively (Table 1). After 12 weeks' treatment, four patients had NETs and four had micronodular ECL-cell hyperplasia [29]. After 52 weeks' treatment, only three patients had NETs.

Biomarkers. After 26 and 52 weeks' netazepide treatment, there were significant reductions in qPCR abundances (normalized to GAPDH) of the biomarkers CgA and HDC, but not MMP-7, relative to baseline of the 12-week study and to the baseline after the period off-treatment (Figure 4).

Discussion

The limitations of the studies were: the open design; the subjective counting of the tumours and measurement of the diameter of the largest one; tumour but not mucosal biopsies may have contributed to tumour clearance in some patients; and the small numbers of patients. Placebo controls seemed unreasonable given that: the studies were exploratory or proof-of-concept; gastric NETs are rare and recognized as an orphan disease in Europe and the USA [31], so the number of eligible patients per centre was limited; and the studies were very demanding for the patient. Also, the gastroscopies were done by only two experienced physicians per centre, and they counted and measured the tumours carefully.

Table 1

Histology of tumour biopsies ($n = 8$ patients)

Patient	Screen	12-week study				52-week study		
		Week 0	Week 6	Week 12	Week 24	Week 0	Week 24	Week 52
1	NET	NET	ECL-M	ECL-M	ECL-M	NET	ECL-M	ECL-M
2	NET	NET	NET	NET	ECL-M	ECL-D	ECL-M	ECL-M
3	NET	ECL-M	ECL-M	ECL-M	ECL-M	ECL-M	ECL-M	ECL-L
4	NET	NET	ECL-M	ECL-M	NET	ECL-M	ECL-M	ECL-M
5	NET	NET	NET	NET	NET	NET	ECL-M	NET
6	NET	NET	NET	ECL-M	NET	NET	NET	NET
7	NET	NET	NET	NET	NET	NET	NET	NET
8	NET	NET	NET	NET	ECL-M	nb	ECL-M	ECL-M

ECL-D, ECL-cell dysplasia; ECL-L, linear ECL cell hyperplasia; ECL-M, micronodular ECL-cell hyperplasia; nb, no biopsy; NET, neuroendocrine tumour

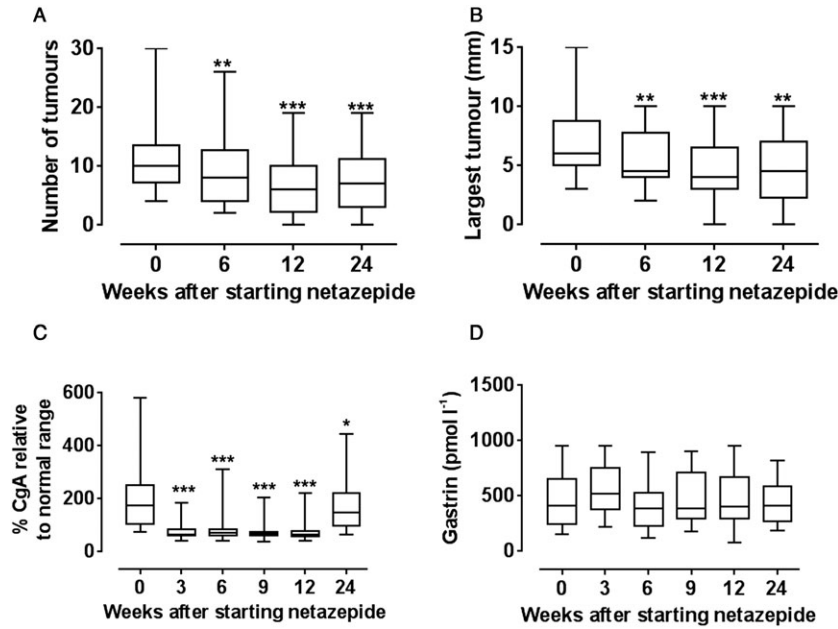


Figure 1

Effect ($n = 16$ patients) of netazepide 50 mg once daily for 12 weeks, and 12 weeks off treatment, on: (A) number of tumours; (B) size of largest tumour; (C) chromogranin A (adjusted); and (D) gastrin. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. (Normal ranges. chromogranin A: Trondheim ≤ 6 nmol l⁻¹; Liverpool ≤ 22 U l⁻¹. Gastrin: < 40 pmol l⁻¹)

