Potential clinical indications for a CCK_2 receptor antagonist

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ABSTRACT

Gastrin controls gastric acid secretion and mucosal cell growth, especially of enterochromaffin-like cells, via gastrin/cholecystokinin-2 receptor (CCK_2R) binding and downstream signalling. Studies in animal models, healthy subjects and patients with gastric neuroendocrine tumours provide compelling evidence to justify developing a CCK_2R antagonist (CCK_2RA) for preventing or treating the trophic effects of hypergastrinaemia or conditions expressing CCK_2R, and with or without a proton pump inhibitor, for treating gastric acid-related conditions. Many compounds have been studied, but most have had problems with potency, selectivity for CCK_2 versus CCK_1 receptor, solubility or oral bioavailability. None has yet been marketed. Netazepide and Z-360 are currently undergoing clinical development, for treatment of gastric neuroendocrine tumours and pancreatic cancer, respectively. There are several other potential indications for a CCK_2RA and an unmet need.

INTRODUCTION

Gastrin is a peptide hormone produced by a single gene and synthesised from preprogastrin, which is processed into progastrin and amidated gastrin peptide fragments, mostly G17 and G34 [1]. All gastrins have a C-terminal amidated tetrapeptide that acts at G protein-coupled CCK_2**R** in the stomach and central and peripheral nervous systems. The C-terminal tetrapeptide is identical to that of another peptide hormone, cholecystokinin, which acts predominantly at CCK_1 receptors in the pancreas, gallbladder and central and peripheral nervous systems.

Food releases gastrin from G cells in the gastric antrum. Gastrin binds to CCK_2R on enterochromaffin-like (ECL) cells in the oxyntic mucosa causing them to synthesise and secrete histamine, which in turn stimulates adjacent parietal cells to secrete acid. Acid secretion is mediated by the proton pump on parietal cells via histamine H_2- and muscarinic M_3-receptors (Figure 1). Gastrin also induces proliferation, migration, differentiation and anti-apoptosis of gastric epithelial cells; regulates various genes in the gastric mucosa; and stimulates paracrine cascades, including cytokines and growth factors [2].

In this article, we describe the potential therapeutic uses of a CCK_2RA. We cite evidence from animal models, healthy subjects, and patients.

TYPES OF CCK_2RA

The discovery that asperlicin, a benzodiazepine-related natural product, had weak affinity for CCK_1 receptors led to invention of various benzodiazepine-derived CCK_2RA, such as L-36,718 (devazepide), L-365,260, CI-988, YM022, Z-360 and YF476 (netazepide), and to other classes of CCK_2RA [3]. Two new classes have recently been invented: JB95008 is a substituted imidazole [4] and JNJ-26070109 is a benzamide derivative [5]. Most CCK_2RA have had problems with potency, selectivity for the CCK_2 versus CCK_1 receptor, solubility or oral bioavailability. No CCK_2RA has yet been developed into a medicine.

CCK_2RA STUDIES IN ANIMAL MODELS

CCK_2RA have been tested in many types of animal model. JNJ-26070109 [5] and netazepide [6] caused dose-dependent inhibition of pentagastrin-stimulated acid secretion in rats and dogs. Netazepide prevented the increases in ECL-cell activity and density, oxyntic mucosal thickness, mucosal histamine decarboxylase (HDC) activity and serum pancreastatin caused by proton pump inhibitor (PPI) induced hypergastrinaemia in rats [7]. Pancreastatin is derived from chromogranin A (CgA), an ECL-cell biomarker. Netazepide not only prevented formation of ECL-cell derived gastric carcinoids accelerated by hypergastrinaemia induced by loxtidine, an insurmountable histamine H_2-receptor antagonist (H_2RA), in Mastomys rodents, but also caused regression of formed lesions [8]. Netazepide and loxtidine had synergistic inhibitory effects on gastric atrophy and cancer in *H. felis* infected transgenic hypergastrinaemic INS-GAS mice, whereas a PPI had no effect [9]. Netazepide also prevented: the inflammatory response of the gastric mucosa to *H. pylori* infection in Mongolian gerbils [10**]; hypergastrinaemia-induced lesions in mice similar to those observed in patients with Barrett's oesophagus [11,12]; and NSAID-induced gastric ulcers in rats as effectively as omeprazole [13]. YM022 [14] and JNJ-26070109 [5] prevented rebound hyper-responsiveness of acid secretion to pentagastrin after stopping omeprazole in rats. Co-administration of a CCK_2RA with an opioid improved anti-nociception and opioid tolerance in animal pain models [15].

