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Trial protocol

Confidential

Trial title	Randomized, placebo-controlled trial of YF476, a gastrin receptor antagonist, in Barrett's esophagus
Short title	YF476 and Barrett's esophagus
Version and date of protocol	Version 23, 13 May 2016
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Phase of trial	Phase II
Place of trial	Columbia University Division of Digestive & Liver Diseases 622 W 168th Street PH 7W-318 New York, NY 10032 USA National Institute for Health Research Clinical Investigation Ward Cambridge University Hospitals NHS Foundation Trust Cambridge Biomedical Campus

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Planned dates of trial	February 2012 – December 2016

1.1 Background

Barrett's Esophagus (BE) is the precursor lesion for the development of esophageal adenocarcinoma (EAC). BE is defined as the presence of intestinal metaplasia in the distal esophagus, and is associated with a 25-30 times increased risk of EAC.[1] Unfortunately, the prognosis of esophageal cancer is poor, with 5-year survival rates of 16.5% in the US.[2]

The development of BE is thought to be caused in part by acid reflux. The change in esophageal lining presumably develops as a result of chronic exposure to refluxed stomach acid and bile, leading to recurrent mucosal injury.[3]

Current management of BE patients consists of endoscopic surveillance and acid suppression.[4] In theory, gastric acid suppression reduces chronic inflammation within the BE segment, which in turn may reduce the risk of progression to EAC. By inhibiting acid secretion and increasing intragastric pH, proton pump inhibitors (PPIs) at therapeutic doses can cause secondary hypergastrinemia. Gastrin exerts trophic effects in BE via cholecystokinin-2 receptor (CCK2R) activation[5], and there is some evidence that hypergastrinemia is associated with an increased risk of progression to high grade dysplasia and cancer.[6]

YF476 is a potent, orally active, highly selective, competitive antagonist of CCK2 receptors.[7] The primary hypothesis for this study is that administration of YF476 to patients with BE will result in a significant decrease in cellular proliferation in the Barrett's mucosa.

1.2 Trial medication

YF476 (25 mg) or matching placebo: one capsule to be taken by mouth once daily for 12 weeks. Study medication will be taken with breakfast except on clinic visit days, when patients will fast overnight and take study medication after completion of study procedures.

1.3 Objectives

Primary:

The primary objective of this study is to determine if administration of YF476 (a CCK2R antagonist) to patients with Barrett's esophagus (BE) decreases tissue Ki67 expression, a marker of cellular proliferation.

Secondary:

The secondary objectives are to assess the effects of YF476 on:

- biomarkers associated with esophageal adenocarcinoma, in particular, cyclooxygease-2 (COX-2), p53, CCK2R and DCAMKL1; and
- fasting serum gastrin, a marker of gastric acid suppression, and plasma chromogranin A (CgA), a marker of ECL cell hyperplasia.

We also aim to determine whether YF476 is safe in patients with BE.

1.4 Type of trial

Phase II, randomised, double-blind, out-patient trial.

1.5 Trial population

a **Total** 20 patients completing the trial.

b **Inclusion criteria**

1. Aged ≥18 years, with histologically confirmed diagnosis of BE without dysplasia. A prior endoscopy with biopsies read as indefinite for dysplasia is permitted if biopsies from the most recent endoscopy prior to study entry demonstrated BE without dysplasia.
2. Minimum of 1 cm circumferential Barrett's mucosa on endoscopy or at least 2 cm maximal contiguous extent of Barrett's mucosa. (Prague criteria C≥1, any M *or* any C, M≥2).
3. Proton pump inhibitor use at least once daily, for at least twelve months prior to enrollment, and stable dose of PPI for the three months before enrollment. Any PPI, dose, and frequency are allowable.
4. ECOG performance status ≤2 and Karnofsky ≥60%; see Appendix A. 5. Normal organ and marrow function, defined as white blood cells (WBC) ≥3 x 10^9/L, absolute neutrophil count ≥1.5 x 10^9/L, platelets ≥100 x 10^9/L, creatinine ≤1.5 mg/dL, total bilirubin ≤1.5 mg/dL, AST ≤100 U/L, ALT ≤100 U/L.
6. Use of adequate contraception during the study as follows.

    • Pre-menopausal women must use a highly effective method of contraception (≤1% failure/year when used correctly), including: ○ intrauterine device (IUD); ○ bilateral tubal occlusion;

    - ○ vasectomised partner – must have had medical assessment of successful surgery; ○ intrauterine hormone-releasing system (IUS);
    - ○ progestogen-only hormonal contraception implants associated with inhibition of ovulation; or
    - ○ hormonal contraception associated with inhibition of ovulation (combined or progestogen-only – includes oral, intravaginal, transdermal and injections).

    • To prevent pregnancy, men with partners of childbearing potential must use a condom or have had a vasectomy (with a medical assessment of successful

surgery). Partners who are not of childbearing potential are defined as: men; post-menopausal women (no menstrual periods for at least 1 year); or women who have no uterus, ovaries or fallopian tubes.

- Women classed as post-menopausal (no menstrual periods for at least 1 year) are not required to use contraception.

7. Willingness to comply with all treatment and follow-up procedures.
8. Ability to understand and the willingness to sign a written informed consent document.
9. Up to date with all age-appropriate cancer screening tests (based on national guidelines.

d **Exclusion criteria**

1. Histologically confirmed BE with high-grade dysplasia.
2. Histologically confirmed diagnosis of invasive carcinoma of the esophagus.
3. Histologically confirmed BE with low-grade dysplasia that has been diagnosed by at least two expert gastrointestinal pathologists.
4. Prior endoscopic therapy for Barrett's esophagus.
5. A relevant history of esophageal or gastric surgery that, in the investigator's opinion, could compromise the objectives of the trial.
6. History of atrophic gastritis, pernicious anemia, or Zollinger-Ellison syndrome.
7. Participation in a trial of an IMP within the previous 28 days.
8. Prolonged QTc interval >450 msec
9. History of allergic reactions attributed to compounds of similar chemical composition to YF476.
10. History or baseline findings of:
    - diabetes mellitus requiring insulin therapy;
    - pancreatitis (baseline amylase and/or lipase ≥ 2.0 x ULN);
    - hepatitis B or hepatitis C;
    - HIV, unless, at the discretion of the PI, with at least 120 days on stable antiretroviral therapy (ART) with undetectable HIV viral load and CD4 >350 cells/mm^3;
    - malabsorption syndrome or inability to swallow or retain oral medicine;
    - major surgery ≤28 days prior to enrollment;
    - ECOG performance status ≥2; or
    - another cancer within 3 years except for basal carcinoma of the skin or cervical carcinoma in-situ.

- Also, any clinically significant and uncontrolled major morbidity including but not limited to: serious cardiac disease (unstable angina, s/p myocardial infarction ≤1 month); respiratory disease (advanced COPD or pulmonary fibrosis); uncontrolled hypertension; active systemic infection; or psychiatric illness/social situations that would limit compliance with study requirements.

11. Certain medicines and herbal remedies (see Appendix B) taken during the 7 days before the start of study drug.
12. A history of cancer >3 years from the time of enrollment, and the patient is not up to date with surveillance for that cancer (based on national guidelines), or has evidence of cancer at the time of enrollment.

1.6 Trial design and methods

Patients will be randomised (1:1 ratio) to receive either 25 mg YF476 or matching placebo. They will attend the clinic 4 or 5 times, and will take up to 18 weeks to complete the trial.