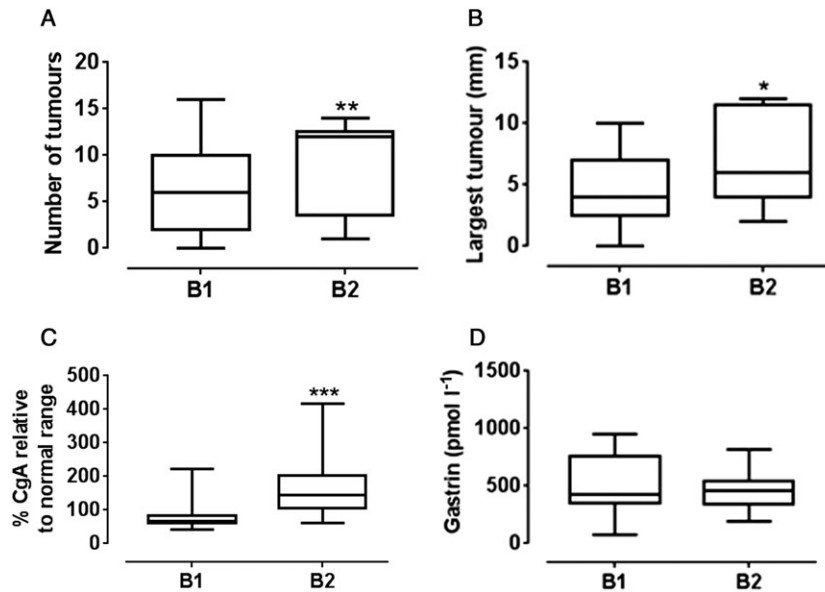


Figure 2

Changes ($n = 13$ patients) during the period off netazepide treatment in: (A). number of tumours; (B). size of the largest tumour; (C). chromogranin A (adjusted); and (D). gastrin. (Normal ranges. chromogranin A: Trondheim ≤ 6 nmol l⁻¹; Liverpool ≤ 22 U l⁻¹; Gastrin: ≤ 40 pmol l⁻¹). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. B1 = end of 12-weeks' netazepide. B2 = start of 52 weeks' netazepide

Furthermore, the histology of the gastric biopsies was assessed blind, and the biomarker measurements were objective.

The clearance of tumours in five of 13 patients during 52 weeks' treatment, and the reduction in circulating CgA in all patients, are consistent with a treatment effect. One

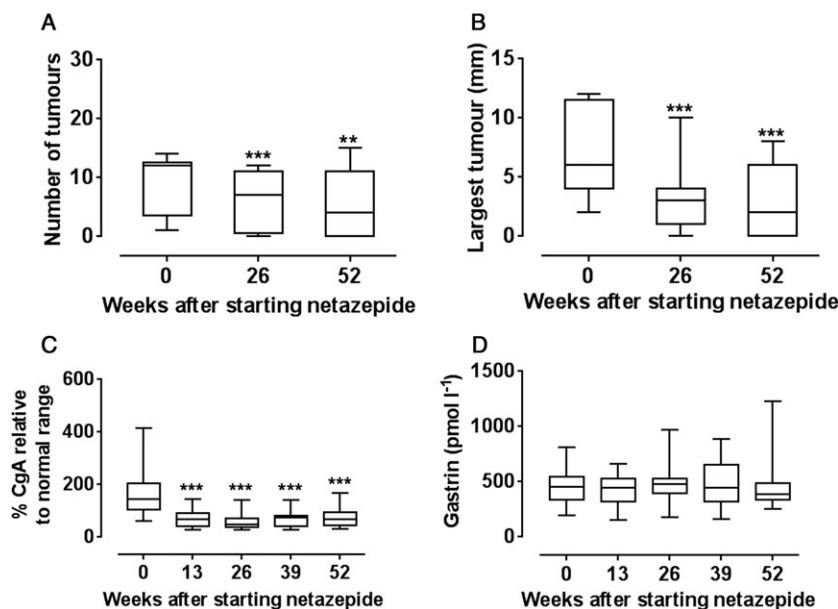


Figure 3

Effect ($n = 13$ patients) of netazepide 25 mg ($n = 5$) or 50 mg ($n = 8$) once daily for 52 weeks on: (A) number of tumours; (B) size of largest tumour; (C) chromogranin A (adjusted); and (D) gastrin. $**P < 0.01$; $***P < 0.001$. (Normal ranges. chromogranin A: Trondheim ≤ 6 nmol l⁻¹; Liverpool ≤ 22 U l⁻¹; Gastrin: ≤ 40 pmol l⁻¹)