CCK_2RA STUDIES IN HEALTHY SUBJECTS

There have been few studies of the clinical pharmacology of CCK_2RA in healthy subjects. L-365,260 produced modest and short lasting inhibition of gastrin-stimulated acid secretion [16]. CI-988 had some effect on CCK4-induced panic symptoms [17]. Single oral doses of netazepide caused dose-dependent increases in basal and food-stimulated gastric pH for more than 24h [18], and dose-dependent inhibition of pentagastrin-induced gastric acid secretion

[19]. Repeated oral doses of netazepide caused persistent inhibition of pentagastrin-induced acid secretion [19,20**], and prevented the increase in plasma CgA resulting from rabeprazole-induced hypergastrinaemia [20**]. Netazepide suppressed acid production as effectively as rabeprazole, and the combination suppressed it more than either treatment alone [20**]. A spray-dried formulation of netazepide had linear pharmacokinetics and oral bioavailability of ~15% [18]. By comparison, bioavailability in rat and dog was 25–50% [6].

POTENTIAL CLINICAL INDICATIONS FOR A CCK_2RA

Thus, animal models and healthy volunteer studies provide compelling evidence for developing a CCK_2RA for clinical use. Potential indications are: hypergastrinaemia; acid-related conditions; *H. pylori* infection; and conditions where CCK_2R are overexpressed (Figure 2). However, to date there have been very few studies in patients.

HYPERGASTRINAEMIA-ASSOCIATED CONDITIONS

There are several causes of hypergastrinaemia, with possible consequences that may be treatable by a CCK_2RA.

1. Chronic atrophic gastritis (CAG) and Zollinger-Ellison syndrome (ZES)

CAG- and ZES hypergastrinaemia can lead to ECL-cell hyperplasia and in some cases gastric neuroendocrine tumours (NETs), called types 1 and 2, respectively [21]. Gastric NETs are rare [22], but have the potential to metastasise [21].

Netazepide once daily for 12 weeks reduced the number of tumours and size of the largest one and normalised circulating CgA in 16 patients with CAG, achlorhydria, hypergastrinaemia and multiple gastric NETs, [23,24**]. After 12weeks off treatment, the number of tumours and size of the largest one remained unchanged but CgA increased again. Netazepide reduced mRNA abundances of CgA and HDC [24**] and reduced miR-222, which was over-expressed, in mucosal biopsies [25**]. miR-222 targets the tumour suppressor and oncogene $p27^{kip1}$ (Figure 3). Serum gastrin did not increase further on netazepide, confirming patients had achlorhydria. Netazepide was safe and well tolerated. Guidelines recommend regular endoscopic surveillance and mapping and biopsy of type 1 and 2 NETs, and if there are concerns, polypectomy to remove the tumours or antrectomy/gastrinoma resection to remove the source of hypergastrinaemia [21]. However, tumours often regrow after polypectomy, and antrectomy carries the risk of morbidity and mortality and isn't always successful. A CCK_2RA is a potential alternative treatment for type 1 NETs, which are gastrin driven and comprise 80% of gastric NETs. Treatment should

probably be continuous otherwise tumours may regrow, even if eradicated. Progress can be monitored by CgA in blood or biomarkers in biopsies. Patients with pernicious anaemia (PA) also have a seven-fold increased risk of developing gastric adenocarcinoma [26]. A CCK_2RA might also help prevent gastric NETs and adenocarcinoma in PA patients. The acid suppressant and anti-trophic effects of a CCK_2RA also make it an ideal potential treatment for ZES patients with type 2 NETs. No trial has been reported.