At **Visit 1**, patients will sign the Informed Consent form. Patients will undergo assessment of eligibility, physical examinations, ECG, urine pregnancy test (premenopausal women only) and safety tests of blood and urine.

Visit 2 can take place on the same day as visit 1, or up to 2 weeks later. At Visit 2, patients will undergo ECG, safety tests of blood and urine, including urine pregnancy test (pre-menopausal women only), unless Visit 2 is on the same day as Visit 1. All patients will have baseline biomarker assessments, baseline esophagogastroduodenoscopy (EGD) and biopsies. If they meet all the eligibility criteria, they will be given a bottle of study medication (25 mg YF476 or placebo) capsules to take once daily, with breakfast, starting the next day. Patients will complete a diary card throughout the treatment period.

The Week 6 visit will be 6 weeks after starting study treatment. Patients will fast before the visit, and will bring their dose of study medication to the clinic, to take after fasting blood tests. First, patients will have safety tests of blood and urine, ECG, and blood taken for biomarkers and trough YF476 concentration. Patients will then take their study medication, and 1 h afterwards will have blood taken for peak YF476 concentration. Patients will be asked about any adverse events and concomitant medications, and will return their study medication bottle for assessment of compliance. They will receive their next bottle of study medication.

The Week 12 visit will be 12 weeks after starting study treatment. Patients will fast before the visit, and will bring their dose of study medication to the clinic, to take after fasting blood tests. First, patients will have safety tests of blood and urine, ECG, and blood taken for biomarkers and trough YF476 concentration, before undergoing EGD assessments and biopsies. Patients will then take their study medication, and 1 h afterwards will have blood taken for peak YF476 concentration.

Follow-up visit will be 4 weeks after the patients have stopped taking study medication.

Patients will have safety tests of blood and urine, ECG, and blood tests for biomarkers. Patients will be asked about any adverse events.

1.7 Methods

- Esophagogastroduodenoscopy (EGD), to enable taking of biopsies for assessment of the primary endpoint, Ki67 expression levels;
- blood sampling for assay of biomarkers and serum for storage;
- diary card for patients to record adverse events, concomitant medication and compliance; and
- physical examination, ECG and safety tests of blood and urine.

1.8 Primary variable

Efficacy:	change in Ki67 expression between baseline (Visit 2) and the Week 12 visit

1.9 Secondary variables

Biomarkers:	change from baseline at Week 12 in biomarkers associated with esophageal adenocarcinoma, in particular, cyclooxygenase-2 (COX-2), P53, CCK2R and DCAMKL1, and in fasting serum gastrin and plasma CgA
Safety:	vital signs, ECG, physical examination, laboratory safety tests: blood tests: red blood cells (RBC), haemoglobin, haematocrit, platelets, white blood cells (WBC, differential: neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils), sodium, potassium, magnesium, chloride, bicarbonate, creatinine, serum glucose, blood urea nitrogen (BUN), alkaline phosphatase, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase/aspartate amino transferase (SGOT/AST), serum glutamic-pyruvic transaminase/ alanine aminotransferase (SGPT/ALT), total bilirubin, total protein, albumin, calcium, amylase, lipase; and urine tests: pH, specific gravity, protein, glucose, ketones, blood, and change in histology.
Tolerability:	adverse events
Pharmacokinetics:	trough and peak concentrations of YF476

STUDY DIAGRAM

Randomized, placebo-controlled trial of a gastrin receptor antagonist, YF476, in Barrett's esophagus

Barrett's esophagus without dysplasia

Proton pump inhibitor use at least once daily, for at least 12 months prior to enrollment, and stable dose of PPI for the 3 months before enrollment

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Informed consent

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Baseline data collection [history + physical, endoscopy + biopsy, blood (safety tests, pregnancy test (pre-menopausal women only) biomarkers), urine]

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Randomization: 1:1 ratio between patients on 25 mg YF476 daily and patients on placebo daily, for 12 weeks

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Follow-up data collection [endoscopy + biopsy, blood (safety tests, biomarkers, YF476 PK), urine, compliance, AEs, SAEs]

↓

Primary Endpoint – Ki67 expression

Secondary endpoints – expression of other biomarkers (including COX-2, p53, CCK2R, DCAMKL1), serum gastrin, plasma CgA and safety.

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List of Abbreviations

ACG	American College of Gastroenterology
ADL	activities of daily living
AE	adverse event
API	active pharmaceutical ingredient
ART	antiretroviral therapy
AUC	area under the curve
BE	Barrett's esophagus
CAP	College of American Pathologists
CCK2R	cholecystokinin 2 receptor
CDE	Common Data Elements
CgA	chromogranin A
CI	confidence interval
CIW	Clinical Investigation Ward
CLIA	Clinical Laboratory Improvement Amendments
cm	centimetre

C_{max}	peak concentration
COX-2	cyclooxygenase-2
CRF	case report form
CTA	clinical trial authorisation
CTCAE	Common Terminology Criteria for Adverse Events
CUMC	Columbia University Medical Centre
CV	Curriculum Vitae
CYP	cytochrome P450 enzyme
DCAMKL-1	doublecortin- and Ca^{2+}/calmodulin-dependent protein kinase-like protein 1
DCP	Division of Cancer Prevention
DSMB	Data Safety Monitoring Board
EAC	esophageal adenocarcinoma
ECL	enterochromaffin-like
EGD	esophagogastroduodenoscopy
FDA	Food and Drug Administration
FWA	Federal-wide assurance
g	gram
GCP	Good Clinical Practice
GERD	gastro-esophageal reflux disesase
GI	gastrointestinal
GMP	Good Manufacturing Practice
GRA	gastrin receptor antagonist
h	hour
H_2RA	histamine-2 receptor antagonist
hERG	human ether-à-go-go related gene
HGD	high grade dysplasia
HIPAA	health insurance portability and accountability act
HMR	Hammersmith Medicines Research
HPMC	hydroxypropylmethylcellulose

IC_{50}	concentration of a drug causing 50% inhibition of a response
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IHC	immunohistochemistry
IMP	investigational medicinal product
IND	Investigational New Drug
IRB	Institutional Review Board
LGD	low grade dysplasia
mg	milligram
MHRA	Medicines and Healthcare products Regulatory Agency
mL	millilitre
mm Hg	millimetres of mercury
NCI	National Cancer Institute
NDA	new drug application
NIHR	National Institute for Health Research
NOAEL	no observable adverse effect level
NSAID	non-steroidal anti-inflammatory drug
ºC	degrees celsius
OHRP	office for human research protections
OR	odds ratio
p53	tumour protein 53
PCR	polymerase chain reaction
PD	pharmacodynamic
pH	negative logarithm to base 10 of the hydrogen ion concentration
PI	principal investigator
PK	pharmacokinetic
PPI	proton pump inhibitor
QA	quality assurance
qRT-PCR	quantitative RT-PCR
QTc	interval of the ECG corrected for heart rate
REC	Research ethics committee

RT-PCR	reverse transcriptase polymerase chain reaction
SAB	Scientific Advisory Board
SAE	serious adverse event
SAS	statistical analysis software
SD	standard deviation
SUSAR	serious unexpected serious adverse reaction
$T_{1/2}$	half-life
T_{max}	time of peak concentration
ΔKi67	change in Ki67 expression
µg	microgram
µmol	micromole

2 Objectives

2.1 Primary objective

The primary objective of this study is to determine if administration of YF476 (a CCK2R antagonist) to patients with Barrett's esophagus (BE) decreases tissue Ki67 expression, a marker of cellular proliferation.

