Trio Medicines Ltd

Clinical trial report

Trial title	Randomized, placebo-controlled trial of netazepide, a gastrin/CCK_2 receptor antagonist, in patients with Barrett's esophagus
Version and date of report	Version A, 23 November 2020
EudraCT number	2014-002418-22
IND#	IND 107354
HMR trial code	10-505
Sponsor trial code	T-016
Investigational product	Netazepide
Trial indication	Barrett's esophagus
Phase of trial	Phase II
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Date first subject screened 15 May 2013

Date of last subject visit 27 December 2017

This trial was conducted in accordance with EU Directive 2001/20/EC, applicable national statutory requirements, and ICH GCP, including the archiving of essential documents. The protocol was approved by the Medicines and Healthcare products Regulatory Agency and an independent recognised research ethics committee before the trial began, and written informed consent was obtained from each subject. This report has been prepared in accordance with ICH E3 and ICH M4E.

Signatures

We, the undersigned, confirm that this report is an accurate record of those parts of the trial for which we were responsible.

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Nov. 29, 2020

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26, Nov 2020

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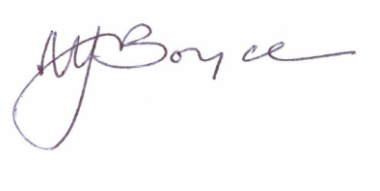

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SYNOPSIS

Background and rationale

Barrett's esophagus (BE) consists of inflammation and thickening of the cells of the mucosa (lining) at the lower end of the esophagus (gullet). The main cause of BE is reflux of acid and bile from the stomach into the esophagus. BE is associated with a 25-30 times increased risk of esophageal cancer. The prognosis of esophageal cancer is poor; the 5-year survival rate is 10–15%.

Current management of BE patients consists of long-term treatment with a proton pump inhibitor (PPI), to suppress gastric acid production, and regular endoscopy (looking down into the esophagus with a pencil-thin tube with a light and camera at the end), to check for early esophageal cancer. However, blocking acid production with a PPI results in a secondary increase in gastrin in the bloodstream (hypergastrinaemia). Gastrin is a hormone that controls acid production and growth of cells in the stomach (ECL cells). Research has shown that BE cells possess gastrin receptors and that hypergastrinaemia is associated with an increased risk of progression of BE to esophageal cancer. Gastrin receptors (CCK_2R) are normally found in the stomach, and not in the esophagus. Hypergastrinaemia also causes inflammation and cancerous changes of cells in animal models of BE.

Netazepide blocks the effects of gastrin. It suppresses gastric acid production as effectively as a PPI and can eradicate stomach tumours (called gastric-NETs) caused by hypergastrinaemia. Therefore, netazepide may prevent progression of BE to esophageal cancer. To date, netazepide has been safe and well-tolerated in studies of over 200 healthy subjects and patients.

Primary objective

- to assess if netazepide can reduce expression of Ki67, a biomarker of cellular proliferation, in patients with BE without dysplasia

Secondary objectives

- to assess the effect of netazepide on biomarkers associated with esophageal cancer, in particular cyclooxygease-2 (COX-2), p53, CCK2R and DCAMKL1
- to assess the effect of netazepide on fasting serum gastrin, a biomarker of PPI-induced gastric acid suppression, and plasma chromogranin A (CgA), a biomarker of growth of cells in the stomach (ECL cells) that possess gastrin receptors
- to assess the safety and tolerability of netazepide in patients with BE

Treatments

Patients were randomised to spray-dried netazepide 25 mg or matching placebo (1:1 ratio), one capsule daily by mouth for 12 weeks. Trio Medicines Ltd, England, supplied the capsules. The batch numbers and expiry dates of netzepide capsules are in Table 1 overleaf.