2. PPI-induced hypergastrinaemia

Although PPIs are safe medicines, PPI-induced hypergastrinaemia has been reported to cause: ECL- and parietal-cell hyperplasia; fundic gland polyps; increased risk of bone fractures; rebound hyperacidity and dyspepsia after PPI withdrawal; and malignant ECL-cell tumours in case reports. A CCK_2RA should be free of those unwanted effects, and should prevent them if co-administered with a PPI.

(a). ECL-cell hyperplasia

In 1,920 patients from 16 studies of patients on long-term PPI therapy, mean gastrin concentrations increased by one to three times upper limit of normal, and ECL-cell hyperplasia increased by 8–52% [27]. There was no evidence of neoplasia in gastric biopsies, and no patient had gastric NETs or adenocarcinoma. *H. pylori* positive patients had a much higher risk of ECL-cell hyperplasia and corpus atrophy. There have been isolated case reports of gastric NETs [28] and gastric cancer [29] in patients with long-term PPI-induced hypergastrinaemia. A CCK_2RA does not cause ECL-cell hyperplasia and prevents PPI-induced ECL-cell hyperplasia in healthy subjects [20**].

(b). Fundic gland polyps

Long-term PPI therapy is associated with increased risk of fundic gland polyps [30*], so they merit a trial of co-administered CCK_2RA.

(c). Bone fractures

Long-term PPI therapy also increases the risk of bone fractures [31]. An effect of PPI-induced hypergastrinaemia on the vacuolar type H^+-ATPase on osteoclasts that mediates acidification of intracellular organelles is a possible cause. Bone fracture risk is also increased in PA patients [32], and osteoporosis risk is increased in CAG patients [33], which excludes a direct effect of PPI on bone, because PA and CAG patients both have hypoacidity and secondary hypergastrinaemia.

H^+/K^+-ATPase β-subunit knock-out mice with hypergastrinaemia developed bone loss and deterioration of bone quality, which was partly prevented by netazepide [34]. Netazepide

caused hypoacidity and hypergastrinaemia in control animals, as expected, but no bone changes, suggesting hypergastrinaemia rather than hypoacidity is the mechanism. Thus, CCK_2RA co-administration might reduce the risk of fracture in PPI-treated patients.

(d). Rebound hyperacidity

Rebound hyperacidity and dyspepsia have been reported after PPI withdrawal [35]. Netazepide did not cause rebound hyperacidity [20**] and would prevent it should it occur after PPI withdrawal.

3. Mutations in KCNQ1, KCN1E and ATP4A genes

Patients with the rare Jervell and Lange-Nielsen syndrome have hypergastrinaemia secondary to hypochlorhydria caused by mutations *of KCNQ1* or *KCNE*1 genes, which are essential for acid secretion. Some patients develop ECL-cell tumours and gastric cancer [36*]. Patients with *ATP4A* gene mutation, which encodes the α-subunit of the proton pump, also have hypergastrinaemia and develop gastric NETs and adenocarcinoma [37*]. Such patients merit a CCK_2RA trial.

4. Cysteamine therapy for cystinosis

Cysteamine slows progression of cystinosis, by removing cystine from cells, but it increases serum gastrin and gastric acid, and is ulcerogenic. Some patients require PPI therapy [38]. A CCK_2RA, with or without a PPI, is a potential therapy.

ACID-RELATED CONDITIONS

CCK_2RA should be free of the unwanted effects of PPI-induced hypergastrinaemia, and if co-administered with a PPI should prevent those unwanted effects, and improve acid control [20**].