2.2 Secondary objectives

The secondary objectives are to assess the effects of YF476 on:

- biomarkers associated with esophageal adenocarcinoma, in particular, cyclooxygease-2 (COX-2), p53, CCK2R and DCAMKL1; and
- fasting serum gastrin, a marker of gastric acid suppression, and plasma chromogranin A (CgA), a marker of ECL cell hyperplasia.

We also aim to determine whether YF476 is safe in patients with BE.

3 Background

3.1 Barrett's esophagus

Barrett's esophagus (BE) is the precursor lesion for the development of esophageal adenocarcinoma (EAC). BE is defined as the presence of intestinal metaplasia in the distal esophagus, and is associated with a 25–30 fold increased risk of EAC.[1] The incidence of EAC has increased five-fold in the past three decades in the United States (US), with similar patterns of incidence observed in most developed western countries.[8] Unfortunately, the prognosis of esophageal cancer is poor, with 5-year survival rates of 16% in the US.[2]

The development of BE is thought to be caused in part by acid reflux. The change in esophageal lining presumably develops as a result of chronic exposure to refluxed acid and bile from the stomach leading to recurrent mucosal injury.[3] The esophageal injury is accompanied by inflammation and, ultimately, a cellular change (metaplasia) to a specialized columnar epithelium. BE-associated intestinal metaplasia can ultimately progress to low grade (LGD) and high grade dysplasia (HGD), states that are associated with elevated risk of progressing to EAC.

Current management of BE patients consists of endoscopic surveillance and acid suppression.[4] For example, current American College of Gastroenterology (ACG) guidelines recommend periodic upper endoscopy with random biopsies of the Barrett's segment in order to assess for the presence of LGD, HGD, or EAC. The purpose of surveillance is to allow for early detection, which may lead to improved patient outcomes. ACG guidelines for the management of BE also recommend gastric acid suppression with proton pump inhibitors (PPIs). In theory, gastric acid suppression reduces chronic inflammation within the BE segment, which in turn may reduce the risk of progression to EAC.

3.2 YF476

Details of YF476 are in the Investigator's Brochure (Trio Medicines Ltd)[7], so only a summary is provided here.

3.2.1 Non-clinical studies

Non-clinical studies have shown YF476 to be a potent, highly selective, and competitive antagonist of gastrin receptors *in vitro* and *in vivo*.[9-11]

Toxicology studies of YF476 consisted of: single- and/or repeat-dose toxicity studies in rats and dogs; toxicokinetics in rats and dogs; and embryo-foetal toxicity studies in rats and rabbits. Other non-clinical studies consisted of: safety pharmacology studies in rats, mice and dogs; genotoxicity studies *in vivo* and *in vitro;* studies of metabolism by cytochrome P450 in human liver microsomes *in vitro*; a study to determine the extent of YF476 protein binding in normal human serum *in vitro*; a study of the effect of YF476 on the hERG ion channel in human embryonic kidney cells *in vitro*: and a study to compare the metabolism of ^{14}C-YF476 in rat, dog and human cells *in vitro*.

In acute toxicity studies in beagle dogs, 100 and 500 mg/kg/day resulted in similar plasma concentrations of YF476, and caused no adverse effects. Therefore, 10, 30 and 100 mg/kg/day were tested in a 13-week toxicity study. Changes in pancreas weight and histopathology were observed in some animals, and serum amylase was elevated in one low-dose male. Although the histological findings resemble nesidioblastosis, a spontaneous finding in laboratory beagles, an effect of YF476 cannot be excluded, so the study did not clearly establish a NOAEL.

A 13-week toxicity study of YF476 doses 100, 300 and 1000 mg/kg/day was done in rats. At 300 and 1000 mg/kg/day, some animals had minor, reversible changes in enzyme activities and heart weight, and discoloured urine and pale faeces. Also, there were minor

histopathological lesions in the heart and pancreas of four treated animals and one control animal. The NOAEL was deemed 100 mg/kg/day.

In the 13-week study in dogs, YF476 had no effect on the ECG, and no effect on the ECG intervals, including QTc. A concentration of YF476 about four times the highest C_{max} in a human subject to date did not affect the hERG channel *in vitro*. Because ~84% of YF476 is bound to serum protein *in vivo*, that represents 25-fold safety cover.

Embryo-foetal studies were done in the rat and rabbit, using YF476 doses of 100, 300 and 1000 mg/kg/day. YF476 had no effect on embryo-foetal survival, growth or development in rabbits. There was slight delay in foetal development in rats given 1000 mg/kg/day, so the NOAEL was deemed 300 mg/kg/day.

The metabolism of parent ^{14}C-YF476 was most efficiently catalysed by human > dog > rat hepatocytes *in vitro*. Phase I and II metabolites were formed. There was no evidence for formation of unique major human metabolites, although the main metabolite was different from that formed by rat and dog. Thus, the rat and dog are appropriate species to assess the potential toxicity of YF476 in humans.

YF476 caused some reversible inhibition of CYP2C8 and CYP3A4 *in vitro*. The likelihood of a drug-drug interaction via those enzymes in trials in patients is remote or possible, respectively, at the proposed doses of YF476.

Non-clinical studies support administration of YF476 to humans for up to 13 weeks. The treatment period for this clinical trial will be 12 weeks.

3.2.2 Phase 1 studies

Trio has completed 11 studies of YF476 in healthy men and women.[7] 184 subjects received YF476: single doses up to 400 mg; up to 25 mg daily for 14 days; 100 mg twice daily for 7 days; 100 mg YF476 daily, either alone or with 20 mg rabeprazole, for 6 weeks; and 25 mg YF476 daily for 14 days either alone or with 40 mg esomeprazole. The outcome measures were: safety; tolerability; pharmacokinetics; and pharmacodynamics (24-h gastric pH; pentagastrin-induced gastric acid secretion; serum gastrin; and markers of ECL-cell hyperplasia). The comparators were placebo, ranitidine, omeprazole and rabeprazole.

Safety and tolerability

In all studies, YF476 was well tolerated. Adverse events were minor, transient, occurred in subjects given placebo or comparators as well as YF476, and were not YF476-dose dependent. YF476 affected neither vital signs, ECG, nor safety tests of blood and urine.

Pharmacodynamics

Single doses caused dose-dependent increases in 24-h gastric pH. The effect lasted much longer than that of a single dose of ranitidine. However, the effect of YF476 on 24-h gastric pH was mostly lost after repeated doses, whereas that of omeprazole persisted. Single doses of YF476 also caused dose-dependent inhibition of pentagastrininduced falls in gastric pH and increases in gastric volume and H^+ output. Again, the effect on gastric pH was mostly lost after repeated doses of YF476. But, the effects on volume and H^+

output persisted. So, gastrin receptors must still be blocked after repeated doses of YF476. In a study of YF476 and rabeprazole, alone and in combination for 6 weeks, YF476:

- was as effective as the PPI in reducing gastric volume and H+ activity;
- was as effective as the PPI in increasing serum gastrin;
- did not increase plasma chromogranin A (CgA), a biomarker for ECL-cell activity, whereas the PPI increased it;
- abolished the PPI-induced increase in CgA;
- augmented gastric acid suppression by the PPI without reducing the increase in gastric pH induced by the PPI; and
- prevented the rebound increase in H^+ activity induced by the PPI.

In conclusion, the combination was more effective than either the PPI or YF476 alone in suppressing gastric acid production. YF476 prevented stimulation of ECL cells and rebound hyperacidity, both of which are caused by PPI-induced hypergastrinaemia. Overall, the effects of YF476 are consistent with antagonism of gastrin receptors.