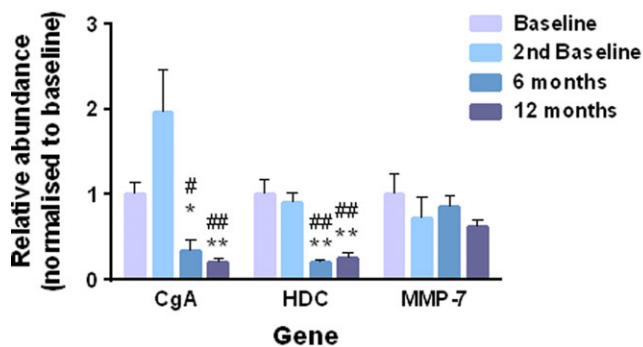


Figure 4

Absolute quantities of abundances of biomarkers in gastric mucosal biopsies ($n = 8$) relative to GAPDH during 52 weeks of netazepide treatment. $* P < 0.05$ and $** P < 0.01$ compared to baseline. $\# P < 0.05$ and $## P < 0.01$ compared to second baseline

patient was left with only one tumour. The reduction in the number of tumours and the size of the largest one in the other patients are supportive evidence of a treatment effect. The reduction in mRNA abundances of the biomarkers CgA and HDC and the changes in the histology in the biopsies of the tumours and flat mucosa, respectively, during both the 12-week [29] and 52-week studies are further evidence of a treatment effect.

While patients were off-treatment for a mean of 14 (range 8–19) months, the number of tumours, size of the largest tumour, circulating CgA and tumour biomarkers all increased again. Circulating CgA is a valid biomarker of ECL-cell activity in patients with type 1 gastric NETs [32–34] and in

healthy subjects [27]. Type 1 gastric NETs are derived from ECL cells, which are also the source of the increased circulating CgA in CAG patients [33]. The normalisation of circulating CgA during both the initial and extended studies is consistent with netazepide inhibiting ECL-cell growth via antagonism of gastrin/CCK₂ receptors on the ECL cells. Circulating CgA increased again during the interval off-treatment, when gastrin/CCK₂ receptors would not have been blocked.

Apart from its well-known effect on gastric acid secretion and its effect on ECL-cell growth via gastrin/CCK₂ receptors, gastrin also has an effect on various cellular mechanisms, including proliferation, apoptosis, migration, differentiation and angiogenesis [35–39]. Proteins such as Reg, MMP-7, MMP-1, and plasminogen activator inhibitor (PAI)-1 and PAI-2 are overexpressed in the stomach of patients with hypergastrinaemia, and may contribute to the formation of gastric tumours [40–43]. Therefore, abundances of MMP-7, PAI-1 and PAI-2, as well as CgA and HDC, were studied in gastric mucosal biopsies as potential biomarkers for type 1 gastric NETs before and during netazepide treatment. In the 12-week study, CgA, HDC and MMP-7 were reduced relative to baseline [29]. They had almost returned to pretreatment levels 12 weeks after the end of treatment. PAI-1 and PAI-2 did not change significantly. In the 52-week study, CgA and HDC were again reduced significantly, at 26 and 52 weeks, but at neither time was there an effect on MMP-7.

The results are strengthened further by a recent report of the effect of netazepide on microRNA-222 (miR-222) in corpus mucosal biopsies and serum during the 12-week study in Liverpool patients [44]. miRNAs are nonprotein coding short RNAs that regulate ~30% of the human genome, and inhibit the translation, increase cleavage or induce degradation

of target mRNAs. One gene can be regulated by several miRNAs, including tumour suppressor genes and oncogenes. There was a small but significant increase in miR-222 expression in mucosal biopsies before netazepide compared to subjects with normal serum gastrin. Expression decreased significantly during netazepide treatment, and returned to baseline after treatment cessation. Serum miR-222 expression was also increased significantly, by 5.7-fold, before netazepide treatment compared to controls. As with mucosal biopsies, expression decreased significantly during netazepide treatment and returned towards baseline after treatment cessation. Gastrin-induced miR-222 overexpression in a human gastric adenocarcinoma cell line transfected with the CCK₂ receptor resulted in suppression of the oncogene p27^{kip1}, which was reversed by pretreatment with netazepide [44]. miR-222 is also dysregulated in gastric adenocarcinoma and in the stomach infected with *H. pylori* [45], and may be a useful biomarker for monitoring gastrin-induced premalignant changes in the stomach [46].