Table 1. Netazepide batches

Patients	Netazepide capsules	
	Batch	Expiry
UK	NT2682	17 Nov 2015
	061/15	31 May 2017
USA	NT0222	12 Apr 2014
	061/15	31 May 2017

Trial design and methods

This was a two-centre, double-blind, phase 2, outpatient trial in 20 patients with BE randomised (1:1 ratio) to netazepide 25 mg or matching placebo. There were 5 or 6 visits. Baseline assessments, which were done at Visit 1 ± Visit 2, were: informed consent; medical history/examination; safety tests of blood and urine; measurement of fasting serum gastrin and plasma CgA; and endoscopy for biopsies of BE cells, to test for Ki67 and other biomarkers. Ki67 density was assessed as the number of Ki67+ cells per mm^2 of BE. Visit 3 (at 4 weeks), Visit 4 (at 8 weeks), and Visit 5 (at 12 weeks) were for measurement of fasting serum gastrin and plasma CgA, and for trough and peak (at 1 h) serum netazepide concentrations. Visit 5 (at 12 weeks) was also for endoscopy for biopsies of BE cells to assay Ki67 and biomarkers associated with esophageal cancer, in particular cyclooxygease-2 (COX-2), p53, CCK_2R and DCAMKL1. Patients were given a container of their treatment at baseline, to start the next day, and at Weeks 4 and 8, to continue treatment. Treatment compliance was assessed by capsule counts at visits. Visit 6 (at 16 weeks, 4 weeks after end of treatment) was a follow-up visit for safety tests of blood and urine, ECG, serum gastrin and plasma CgA concentrations, and adverse events.

After 6 patients (3 per treatment group) had their Week 4 and 8 visits, the protocol was amended so that those visits were replaced by single visit at Week 6 in order to reduce the total number of visits.