Pharmacokinetics

The pharmacokinetic profile of YF476 varied among the 1996, 2001, 2005 and 2009 capsule formulations. The 2001 and 2005 formulations were crystalline, and the 1996 and 2009 formulations were spray-dried. The spray-dried formulations had the most favourable profile. In a single-dose study of 50–400 mg of the current (2009) formulation of YF476, absorption was rapid, T_{max} was 1.5–2 h, $T_{½}$ varied with dose, and C_{max} and AUC_{0-24} were not proportional to dose. $T_{½}$ after 100 mg YF476 was about 7 h. Food increased C_{max} and $AUC_{0–24}$ by 1.9 and 1.6-fold, respectively. The absolute bioavailability was about 16% in a study of 100 mg 'cold' YF476 by mouth after fasting and 15 μg ^{14}C-YF476 intravenously. Protein binding in human serum was about 84%. In a 7-day study of 100 mg YF476 twice daily of the 1996 formulation, YF476 reached steady state by Day 3 and did not accumulate by Day 7. Even though the crystalline formulations were much less bioavailable than the spray-dried formulations, the pharmacodynamic results were still favourable, which suggests that YF476 is a potent gastrin antagonist in man.

3.3 Rationale

As described above, clinical guidelines for the management of BE include gastric acid suppression with chronic PPI therapy. Acid reflux appears to have an important causal role in the development of BE, and a history of reflux symptoms is associated with EAC.[12] However, data are inconclusive with regard to acid suppression and EAC risk. In a retrospective study of veterans with BE, PPI compared with histamine-H_2 antagonists or no acid suppression was associated with a lower risk of progression to dysplasia.[13] In contrast, other studies suggest that PPIs may be associated with an *increased* risk for developing EAC,[14] and Feagins *et al* reported anti-proliferative effects of acid exposure in non-dysplastic BE cells.[15]

By inhibiting acid secretion, PPIs at therapeutic doses can cause secondary hypergastrinemia. Gastrin exerts trophic effects in BE via CCK2R activation.[5] There is increased expression of CCK2R in BE compared to squamous epithelium, and gastrin can stimulate proliferation, anti-apoptosis, COX-2 expression, and loss of cell-cell adhesion in BE cells.[5, 16] The chief investigator's group recently reported that BE patients with high serum gastrin levels had significantly increased odds of a history of high grade dysplasia (HGD) or EAC.[6] In that cross-sectional study, they enrolled 95 patients with BE (with and without dysplasia or EAC) and GERD controls on chronic PPI therapy. Subjects in the highest serum gastrin quartile had significantly increased odds of a history of advanced neoplasia (adjusted OR 5.46, 95% CI 1.20–24.8). The group also analyzed 19 samples from non-dysplastic Barrett's epithelium, and found marked elevations in Ki67 expression, with a mean Ki67 index of 48.3% (SD 16.0%). They also found a moderate correlation between serum gastrin and tissue Ki67 index (r=0.64, p=0.05) in a subset of ten patients [17].

Those studies support the notion that gastrin may promote carcinogenesis in BE, and that these potentially pro-neoplastic effects occur via activation of the CCK2 receptor. We therefore propose the following clinical trial to analyze the effects of a CCK2 receptor antagonist in patients with BE. The primary hypothesis is that administration of YF476, a CCK2 receptor antagonist, in patients with BE will result in a significant decrease in cellular proliferation in the Barrett's mucosa.

4 Summary of study plan

4.1 Study design

We propose a randomized, double-blind, placebo-controlled pilot study of YF476, a cholecystokinin-2 receptor antagonist, in patients with Barrett's esophagus without dysplasia. Patients will take YF476 or placebo for 12 weeks.

There are 2 research sites, Columbia University and the University of Cambridge. We aim for 20 patients to complete the study. Patients will be randomized (1:1 ratio) to treatment with YF476 or placebo.

Patients will make up to 5 visits to the research site (Visits 1 and 2 may be combined, or up to 2 weeks apart. See schedule of events in Section 8.1.):

- Registration (Visit 1)
- Baseline (Visit 2 – start of treatment phase)
- Study treatment (6 weeks after the start of the treatment phase). • End of study treatment (after 12 weeks' treatment)
- Follow-up (4 weeks after the end of treatment).

After giving informed consent (Visit 1) and after the initial endoscopy (Visit 2), patients will be given one bottle of YF476 or placebo. They will be given a study bottle again at the Week 6 visit.

The primary endpoint of the study (Ki67 expression) will be assessed using endoscopic biopsies taken at baseline and at 12 weeks.

Esophageal tissue from endoscopic biopsies will be evaluated for Ki67 expression (primary endpoint) and expression of additional markers potentially associated with

esophageal neoplasia (secondary endpoints). Expression of these markers will be assessed by immunohistochemistry (IHC) and reverse transcriptase-PCR (RT-PCR), as appropriate.

Blood samples will be taken at Visit 2, the Week 6 visit and the Week 12 visit for measurement of serum gastrin and plasma CgA, and at the Week 6 and Week 12 visits for trough and peak YF476 concentrations.

Safety tests and pregnancy tests (pre-menopausal women only) will be done at each visit. Patients will complete a diary card daily to record study drug, concomitant medication and adverse events. Adverse events will be assessed 6 and 12 weeks after starting study medication, and 4 weeks after finishing study medication. Blood and urine samples will also be collected at these visits to assess for hematologic, metabolic, or other disturbances that may be due to study drug. The duration of the study for each individual patient will be between 16 and 18 weeks.

4.2 Discussion of study design

Patient population

In this first trial of YF476 in patients with BE, we will study only those without dysplasia. BE patients without dysplasia are all placed on PPIs for acid suppression and undergo lifelong endoscopic surveillance. Therefore, this population will be most vulnerable to any long-term adverse effects of increased gastrin exposure, and represents the group of patients who could potentially benefit the most from a gastrin receptor antagonist. The standard of care for patients with high grade dysplasia (HGD) at the study institutions consists of endoscopic ablative therapy and not continued endoscopic surveillance. The diagnosis of low grade dysplasia (LGD) is confounded by extremely poor interobserver reliability, even among expert pathologists. Additionally, ablative therapy is also frequently recommended for LGD patients. Therefore, BE patients with LGD and HGD are not ideal candidates for chemoprevention studies in BE. *Dose regimen*

The dose regimen of 25 mg YF476 or matching placebo once daily has been chosen based on a previous study of YF476 (T-013), which showed that a dose as low as 5 mg YF476 suppressed the increase in CgA, a marker of ECL-cell activity, induced by PPI treatment, in healthy subjects. The patients in the proposed T-016 study will have had prolonged hypergastrinaemia, so a dose of 25 mg YF476 is appropriate. A placebocontrolled design is appropriate for this study as there is no alternative treatment known to reduce the levels of Ki67 expression in patients with BE.

Efficacy tests

We will use Ki67 index as the primary endpoint, as it is a reliable and reproducible marker of ongoing cellular proliferation. Quantitative assessment of the number of proliferating

(Ki67+) epithelial cells is easiest and most reliable in immunohistochemistry (IHC). We will also take blood samples to assay fasting serum gastrin and plasma CgA, and trough and peak YF476 concentrations.

4.3 Risk-benefit analysis

What are the potential risks of harm to the trials patients from YF476 and from the trial procedures, and how might they be mitigated?