H. PYLORI INFECTION

H. pylori is a risk factor for peptic ulcer disease and gastric cancer. Netazepide completely prevented the inflammatory response of gastric mucosa to *H. pylori* in Mongolian gerbils [10**]. Therefore, a CCK_2RA with or without a PPI could be a useful addition to antibiotic regimens for *H. pylori* eradication. However, this has yet to be tested in patients.

CONDITIONS ASSOCIATED WITH CCK_2R EXPRESSION

CCK_2 receptors are expressed on cells of some patients with pancreatic cancer [39], Barrett's oesophagus [40], gastrointestinal stromal tumours [41], gastric adenocarcinoma [42], and other cancers. Therefore, a CCK_2RA may have therapeutic benefit.

1. Pancreatic adenocarcinoma

JB95008 prolonged life in a small placebo-controlled trial, but a second trial showed no difference between JB95008 and 5-fluorouracil. Development was stopped because JB95008 required intravenous infusion due to poor oral bioavailability [4]. In a placebo-controlled trial of gemcitabine with or without Z-360, there were no differences between treatments [43].

2. Barrett's oesophagus

PPI-induced hypergastrinaemia is associated with advanced oesophageal adenocarcinoma in patients with Barrett's oesophagus [44]. CCK_2RA co-administration should increase acid suppression and inhibit any trophic effect of PPI-induced hypergastrinaemia. A CCK_2RA alone might be adequate. A trial of the effect of netazepide on biomarkers in Barrett's patients is in progress [Abrams J, *https://clinicaltrials.gov*, NCT01298999].

3. Gastrointestinal stromal tumour (GIST)

GISTs are removed surgically if localised or treated with a tyrosine kinase inhibitor if metastatic, but they can 'escape'. Gastrin increases tumour volume and cell proliferation and stimulates kinase pathways in GIST xenografts, which express CCK_2 receptors [41]. A trial of a CCK_2RA is therefore justified.

4. Gastric adenocarcinoma

Many patients have raised serum gastrin. Alpha-amidated gastrins and their receptors are detectable in 80% [42]. It is not clear whether long-term PPI therapy increases the risk of gastric adenocarcinoma [30]. Patients usually present late, so a potential therapeutic effect of a CCK_2RA seems unlikely.

CENTRAL AND PERIPHERAL NERVOUS SYSTEM DISORDERS

L-365,260 and CI-988 lacked effect in patients with panic disorder [45]. L-365,260 did not augment the analgesic effect of morphine in neuropathic pain [15], but Z-360 may have a role in pancreatic cancer pain [43,46].

CONCLUSIONS AND FUTURE WORK

The hormone gastrin regulates several essential biological processes, including gastric acid secretion, mucosal remodelling and proliferation via CCK_2 receptors. Hypergastrinaemia dysregulates those processes and contributes to development of various diseases. CCK_2RA inhibit CCK_2R receptor binding and have the potential to reduce pathological downstream signalling, particularly of ECL cells.

More is known about the role of gastrin in health and disease [1,2] since the last review in this journal [47], and there is further evidence of the malignant potential of hypergastrinaemia [36*,37*]. miR-222, which targets the tumour suppressor and oncogene $p27^{kip1}$, is a potential biomarker for monitoring gastrin-induced premalignant changes in the stomach. There is increasing evidence of involvement of CCK_2R in progression of Barrett's oesophagus [11,12,40,44].

Only netazepide and Z-360 are currently in clinical development in patients, for treatment of type 1 gastric NETs [23,24**] and pancreatic cancer [43,46], respectively. Ceclazepide (TR2-A) is a new benzodiazepine-derived CCK_2RA and prodrug that has advantages over netazepide in terms of selectivity, solubility and bioavailability, and is in pre-clinical development [48].

CONFLICTS OF INTEREST

MB owns HMR and the netazepide and ceclazepide patents. DMP has received HMR funding to study CCK_2RA.