Inclusion criteria

Aged ≥ 18 years; histologically confirmed diagnosis of BE; have taken a PPI at least once daily for the past 12 months; stable dose of PPI for the past 3 months; and otherwise in good health.

Sample size and statistics

Sample size was based on previous studies of Ki67. Twenty BE patients (10 per group) were judged enough to detect a 25% difference in Ki67 between treatments. The data were analysed by Columbia University.

The primary outcome measure was change in Ki67 expression (ΔKi67) between baseline and Week 12 visit. The secondary outcome measures were changes between baseline and Week 12 visit in biomarkers associated with EC, and serum gastrin and plasma CgA concentrations. Changes in gene expression were assessed by RNA sequencing. The treatments were compared by t-tests.

Results

Twenty-four patients were randomised and 20 completed the study; 10 received netazepide and 10 received placebo. None of the 4 withdrawals was deemed related to treatment. Treatment compliance was 97%, which is high.

Netazepide concentrations

The protocol was amended so that Weeks 4 and 8 visits were replaced by one visit at Week 6. Some of the required blood samples were not taken (Table 2). Most pre-dose samples were below the limit of quantification (1 ng/mL) for netazepide, and concentrations at 1 h after dosing were very variable. Peak concentrations in previous studies was about 1.5 h.

Table 2. Netazepide plasma concentrations (ng/mL) at 1 h after dosing

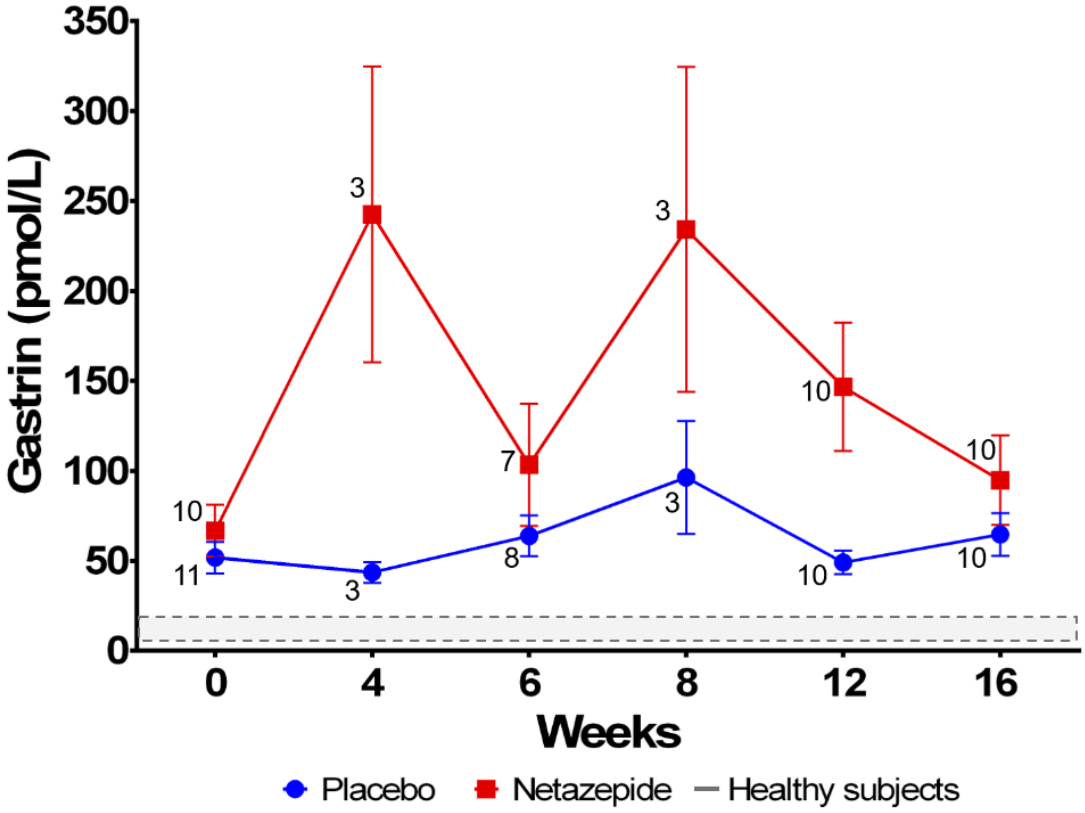
Visit	n	Mean	SD	Range
Week 4	3	38.9	40.1	14.3-85.1
Week 6	6	99.1	63.6	3.3-172.8
Week 8	3	102.6	55.1	58.2-164.2
Week 12	8	72.1	72.2	3.0-224.2