Our rationale for pooling the results is as follows. The protocol for the 12-week and 52-week studies was the same for both centres, apart from the reduction in netazepide dose from 50 mg to 25 mg daily in the 52-week study at the Trondheim centre, for reasons explained earlier, and the two centres used different assays for circulating CgA. Also, the Liverpool centre did a more detailed assessment of mucosal and blood biomarkers in the 52-week study. The results of the 12-week studies, which are published elsewhere [28, 29], were remarkably similar for the two centres. Each centre was independent, and there was no contact between them during either of the studies. The effects of netazepide for 52 weeks on tumour number and size and circulating CgA and gastrin were also similar for the two centres, despite the difference in doses. Circulating CgA was reduced by netazepide to within the normal range in both centres and in both studies, so it seemed reasonable to adjust measurements to the upper limit of normal. Patients in the Trondheim centre were given the lower dose of netazepide because NoMA was more receptive to allowing a lower dose for long-term treatment, and netazepide 25 mg of a similar formulation was top of the dose–response curve for increasing gastric pH in healthy subjects [47]. Netazepide is a competitive antagonist at CCK₂ receptors [22, 26], so patients with hypergastrinaemia may require adjustment of netazepide dose according to their serum gastrin concentration. Finding the dose for phase 3 studies is the most challenging aspect of proof-of-concept studies: 25 mg once daily was enough for patients in one centre, and might be enough for all patients with type 1 gastric NETs.

In animal models [24] and healthy subjects [27], acid suppression by netazepide leads to a secondary increase in serum gastrin, which is 'harmless' because the gastrin/CCK₂ receptors are blocked. Netazepide did not increase serum gastrin further in the CAG patients, confirming that they had achlorhydria as a result of parietal-cell atrophy. In other words, their serum gastrin was already maximally increased.

Netazepide has so far been safe and well tolerated in clinical trials. To date, about 220 healthy subjects have taken netazepide by mouth for up to 6 weeks, and 27 patients have taken it for 3–36 months. Adverse events have been minor, transient, independent of netazepide dose, and as common

in placebo or comparator groups. Plasma netazepide levels at 1 h after 25 or 50 mg doses daily for 12 weeks in CAG patients were within the range seen with those doses at that time in studies of healthy subjects that used a similar formulation [47]. Thus, achlorhydria does not appear to affect the bioavailability of netazepide in CAG patients.

Patients with pernicious anaemia, one of the possible presentations of type 1 gastric NETs, have a nearly seven-fold increased risk of gastric adenocarcinoma [48]. Children with hypergastrinaemia caused by genetic mutations of *KCNQ1* or *KCNE1* [49–52] or *ATP4A* [53], genes that control acid secretion by the parietal cell, not only develop gastric NETs, but also have a high risk of gastric adenocarcinoma. Some patients with those genetic mutations have needed gastrectomy. These reports all support the concept that hypergastrinaemia has malignant potential. Indeed, all gastric NETs have the potential to metastasize, especially ones that are >2 cm in size, infiltrate the *muscularis propria*, are angioinvasive and/or are G2 grade [10]. The percentage of type 1 gastric NETs that metastasize may be an underestimate [31].

A survey of cancer registries and a PubMed search yielded a prevalence rate for gastric NETs of 0.32 (range 0.09–0.92), 0.17 and 0.05 per 10 000 population of 10 European countries, USA and Japan, respectively [31]. There has been a 10–20 fold increase in the prevalence of gastric NETs in the USA, Japan and the countries of Europe in recent decades. The reasons for the increases are unknown, but they may include improvements in clinical practice, such as diagnostic gastroscopy and biopsies, and greater awareness of the condition [54, 55].