HIGHLIGHTS

- Gastrin controls gastric acid secretion and mucosal cell growth via CCK_2R.
- CCK_2RA can prevent/treat consequences of hypergastrinaemia or acid-related diseases.
- CCK_2RA can eradicate type 1 NETs and reduce miR-222, which targets $p27^{kip1}$.
- miR-222 is a potential biomarker for gastrin-induced gastric premalignant changes.
- There are many other potential clinical indications for a CCK_2RA, and an unmet need.

FIGURE LEGENDS

Figure 1. Control of gastric acid secretion

Figure 2. The main causes of hypergastrinaemia, conditions associated with increased CCK_2R expression, and status of CCK_2RA tested in patients.

1. Netazepide (YF476): Reduced number and size of the largest tumour and normalised CgA in patients with type-1 gastric NETs [23,24]. Still in development.
2. Z-360: No evidence of efficacy when added to gemcitabine, apart from apparent reduction in pain, in patients with pancreatic cancer [43]. Still in development.
3. JB95008: Intravenous infusion prolonged life significantly in patients with pancreatic cancer, but no difference when compared with 5-FU [4]. Development stopped because of poor oral bioavailability.
4. L-365,320: No effect in patients with panic disorder [45]. No augmentation of analgesic effect of morphine in patients with neuropathic pain [15]. Caused arrhythmias; development stopped [3]
5. Cl-988: No effect in patients with panic disorder [45]. Development stopped [3].

Figure 3. A schematic diagram of the signalling pathways that are activated by CCK_2R binding. miR-222 transcription is increased via the PKC and PI3K pathways and partially via the MAPK pathway. The pri(mary)-miR-222 transcript is cleaved by the RNAse II enzyme Drosha into a hairpin structure (pre-miR-222) which is transported from the nucleus to the cytoplasm via exportin-5. Pre-miR-222 is cleaved by a second RNase II enzyme, Dicer, into mature miR-222 which associates with RISC to target imperfect complementary mRNA sequences. Mature miR-222 inhibits the translation of $p27^{kip1}$ which increases cell migration and epithelial mesenchymal transition in AGS_{GR} cells.
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Figure 1