Fasting gastrin concentrations

There was no difference between treatments with respect to fasting gastrin at baseline (netazepide 66.7 ± SEM 14.4 pmol/L; placebo 51.8 ± SEM 8.8 pmol/L; p=0.58) and fasting CgA (netazepide 14.5 ± SEM 6.1 nmol/L; placebo 9.1 ± SEM 1.5 nmol/L; p=0.46). Mean fasting gastrin was higher than in healthy subjects (mean 12.3 ± SD 6.4 pmol/L), which is consistent with PPI-induced hypergastrinaemia (Figure 1). Furthermore, mean fasting gastrin was significantly higher in those on twice daily PPI (twice daily: mean 75.4 ± SEM 14.3 pmol/L; once daily: mean 44.0 ± SEM 6.6 pmol/L; p=0.03). But there was no difference between the two groups (p=0.58).

Figure 1. Mean (±SEM) fasting serum gastrin concentrations (pmol/L)

The number of patients sampled at each time point are shown. The reason that there are only 3 patients at Weeks 4 and 8 is that the protocol was amended to replace those visits by one visit at Week 6.
The dotted lines are the range of fasting gastrin concentrations in healthy subjects calculated from previous Trio studies.

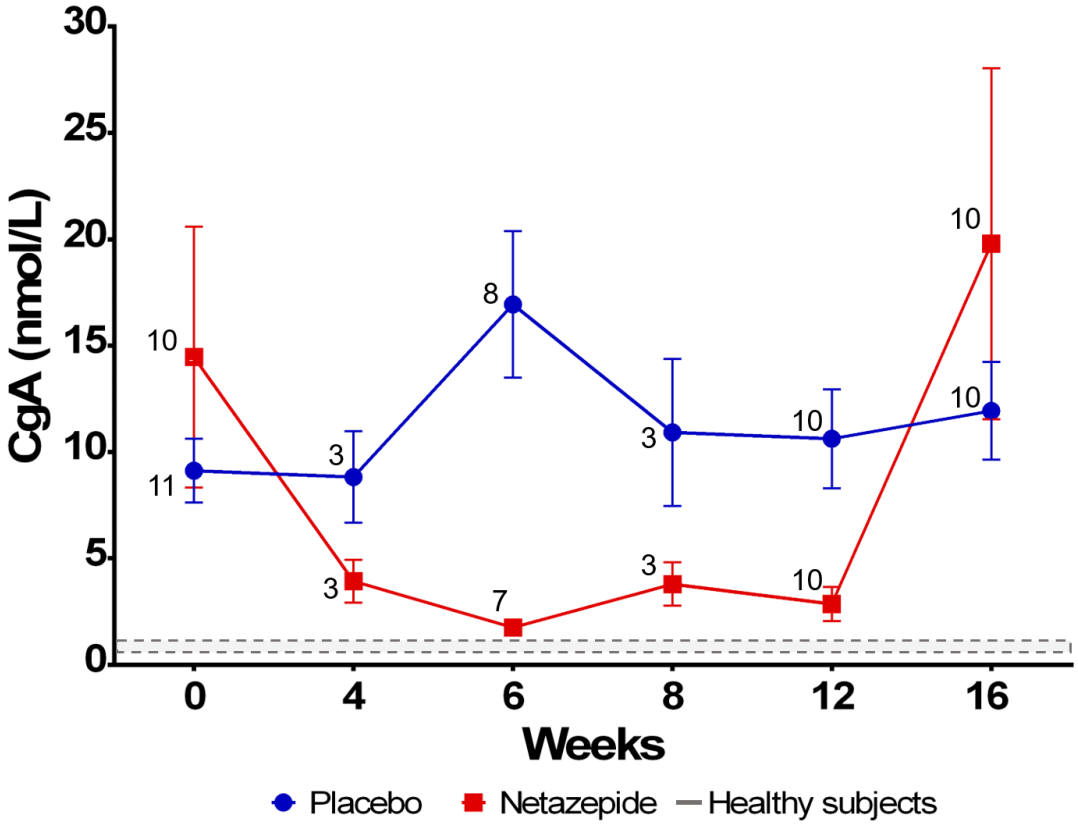


Fasting chromogranin A concentrations

There was no difference between treatments with respect to fasting CgA at baseline (netazepide 14.5 ± SEM 6.1 nmol/L; placebo 9.1 ± SEM 1.5 nmol/L; p=0.46). As expected, mean values were significantly higher compared to those of healthy subjects (0.9 ± SEM 0.3 nmol/L) (Figure 2). In addition, like baseline gastrin, baseline CgA was significantly higher in twice daily PPI users (twice daily: mean 17.3 ± SEM 5.9 nmol/L; once daily: mean 6.5 ± SEM 0.9 nmol/L; p=0.049).

Figure 2. Mean (±SEM) fasting plasma CgA concentrations (nmol/L)

The number of patients sampled at each time point are shown. The reason that there are only 3 patients at Weeks 4 and 8 is that the protocol was amended to replace those visits by one visit at Week 6.
The dotted lines are the range of fasting CgA concentrations in healthy subjects calculated from previous Trio studies.