Reported adverse events and potential risks

In all studies in healthy volunteers, YF476 was well tolerated. Adverse events were minor, transient, occurred in subjects given placebo or comparators as well as YF476, and were not YF476-dose dependent. YF476 did not affect: vital signs; ECG; or safety tests of blood and urine. There was no evidence of effect on QT interval in human studies, but *in vitro* studies showed that YF476 has a low potential for prolonging QT interval (See Section 3.2.1). So, until we have more information about YF476, patients with prolonged QT interval are excluded from clinical trials.

The adverse effects that occurred at a frequency of 10% or greater were: abdominal distention, diarrhea, abdominal pain, nausea, and headache. None of these adverse effects were observed with greater frequency as compared to placebo or comparators. Because of the pancreatic findings in the 13-week toxicology study of dogs (Section 3.2.1), serum amylase and lipase will be monitored at each visit. Also, any patient who complains of abdominal discomfort at any time during the study will be seen immediately by the investigator or delegate and evaluated with history, physical examination, and laboratory tests, including serum amylase and lipase, and other appropriate tests, including radiological investigations. *Risk from concomitant medications*

In vitro studies have shown that the likelihood of a drug-drug interaction in patients *in vivo* is:

- CYP2C8: possible for 50–400 mg YF476
- CYP3A4 (testosterone): remote for 50 & 100 mg YF476, and possible for 200 & 400 mg
- CYP3A4 (midazolam): remote for 50 mg YF476, and possible for 100–400 mg. Although YF476 caused some irreversible inhibition of CYP3A4, the degree of inhibition makes a drug-drug interaction in patients *in vivo* unlikely. And, a clinical study (T-007) of YF476 and midazolam (a substrate of CYP3A4) alone and in combination showed that neither medicine affects the pharmacokinetics of the other (see YF476 Investigator's brochure for further details).

Many medicines are metabolised via CYP3A4/5. Very few are metabolised via CYP2C8. Examples of the main types of medicine that are metabolised by CYP3A4/5

and CYP2C8 are listed in Appendix B. Guidance about assessing the risk and managing drug-drug interactions is also provided in Appendix B. All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the patient will

be documented in the CRF at baseline, and the patient will record any new concomitant medications in the diary card, which will be checked at each visit. *Risk from study procedures: endoscopy*

Patients who take part in the trial must undergo endoscopy at baseline and at the Week 12 visit. All patients in the study will have had at least one endoscopy before the trial. Most gastroscopies are done without any problem. Some people have a sore throat for a day or so afterwards. Patients may feel tired or drowsy for several hours after sedation. There is a slightly increased risk of developing a chest infection after endoscopy. Rarely, the endoscope damages the esophagus, causing bleeding or infection. Patients will be given information on how to prepare for their endoscopy according to local procedures.

Overall, the potential benefits to the trial patients of taking part in the trial greatly outweigh the risks of any harm.

5 Patient selection

5.1 Inclusion criteria

1. Aged ≥ 18 years, with histologically confirmed diagnosis of BE, defined as endoscopically-suspected BE with intestinal metaplasia present on esophageal biopsies, without dysplasia. A prior endoscopy with biopsies read as indefinite for dysplasia is permitted if biopsies from the most recent endoscopy prior to study entry demonstrated BE without dysplasia. The most recent endoscopy documenting BE must have been performed no more than three (3) years from the time of enrollment.
2. Minimum of 1 cm circumferential Barrett's mucosa on endoscopy or at least 2 cm maximal contiguous extent of Barrett's mucosa. (Prague criteria C≥1, any M *or* any C, M≥2).
3. Proton pump inhibitor use at least once daily, for at least twelve months prior to enrollment, and stable dose of PPI for the three months before enrollment. Any PPI, dose, and frequency are allowable.
4. ECOG performance status ≤2 and Karnofsky ≥60%; see Appendix A.
5. Participants must have normal organ and marrow function, defined as white blood cells (WBC) ≥3 x 10⁹/L, absolute neutrophil count ≥1.5 x 10^9/L, platelets ≥100 x 10^9/L, creatinine ≤1.5 mg/dL, total bilirubin ≤1.5 mg/dL, AST ≤100 U/L, ALT ≤100 U/L.
6. The effects of YF476 on the developing human fetus at the recommended therapeutic dose are unknown. All patients must agree to use adequate contraception for the duration of study participation as follows.
    - Pre-menopausal women must use a highly effective method of contraception (≤1% failure/year when used correctly), including:
        - intrauterine device (IUD); ○ bilateral tubal occlusion;

- vasectomised partner – must have had medical assessment of successful surgery;
- intrauterine hormone-releasing system (IUS);
- progestogen-only hormonal contraception implants associated with inhibition of ovulation; or
- hormonal contraception associated with inhibition of ovulation (combined or progestogen-only – includes oral, intravaginal, transdermal and injections).

- To prevent pregnancy, men with partners of childbearing potential must use a condom or have had a vasectomy. Partners who are not of childbearing potential are defined as: men; post-menopausal women (no menstrual periods for at least 1 year); or women who have no uterus, ovaries or fallopian tubes.
- Women classed as post-menopausal (no menstrual periods for at least 1 year) are not required to use contraception.

7. Willingness to comply with all treatment and follow-up procedures.
8. Ability to understand, and the willingness to sign, a written informed consent document.
9. Up to date with all age-appropriate cancer screening tests (based on national guidelines.

5.2 Exclusion criteria

1. Histologically confirmed BE with high-grade dysplasia.
2. Histologically confirmed diagnosis of invasive carcinoma of the esophagus.
3. Histologically confirmed BE with low-grade dysplasia that has been diagnosed by at least two expert gastrointestinal pathologists.
4. Prior endoscopic therapy for Barrett's esophagus, including (but not limited to) mucosal resection, radiofrequency ablation, and photodynamic therapy.
5. A relevant history of esophageal or gastric surgery that, in the investigator's opinion, could compromise the objectives of the trial.
6. History of atrophic gastritis, pernicious anemia, or Zollinger-Ellison syndrome.
7. Participation in a trial of an IMP within the previous 28 days.
8. Prolonged QTc interval (>450 msec)
9. History of allergic reactions attributed to compounds of similar chemical composition to YF476.
10. History or baseline findings of:
    - diabetes mellitus requiring insulin therapy;
    - pancreatitis (baseline amylase and/or lipase ≥ 2.0 x ULN);
    - hepatitis B or hepatitis C;

- HIV, unless, at the discretion of the PI, with at least 120 days on stable ART with undetectable HIV viral load and CD4 >350 cells/mm^3;
- malabsorption syndrome or inability to swallow or retain oral medicine;
- major surgery ≤28 days prior to enrollment;
- ECOG performance status ≥2; or
- cancer within 3 years except for basal carcinoma of the skin or cervical carcinoma in-situ.
- Also, any clinically significant and uncontrolled major morbidity including but not limited to: serious cardiac disease (unstable angina, s/p myocardial infarction ≤1 month); respiratory disease (advanced COPD or pulmonary fibrosis); uncontrolled hypertension; active systemic infection; or psychiatric illness/social situations that would limit compliance with study requirements.

11. Certain medicines and herbal remedies (see Appendix B) taken during the 7 days before the start of study drug.
12. A history of cancer >3 years from the time of enrollment, and the patient is not up to date with surveillance for that cancer (based on national guidelines), or has evidence of cancer at the time of enrollment.

5.3 Inclusion of women and minorities

Men and women of all races and ethnic groups are eligible for this trial. However, the described epidemiology of Barrett's esophagus is such that the majority of study patients are likely to be white males.