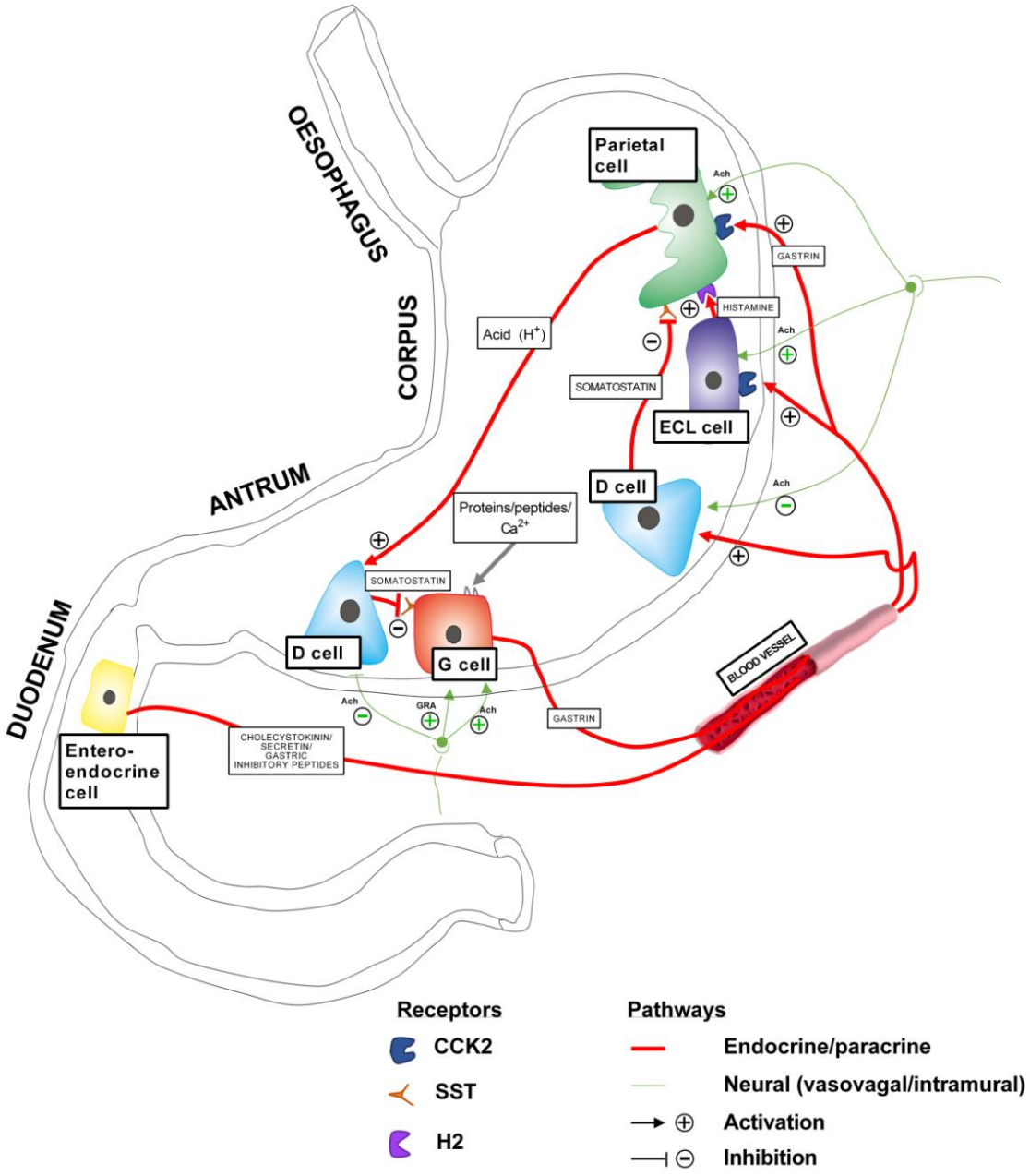
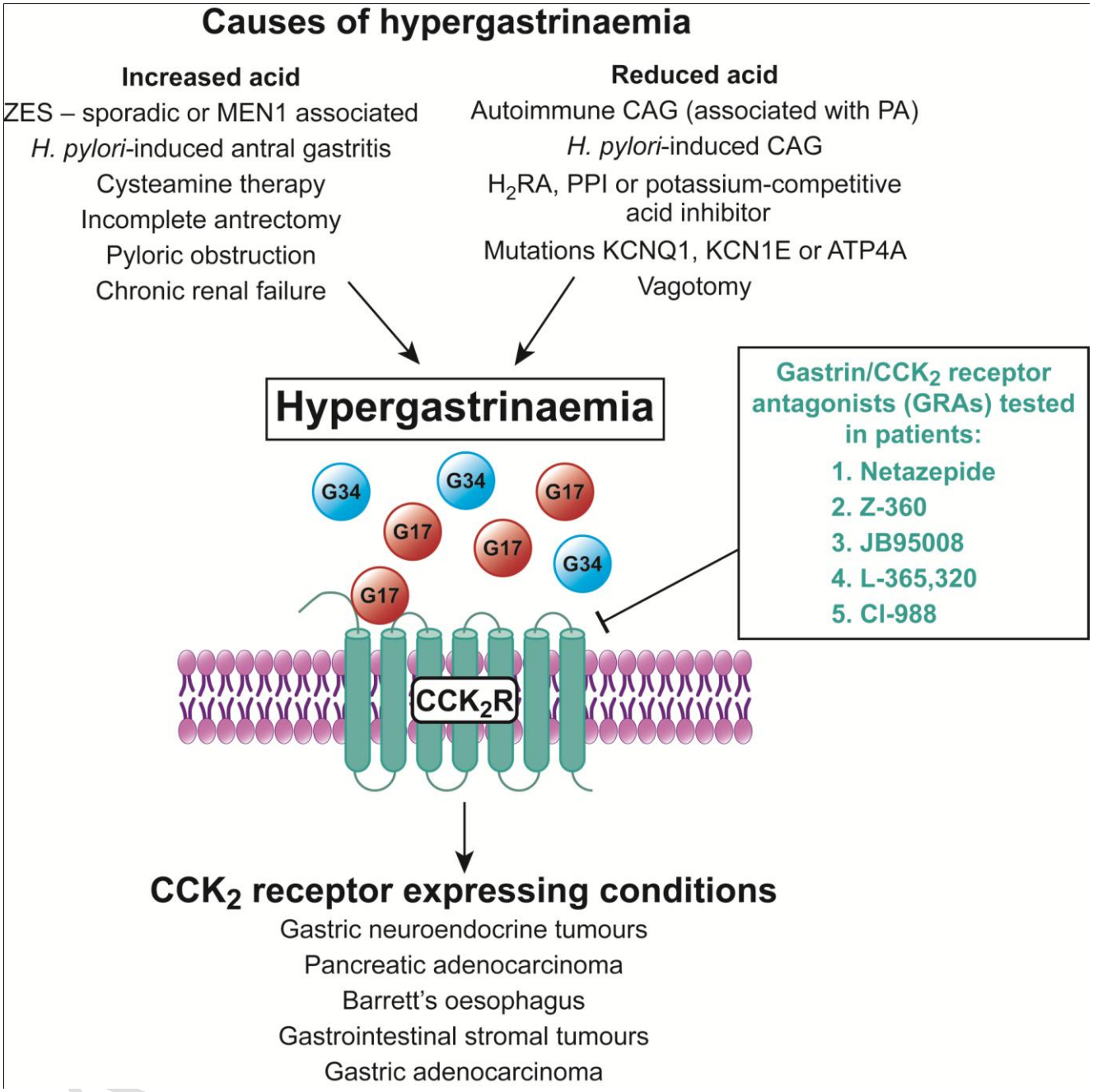