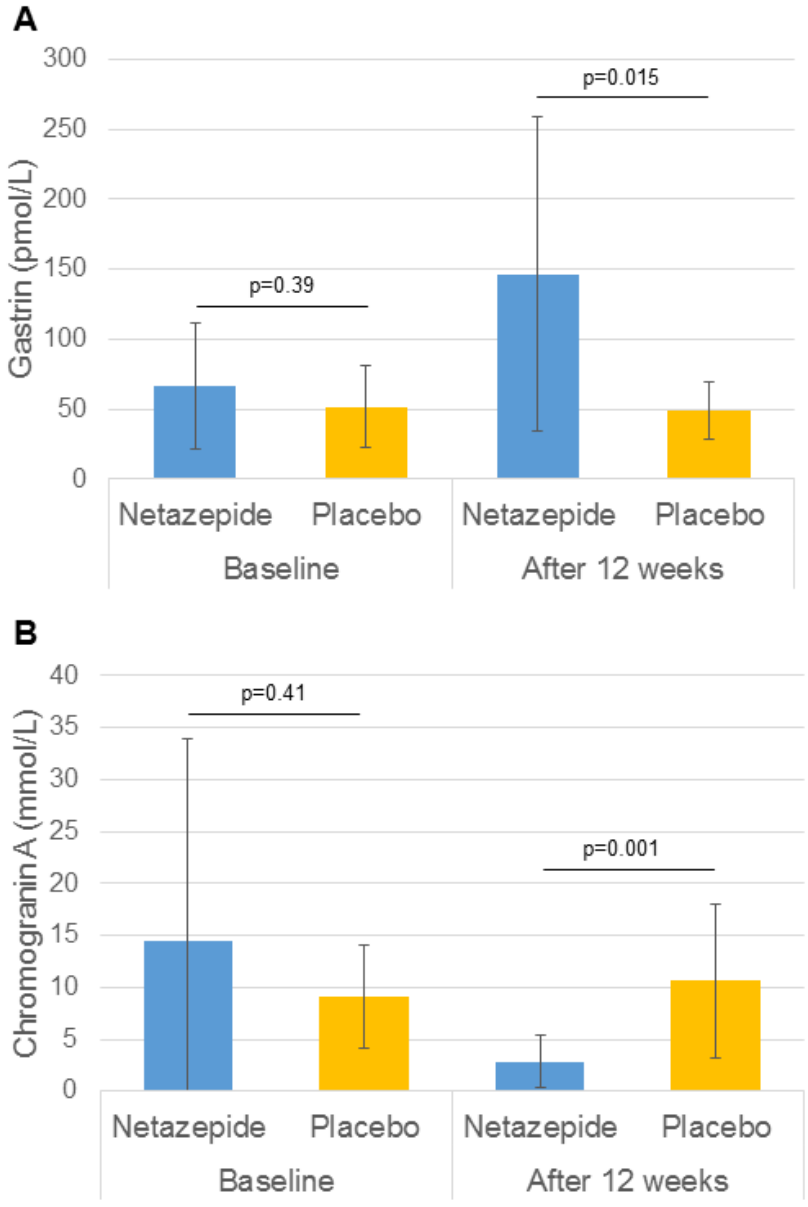


Effect of netazepide on fasting gastrin and CgA

At 12 weeks of treatment, patients on netazepide had significantly increased serum gastrin (p=0.015) and decreased plasma CgA (p=0.001) compared to those on placebo (Figure 3). The mean differences from baseline for the netazepide and placebo groups for serum gastrin was 80 and –6 pmol/L, respectively, and for plasma CgA was 11.6 and 1.4 nmol/L, respectively.

Figure 3. A. Mean (± SD) serum gastrin and B. Mean plasma CgA (± SD)

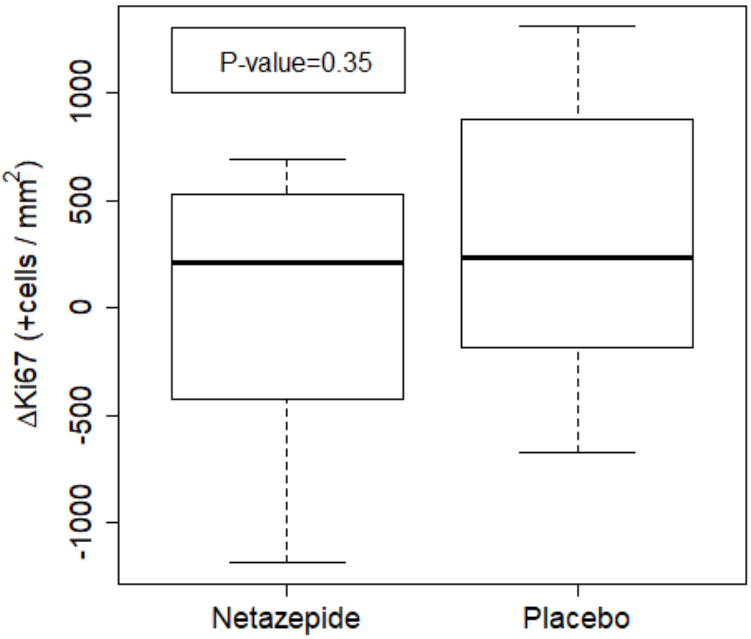
After 12 weeks, netazepide increased gastrin (p=0.015) and reduced CgA (p=0.001) significantly compared to placebo (Abrams *et al* 2020).



Effect of netazepide on Ki67 density

There was no significant difference in baseline Ki67 density between netazepide and placebo (1539 cells/ mm2, SD 514 vs. 1556 cells/mm^2, SD 622, respectively; p=0.95). But there was a correlation between baseline serum gastrin and change in cell proliferation, ΔKi67, which was not significant (r= 0.55, p=0.099). There was a difference between treatments in the primary outcome measure, ΔKi67 (netazepide: +35.6 cells/mm^2, SD 620.7; placebo: +307.8 cells/mm^2, SD 640.3; p=0.35), but it was not significant (Figure 5).