5.4 Recruitment and retention plan

There will be 2 study sites: Columbia University Medical Center (CUMC), New York, US, and the National Institute for Health Research (NIHR) Clinical Investigation Ward (CIW), Cambridge University Hospitals, Cambridge, UK. Eligible patients will be recruited from the clinical practices of the study investigators (JAA, CJL, JMP, RF). All of the study investigators see a large volume of patients referred for management and treatment of Barrett's esophagus. We aim for 20 patients to complete the trial. Recruitment progress will be assessed on an ongoing basis. Screening and enrollment logs will be regularly reviewed by the investigators. Patient withdrawals will be carefully monitored. Methods for patient retention will be implemented, including the maintenance of regular contact with study patients. The study staff will meet regularly to review issues related to recruitment and retention, identify areas of improvement, and implement appropriate strategies to address any deficiencies. Additional recruitment methods such as advertising may be performed by each individual site with the approval of that site's Institutional Review Board or REC.

Withdrawals will be replaced at the discretion of the investigator.

6 Administration of study medication

Study medication will be dispensed on an outpatient basis, and self-administered by patients. Reported adverse events and potential risks are described in Section 4.3.

6.1 Dose regimen and dose groups

- Patients will take 25 mg YF476 (1 x 25 mg capsule) or matching placebo capsules, by mouth once daily.
- The treatment period for this trial will be 12 weeks.

6.2 YF476 administration

- Each study patient will self-administer the YF476 or placebo.
- The study medication will be dispensed into bottles: 46 capsules of 25 mg YF476 or placebo per bottle. 1 bottle will be dispensed to participants at the baseline visit (Visit 2) and at the Week 6 visit.
- Study medication should be taken once daily, in the morning, with breakfast. But, if a patient takes the study medication without food, or at a different time of the day, it will not be recorded as a protocol deviation. Missed doses will be recorded as protocol deviations.

6.3 Run-in procedures

No run-in phase is planned for this study.

6.4 Contraindications

Contraindications to taking YF476 include prolonged QTc interval, pregnancy or breastfeeding. Patients with prolonged QTc interval (>450 msec) are excluded. Women of childbearing potential will be included in the study only if they are using adequate contraception and will be advised by the recruiting physician and in the information and consent form about the risks of becoming pregnant while taking part in the study. Pregnancy tests will be done at each visit.

6.5 Concomitant medications

All study patients will take proton pump inhibitors at least once daily. Any formulation of PPI is permitted during the study period.

All medications (prescription and over-the-counter), vitamin and mineral supplements, and/or herbs taken by the participant will be documented on the concomitant medication CRF and will include: 1) start and stop date, dose and route of administration, and indication. Medications taken for a procedure (eg: biopsy) should also be included.

CYP 450 enzymes

Studies were done to assess the potential of YF476 to inhibit cytochrome P450 enzymes *in vitro*. For details, see Appendix B – drug interactions, and the YF476 Investigator's Brochure. The likelihood of a drug-drug interaction in patients *in vivo* is:

- CYP2C8: possible for 50–400 mg YF476

- CYP3A4 (testosterone): remote for 50 & 100 mg YF476, and possible for 200 & 400 mg
- CYP3A4 (midazolam): remote for 50 mg YF476, and possible for 100–400 mg.

Although YF476 caused some irreversible inhibition of CYP3A4 *in vitro*, the degree of inhibition makes a drug-drug interaction in patients *in vivo* unlikely. And, a clinical study (T-007) of YF476 and midazolam (a substrate of CYP3A4) alone and in combination showed that neither medicine affects the pharmacokinetics of the other. For details, see the Netazepide (YF476) Investigator's Brochure.

Many medicines are metabolised via CYP3A4/5. Very few are metabolised via CYP2C8. Examples of the main types of medicine that are metabolised by CYP3A4/5 and CYP2C8 are listed in Appendix B. Guidance about assessing the risk and managing drug-drug interactions is also provided in Appendix B.

Drugs that are substrates for, or inhibitors or inducers of CYP3A4/5 and CYP2C8 are not prohibited, but the patients must be monitored carefully, and the investigator must be informed of any new concomitant medications via the patient's diary card. Midazolam may be used during endoscopy. It was used safely before gastroscopy in a study of YF476 in healthy subjects.

6.6 Dose modification

Dose modifications of study drug will not be performed. If drug-related toxicity is suspected, then treatment will be discontinued.

6.7 Compliance

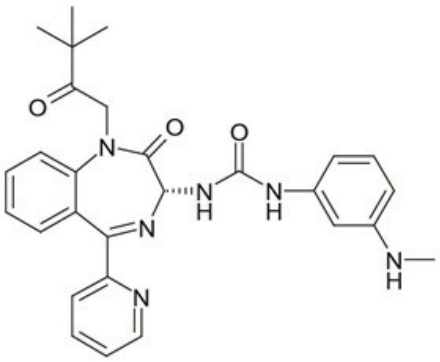
- All randomized patients will be eligible for statistical analysis. Patients will be analyzed on an intent-to-treat basis, and also per protocol.
- Compliance will be measured by capsule counts at the Week 6 and Week 12 visits, and by review of patient diary cards.

7 Pharmaceutical information

YF476 (IND # 107354, IND Sponsor: Trio Medicines Ltd)

7.1 Active pharmaceutical ingredient (API): YF476

International non-proprietary name (INN): netazepide **Structural formula:**



Chemical formula: (*R*)-1-[2,3-dihydro-2-oxo-1-pivaloylmethyl-5- (2-pyridyl)-1*H* -1,4-benzodiazepin-3-yl]-3-(3-methylaminophenyl)urea

Molecular formula: $C_{28}H_{30}N_6O_3$ **Molecular weight:** 498.58

Description: white crystalline powder

Purity: 99.9%

Optical rotation: $[\alpha]D_{20}$ = +124 ° (Acetone, 0.5%)

Acid dissociation constant (pKa): 2.53, 4.59

Hygroscopicity: <0.3% at room temperature, and 93% relative humidity (7 days)

Stability: Chemically stable for at least 9.5 years at room temperature, protected from light.

7.2 Formulated intermediate: spray-dried YF476

YF476 and hydroxypropylmethylcellulose (HPMC) (YF476:HPMC ratio 1:3.5) were dissolved in dichloromethane/isopropanol (7:2 v/v). The solution was spray-dried, and at all stages of production, the solution and resulting spray-dried powder were shielded from light. The formulated intermediate was characterised as follows.

Description: white to off-white powder

Structural configuration: amorphous by x-ray powder diffraction

YF476 purity: ≥95% of the theoretical purity (adjusted for water)

Water content: ≤7.5%

Residual dichloromethane: ≤2000 ppm

Residual isopropanol: ≤10000 ppm

Stability: Chemically and physically stable at 2–8 °C for at least 4 years. Stability studies are ongoing.

7.3 Final formulation

The co-formulation of spray-dried YF476 and HPMC is blended with starch, and placed into hard gelatine capsules at the Hammersmith Medicines Research (HMR) Pharmacy. Each capsule contains 25 mg YF476. Matching placebo capsules, containing starch only, will also be prepared. The sponsor will provide a certificate of analysis for the test product.

7.4 Packaging and labelling

For the CUMC, HMR Pharmacy will provide bulk supplies of investigational medicinal product (IMP) in accordance with The Rules Governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice (GMP) [19], and with HMR's Manufacturing Authorisation for IMPs (MIA(IMP)). The trial medication will be dispensed and labelled by the site pharmacy in accordance with United States Code of Federal Regulations governing labelling of investigational new drugs (21CFR 312.6).