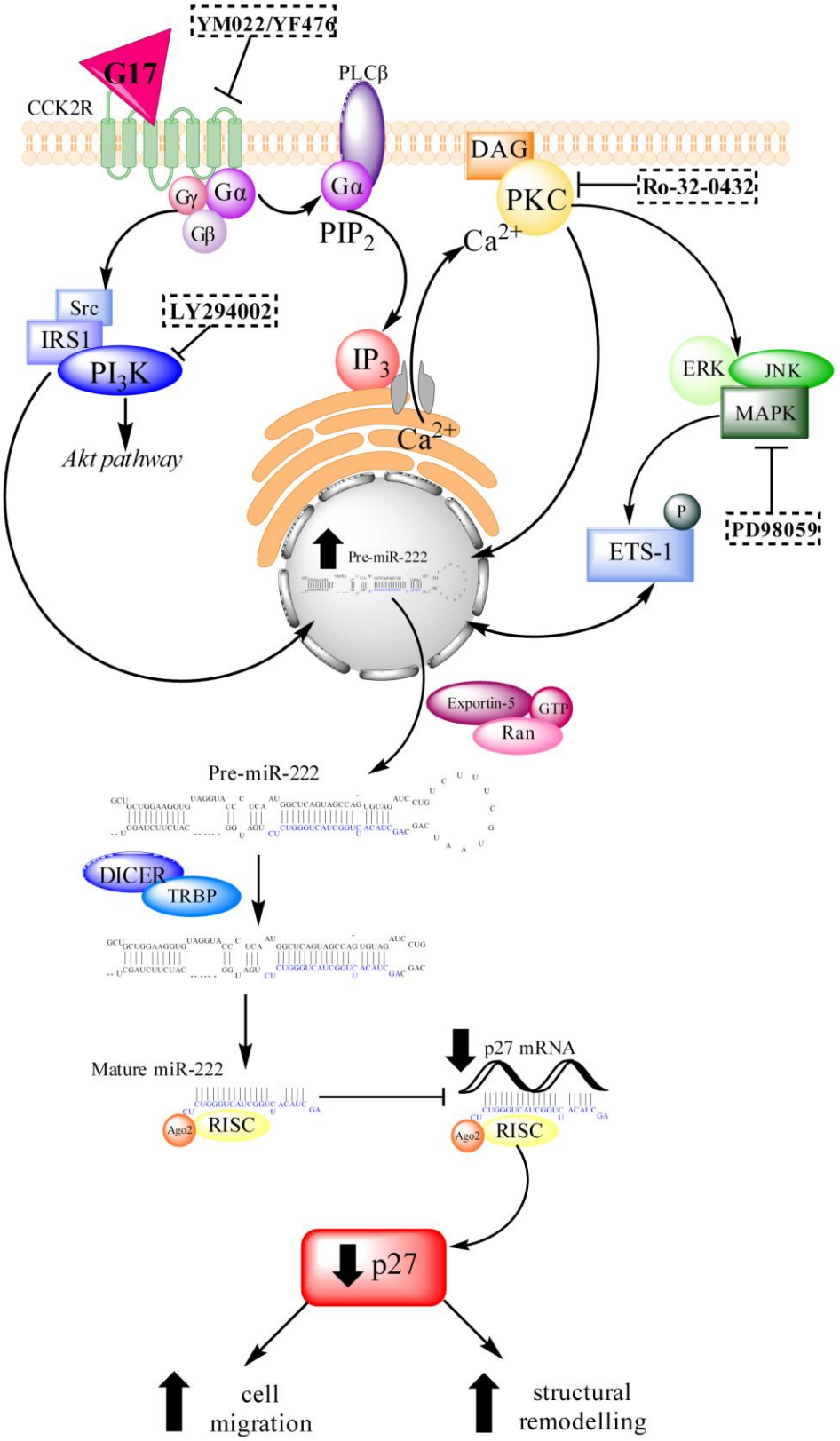