Figure 5. Box whisker plots of ΔKi67 expression in BE epithelium (+cells/mm^2) after 12 weeks' treatment with netazepide compared with placebo



Effect of netazepide on gene expression

There was no significant difference in COX-2, p53, CCK_2R and DCAMKL1 expression between netazepide and placebo groups. However, in global gene expression analyses, 62 genes were differentially expressed in the netazepide group compared with those in the placebo group. In particular, netazepide increased the expression of genes related to gastric phenotypes (TFF2, MUC5B) and certain cancer-associated markers (REG, PAX9, MUC1) and decreased the expression of intestinal markers (MUC2, FABP1, FABP2, CDX1). In pathway analyses, netazepide was associated with upregulation of IL-1, NOD1/2, interferon alpha/beta, and interferon gamma signalling pathways, and downregulation of pathways associated with DNA replication and repair.

Safety and tolerability

There were no clinically significant changes in vital signs, ECG, and safety tests of blood and urine. Table 3 is a summary of the total number of treatment-emergent adverse events (TEAEs) and the number deemed to be possibly related to netazepide. More TEAEs were attributed to netazepide than placebo. However, all TEAEs were mild-to-moderate in severity, and overall netazepide was safe and well tolerated.

Table 3. Treatment-emergent adverse events

	Placebo N=11	Netazepide N=13
Total number of TEAEs	21	25
Number deemed possibly related to netazepide	2	9

Discussion

This was one of the earliest studies of netazepide in patients and really the first in the USA. At that time, 12 weeks was the longest period of netazepide treatment covered by the toxicology studies. It was a demanding study for patients. There were several outpatient visits – largely to monitor safety because of the paucity of safety data from repeated doses of netazepide in patients at the time – two upper endoscopies, and an equal chance of being allocated to placebo. Understandably, patient recruitment was slow. Therefore, visits at Weeks 4 and 8 were combined into a single visit at Week 6, and later a second site in the UK was added, which contributed 9 of the 20 patients who completed the study.

As expected, fasting serum gastrin at baseline on PPI alone was several-fold higher than normal. Thus, all patients had hypergastrinaemia. Furthermore, fasting gastrin was increased in patients on twice-daily PPI compared with those on once-daily PPI. Fasting plasma CgA at baseline was also several-fold higher than normal, as in previous PPI studies (Sanduleanu *et al* 2001; Boyce *et al* 2015). Also, like gastrin, fasting CgA was increased in patients on twice-daily PPI compared with those on once-daily PPI.

Also as expected, at 12 weeks after addition of netazepide to the PPI there had been a further increase in serum gastrin, and plasma CgA was low. Those findings are consistent with the combination causing greater suppression of gastric acid than the PPI alone, and with netazepide blocking the trophic effect of PPI-induced hypergastrinaemia on ECL cells, as in previous studies (Boyce *et al* 2015).

Thus, netazepide 25 mg was a pharmacologically active dose in this study, similar to previous studies. However, although compared with placebo ΔKi67 density of BE biopsies was less after addition of netazepide to the PPI for 12 weeks, the difference was not significant. Also, there was no difference between treatments for COX-2, p53, CCK_2R and DCAMKL1. The lack of effect of netazepide on COX-2 is in contrast to the inhibition by the gastrin/CCK_2 receptor antagonist YM022 in gastrin-induced up-regulation of COX-2 in BE cells *in vitro*

(Abdalla *et al* 2004) and of the beneficial effect of aspirin, a COX-2 inhibitor, in BE patients in the AspECT trial (Jankowski *et al* 2018). Many genes were differentially expressed in the netazepide group compared with those in the placebo group. Netazepide was associated with increased expression of some genes and decreased expression of others. Likewise, netazepide was associated with upregulation of some signalling pathways and downregulation of others. It is difficult to know what overall effect those various changes might have had on BE cells. Trefoil factor family (TFF) peptides and secretory mucins (MUC) are thought to have a beneficial effect on BE cells, so the increases in TFF2 and MUC5B associated with netazepide treatment may be the most relevant (Fabisiak *et al* 2019; Paterson *et al* 2020).

Conclusions

Overall, the results of this exploratory study do not exclude a treatment difference. The number of patients was small and netazepide treatment was of short duration compared with the many weeks required to eradicate gastric neuroendocrine tumours in patients with hypergastrinaemia secondary to chronic atrophic gastritis (Boyce *et al* 2017).

A longer period of netazepide treatment, a larger number of patients with BE, a less demanding protocol, and use of the simpler Cytosponge device for collecting samples of BE cells (Januszewicz *et al* 2019) rather than upper endoscopy, which patients dislike, may yield a different result.

References

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