For NIHR CIW, the trial medication will be packaged and labelled by the HMR Pharmacy, in accordance with The Rules Governing Medicinal Products in the European Union, Volume 4: Good Manufacturing Practice (GMP)[19]], and with HMR's Manufacturing Authorisation for IMPs (MIA(IMP)). The IMP labels will include the information required by Annex 13 to GMP.

7.5 Storage, accountability and dispensing of study medication

The HMR Pharmacy will dispatch the study medication to the site Pharmacy by secure temperature-controlled courier after ethical and regulatory approvals of the trial have been obtained. The site Pharmacy will record and acknowledge in writing the receipt of all supplies of study medication. The study medication will be stored securely at 2 to 8°C, protected from moisture and light, accessible only to those individuals authorised by the investigator to dispense the IMP.

Each site pharmacy will dispense study drug (or placebo) for trial patients in accordance with the randomization code. The labels on bottles given to trial patients will not indicate whether the capsules are study drug or placebo, so as to maintain blindness among the study investigators and study patients.

The site Pharmacy will maintain a careful record of the inventory and disposition of all medication received from the HMR Pharmacy. The site Pharmacy will maintain adequate records of receipt, dispensing and final disposition of YF476 and placebo. The receipt record will include from whom the study medication was received and to whom study medication was distributed, date, quantity and batch or lot number. The dispensing record will be blinded, and will include quantities and the dates YF476 or placebo was dispensed

to and returned by each participant. Dr. Julian A. Abrams will be responsible for study medication accountability for participants at CUMC and Prof. Rebecca Fitzgerald will be responsible for study medication accountability for participants at NIHR CIW.

At the end of the trial, all unused study medication supplies will be returned to the HMR Pharmacy.

7.6 Registration and randomization

- A member of the HMR Statistics and Data Management Department will generate a randomization list for each site. Patient numbers will be allocated to eligible patients immediately before the EGD and biopsy at Visit 2 (Baseline). Patients enrolled at CUMC will be allocated patient numbers 01–24. Patients enrolled at NIHR CIW will be allocated patient numbers 1001–1024. The lists are independent of each other. At both sites, patients on PPI once daily will be given the next available number from the top of the list, and patients on PPI twice daily will be given the next available number from the bottom of the list.
- At each site, the site coordinators will maintain a site-specific list of participants, including patient codes, start date, and duration of treatment. Every time a patient is recruited at one of the sites, the coordinator for that site will inform the chief investigator (if applicable) and the coordinator for the other site. It is the chief investigator's responsibility to ensure that the total number of study patients does not exceed the target.

The randomization list will specify whether the patient receives a dose of YF476 or placebo. Fixed allocation randomization will be performed, with equal allocation (1:1) between the 25 mg YF476 and placebo groups. Blocked randomization will be used. Randomization will be stratified by PPI dose and by site. The site pharmacy will dispense YF476 or placebo according to the randomization list. The study investigators and the study patients will remain blinded.

7.7 Blinding and unblinding methods

- Bottles containing study medication for individual patients will not be labelled with any information to indicate whether the capsules are YF476 or placebo.
- Patients will be blinded to study treatment.
- One member of the HMR Statistics and Data Management Department will not be blinded. That person will generate the randomization code. The Columbia University biostatistician will be blinded.
- All Investigators will be blinded to study treatment.
- For each site, there will be a blinded monitor and an unblinded monitor. The unblinded monitor will review dispensing records at the site Pharmacy.
- Pharmacy staff will not be blinded.
- The Data and Safety Monitoring Board will not be blinded.

- The blind will be broken by the investigator or delegate in the following circumstances:
    - If unblinding is required in the interest of the safety of a subject, an investigator will discuss the matter with the sponsor before opening the individual code-break envelope for that subject. In a medical emergency, the principal investigator or delegate may open the individual code-break envelope for that subject without prior consultation with the sponsor. In that event, the principal investigator or delegate will notify the sponsor as soon as possible that the randomisation code has been broken for the subject.
    - The blind will be broken for the entire study population at the completion of the study, after the statistical analysis plan (SAP) has been signed and the database has been locked.

7.8 YF476 destruction/disposal

At the completion of investigation, the site Pharmacy will return all unused YF476 and placebo capsules to the HMR Pharmacy.

8 Clinical evaluations and procedures

8.1 Schedule of events

	Baseline Testing/ Prestudy Evaluation		Study treatment		Follow-up period
	Visit 1[1]	**Visit 2**	**Week 6 visit**	**Week 12 visit**	**Follow–up**
Evaluation/ Procedure	Registration	Randomization, & Endoscopy	Week 6	Week 12	Follow Up Visit 4 weeks after stopping treatment
Informed consent	X				
Assess eligibility	X				
Central pathology review[2]	X				
Medical history	X				
Physical exam	X				
Vital signs	X	X[5]	X	X	X
Height and weight/ waist circumference	X				
Smoking history	X				
Medication history	X				
Blood and urine collection for safety tests	X	X[5]	X	X	X
Urine pregnancy test[4]	X	X[5]	X	X	X
ECG	X	X[5]	X	X	X
Blood for YF476 assay[3]			X	X	
Blood for assay of fasting serum gastrin and plasma CgA, and serum for storage		X	X	X	X
EGD (Upper GI endoscopy)		X		X	
Biopsies		X		X	
Concomitant medication assessment		X	X	X	
Dispense study medication		X	X		
Collect study medication			X	X	
Compliance assessment			X	X	
AE assessment		X	X	X	X

1. There may be up to a 2-week gap between Visits 1 and 2.
2. Review of patient's history to obtain histological confirmation of the patient's eligibility to participate in the trial.
3. Plasma YF476 assay: patients fast overnight. For the Week 6 and 12 visits, patients bring their container of study medication with them, and take study medication only after collection of blood samples for trough YF476, fasting serum gastrin and CgA levels, and endoscopy (if applicable). Patients stay for 1 h after dosing for collection of a blood sample for peak YF476 level.
4. Pre-menopausal women only.
5. These tests may be omitted if Visit 2 occurs on the same day as Visit 1.

8.2 Baseline testing and pre-study evaluation

Visit 1: registration

Patients will attend the clinic and complete the informed consent form. The investigator will complete the following evaluation for each patient, and enter the data from this visit, and all subsequent visits, on the appropriate page of the CRF:

- review of most recent endoscopy (within 3 years) to confirm diagnosis of Barrett's esophagus with no dysplasia;
- review of medication history to determine that participants meet the inclusion criteria (daily PPI use for at least the most recent 12 months before study entry, and stable PPI dose for at least the most recent 3 months before study entry);
- medical history, physical examination, ECG and vital signs; • blood and urine samples for laboratory safety tests; and
- urine pregnancy test (pre-menopausal women only).

Visit 2: baseline

Visit 2 may occur on the same day or up to 2 weeks after Visit 1. The patient will fast for 6 hours before the study visit. The investigator will complete the following evaluation for each patient, and enter the data from this visit, and all subsequent visits, on the appropriate page of the CRF. If Visits 1 and 2 are combined, only 1 blood sample (safety tests, fasting serum gastrin, plasma CgA and serum for storage) and 1 urine sample will be taken, and ECG, vital signs and pregnancy tests will be done only once.

- blood and urine samples for laboratory safety tests, urine pregnancy test (premenopausal women only), ECG and vital signs;
- blood samples for fasting serum gastrin, plasma CgA and serum for storage;
- endoscopy and biopsies; and
- randomization to 25 mg YF476 or placebo, and dispensing of study medication (a 42-day supply, plus overage: 1 bottle of 25 mg YF476 or placebo containing 46 capsules).