In the absence of a licensed medical treatment for type 1 gastric NETs, SST analogues, such as octreotide and lanreotide, are sometimes used off-label [14–19], despite neither ENETS nor NANETS recommending them for that purpose [10, 20]. SST (somatotropin release inhibiting factor) is an abundant neuropeptide, which acts on five subtypes of SST receptor (SST₁–SST₅). Activation of those receptors produces a wide range of physiological effects throughout the body, including inhibition of secretion of many hormones, modulation of neurotransmission, inhibition of cell proliferation and smooth muscle contractility [5]. SST analogues are licensed only for treatment of patients with: acromegaly; symptoms associated with functional gastroenteropancreatic NETs, such as carcinoid tumours, insulinomas and glucagonomas; and progression of well-differentiated advanced NETs [56, 57]. An estimated 80% of those tumours express SST receptors. In clinical trials of octreotide, very common (≥1/10) adverse drug reactions (ADR), based on the CIOMS III convention, included: diarrhoea, headache, gall stones and hyperglycaemia, and common ADR (≥1/100 to <1/10) included: steatorrhoea, dizziness, hypothyroidism, cholecystitis, dyspnoea, hypoglycaemia and bradycardia [56]. Glucose and thyroid function monitoring, and ultrasonic examination of the gallbladder for gall stones before and every 6 months after the start of treatment, are recommended. However, SST analogues have been generally well tolerated when used off-label in patients with type 1 gastric NETs to inhibit gastrin release, reduce ECL-cell hyperplasia and to reduce the tumour load [14–19]. The tumours regrow when treatment is stopped.

Of 254 consecutive patients with type 1 gastric NETs from five countries, 20 (7.9%) had metastases to lymph nodes or liver at presentation [58]. Of those 20 patients, 10 underwent gastrectomy and lymph node dissection, four underwent antrectomy and wedge resection, and one underwent only primary tumour biopsy. Five patients were treated with SST analogues; one had a complete response, and the disease stabilized in another. The other three patients had liver metastases, which progressed. All 20 patients were alive after follow-up for a mean 83 (range 12–360) months. SST analogues were generally well tolerated, apart from deterioration in control of one patient's diabetes.

Thus, there is evidence that SST analogues are an effective medical treatment for patients with type 1 gastric NETs, perhaps even ones that have metastasized. However, they are not licensed for that purpose and there have been no controlled trials. Furthermore, their ADR profile is unfavourable [56], they must be given by injection, and they are expensive [19]. An orally active gastrin/CCK₂ receptor antagonist may offer advantages over an SST analogue in the treatment of type 1 gastric NETs. Further studies are required to confirm whether that is so.

Conclusions

- This study confirms that type 1 gastric NETs are gastrin driven. A gastrin/CCK₂ receptor antagonist such as netazepide is a potential targeted treatment for type 1 gastric NETs, and an alternative to regular gastroscopy or surgery. But that would need confirmation in a placebo-controlled study in a larger number of patients. Given the rarity of the tumours, the trial would need to be multicentre and international.
- Treatment of type 1 gastric NETs with a gastrin/CCK₂ antagonist should be continuous, because they will eventually regrow if treatment is stopped. Measuring circulating biomarkers CgA or miR-222 is a simple way to monitor treatment. Measuring gastric mucosal biomarkers CgA, HDC or miR-222 is an alternative method.
- A longer trial is required to assess whether a gastrin/CCK₂ receptor antagonist can clear type 1 gastric NETs in all patients. The capacity of atrophic gastric mucosa to remodel itself may be limited, and a gastrin/CCK₂ receptor antagonist may not be capable of causing complete regression of large type 1 gastric NETs that have been present for many years. However, it might reduce the risk of invasion/metastasis.

Competing Interests

All authors have completed the Unified Competing Interest form at http://www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author). None of the authors has any conflict of interest. The Liverpool centre was funded by Trio, a subsidiary of Hammersmith Medicines Research (HMR), a contract research organisation. The biopsy work was part-funded by a grant from UKINETS (TRANSNETS). Trio monitored the study and measured

serum gastrin, plasma CgA for the Liverpool centre and plasma netazepide for both centres. M.B. is an employee of HMR, and owns both companies. Trio holds the patent for netazepide.

Contributors

M.B. designed the studies and wrote the draft manuscript. D. M.P., A.R.M., L.S. and R.F. did the gastroscopies. F.C. did the histopathology. B.N.P. and A.V. did the qPCR. All authors contributed to and approved the final version of the manuscript.

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Supporting Information

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Table S1 12-week studies ($n = 16$ patients): A. number of tumours; B. size of largest tumour (mm); C. CgA (adjusted); and D. gastrin (pmol l^{-1})

Table S2 52-week studies ($n = 13$ patients): A. number of tumours; B. size of largest tumour (mm); C. CgA (adjusted); and D. gastrin (pmol l^{-1})