OESOPHAGUS
CORPUS
ANTRUM
DUODENUM
Parietal cell
Ach
GASTRIN
HISTAMINE
Acid (H+)
SOMATOSTATIN
ECL cell
D cell
Proteins/peptides/ Ca2+
SOMATOSTATIN
D cell
G cell
BLOOD VESSEL
GRA
GASTRIN
Entero-endocrine cell
CHOLECYSTOKININ/ SECRETIN/ GASTRIC INHIBITORY PEPTIDES
Receptors
CCK2
SST
H2
Pathways
Endocrine/paracrine
Neural (vasovagal/intramural)
Activation
Inhibition

Figure 2



1. **Netazepide (YF476):** Reduced number and size of the largest tumour and normalised CgA in patients with type-1 gastric NETs [23,24]. Still in development.
2. **Z-360:** No evidence of efficacy when added to gemcitabine, apart from apparent reduction in pain, in patients with pancreatic cancer [43]. Still in development.
3. **JB95008:** Intravenous infusion prolonged life significantly in patients with pancreatic cancer, but no difference when compared with 5-FU [4]. Development stopped because of poor oral bioavailability.
4. **L-365,320:** No effect in patients with panic disorder [45], no augmentation of analgesic effect of morphine in patients with neuropathic pain [15]. Caused arrhythmias; development stopped [3].
5. **CI-988:** No effect in patients with panic disorder [45]. Development stopped [3].

Figure 3


YM022/YF476
G17
CCK2R
PLCβ
Gγ
Gα
Gβ
Gα
PIP2
DAG
PKC
Ro-32-0432
Ca2+
Src
IRS1
PI3K
LY294002
Akt pathway
IP3
Ca2+
ERK
JNK
MAPK
PD98059
P
ETS-1
Pre-miR-222
Exportin-5
GTP
Ran
Pre-miR-222
DICER
TRBP
Mature miR-222
Ago2
RISC
p27 mRNA
Ago2
RISC
p27
cell migration
structural remodelling