The investigator will then:

- give the patient a diary card;
- tell the patient to start taking their study medication the day after Visit 2;
- remind the patient to fast before the Week 6 visit (6 weeks after starting study medication); and
- remind the patient not to take their study medication at home on the day of the Week 6 visit, but instead to bring their container of study medication with them to the visit, and take their dose at the clinic, after the fasting blood tests.

8.3 Evaluations during study treatment

Week 6 visit: after 6 weeks of study treatment The investigator will:

- check that the patient has fasted;
- collect the diary card, and issue a new one;
- check the diary card for compliance with treatment, and for adverse events and concomitant medication;
- collect the study medication bottle and assess compliance;
- do an ECG and vital signs;
- take blood and urine for laboratory safety tests and urine pregnancy testing (pre-menopausal women only);
- take blood samples for fasting serum gastrin, plasma CgA, trough YF476 concentration and serum for storage;
- after taking the fasting blood samples, give the patient their dose of study medication, wait 1 h, then collect blood for assay of peak concentration of YF476;
- issue a new container of study medication, to be taken as before;
- remind the patient to fast before the next visit; and
- remind the patient not to take their study medication at home on the day of the Week 12 visit, but instead to bring their container of study medication with them to the visit, and take their dose at the clinic, after the fasting blood tests and endoscopy.

8.4 Evaluations at completion of study treatment

Week 12 visit: after 12 weeks of study treatment The investigator will:

- check that the patient has fasted;
- collect the diary card;
- check the diary card for compliance with treatment, and for adverse events and concomitant medication;
- collect the study medication bottle and assess compliance;
- do an ECG and vital signs;
- take blood and urine for laboratory safety tests and urine pregnancy testing (premenopausal women only);
- take blood samples for fasting serum gastrin, plasma CgA, trough YF476 concentration and serum for storage;
- do an endoscopy and biopsies; and

- after the endoscopy, give the patient their dose of study medication, wait 1 h, then collect blood for assay of peak concentration of YF476.

8.5 Post-treatment follow-up period

Follow-up: 4 weeks after stopping study treatment

The patient will return 4 weeks after the end of the treatment period. The investigator will:

- assess the patient for any delayed adverse events;
- do an ECG and vital signs;
- take blood and urine for laboratory safety tests and urine pregnancy testing (premenopausal women only); and
- take blood samples for fasting serum gastrin, plasma CgA, and serum for storage.

8.6 Flexibility of study days

There will be a ±4 day window for clinic visits.

8.7 Withdrawal of patients from the trial

8.7.1 Termination of study medication

Patients may stop taking study medication for the following reasons.

- When a patient has completed the protocol-prescribed treatment, we will not provide any further study medication.
- If a patient has an adverse event, grade 2 or greater (see section 11.1), the patient or investigator may choose to terminate study treatment.
- A patient might be non-compliant and stop taking the protocol-prescribed treatment; or the investigator may choose to terminate study treatment.
- The investigator may terminate study treatment because of a medical contraindication.
- The investigator may terminate study treatment because of evidence of serious interactions with medications metabolized by CYP3A4/5 or CYP2C8, (see Appendix B for list of relevant medications).

In all cases listed above, patients will continue to be followed, if possible, for safety reasons and in order to collect endpoint data according to the schedule of events.

8.7.2 Termination of study participation

Patients may go ‘off-study’ (ie leave the study with no follow-up possible) for the following reasons.

- The protocol treatment and any protocol-required follow-up period is completed.
- If a patient has an adverse event, grade 2 or greater (see section 11.1), the patient may choose to terminate study participation.

- A patient might be lost to follow-up, if the investigator is unable to establish contact with him or her.
- A patient might be non-compliant and not attend the clinic visits (including the follow-up visit).
- The investigator may terminate study participation because of a medical contraindication.
- A patient may withdraw his or her consent to participate in the study.
- Determination of ineligibility, not discovered before enrollment. In particular; patients will be withdrawn from the study if their baseline endoscopy (Visit 2): ○ does not show any intestinal metaplasia; or
  - ○ biopsies are read as indefinite for dysplasia, low grade dysplasia, high grade dysplasia, or adenocarcinoma.
- Death.

8.8 Study termination

Enrollment of patients onto the study will be terminated when at least 20 participants have completed 12 weeks of treatment, undergone a post-treatment endoscopy, and returned for the follow-up visit (4 weeks after stopping study medication). The study will end when the analysis of all biopsy samples is complete.

9 Criteria for evaluation and endpoint definition

9.1 Primary endpoint

- Change in Ki67 expression (ΔKi67) between baseline (Visit 2) and the Week 12 visit

9.2 Secondary endpoints

- Biomarkers
  - ○ Change from baseline at Week 12 in biomarkers associated with esophageal adenocarcinoma, in particular:
    - Change in CCK2R mRNA levels (measured by qRT-PCR)
    - Change in DCAMKL1 expression (measured by IHC)
    - Change in DCAMKL1 mRNA levels (qRT-PCR)
    - Change in COX-2 mRNA levels (qRT-PCR)
    - Change in p53 expression (IHC) ○ Reduction in PPI-induced increase in plasma CgA ○ Increase in PPI-induced hypergastrinaemia
- Safety
  - ○ Vital signs, ECG, physical examination, laboratory safety tests: blood tests: red blood cells (RBC), haemoglobin, haematocrit, platelets, white blood cells

(WBC, differential: neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils), sodium, potassium, magnesium, chloride, bicarbonate, creatinine, serum glucose, blood urea nitrogen (BUN), alkaline phosphatase, lactate dehydrogenase (LDH), serum glutamic-oxaloacetic transaminase/aspartate amino transferase (SGOT/AST), serum glutamicpyruvic transaminase/ alanine aminotransferase (SGPT/ALT), total bilirubin, total protein, albumin, calcium, amylase, lipase; and urine tests: pH, specific gravity, protein, glucose, ketones, blood.

- Change in histology

- Tolerability: assessment of adverse events.
- Pharmacokinetics: through and peak concentration of YF476

10 Methods

10.1 Methods for clinical procedures

Upper endoscopy with biopsies will be performed at baseline (Visit 2) and at the Week 12 visit, after 12 weeks' treatment with study medication. Patients will fast before the endoscopy, and will need to come accompanied to the site endoscopy suite, according to hospital practice and regulations. Upon arrival at the endoscopy suite, a peripheral intravenous line will be inserted. Informed consent will be obtained for the endoscopy prior to starting the procedure. In the procedure room, a local anaesthetic (xylocaine) will be sprayed in the back of the mouth to diminish the gag reflex. Subsequently, intravenous medication (specifically, midazolam, fentanyl, and/or propofol) will be administered in order to achieve moderate sedation. Once the patient has been sedated, the upper endoscope will be inserted into the esophagus and advanced to the second portion of the duodenum. The following represents standard clinical care for Barrett's esophagus: 1) the area of Barrett's esophagus will be carefully inspected for any focal nodules or mucosal irregularities that could represent the presence of dysplasia; 2) if any such abnormalities are noted, then targeted biopsies of these areas will be taken; 3) irrespective of the presence of such abnormalities, random 4-quadrant biopsies will be taken every 1–2 cm along the length of BE. For study purposes, 6 additional biopsies will be taken and snap frozen for qRT-PCR analyses: GE junction (top of the gastric folds; 2 biopsies); mid-Barrett's segment (2 biopsies); normalappearing esophageal mucosa (2 cm above the maximal extent of BE; 2 biopsies).

10.2 Rationale for laboratory methods

In general, it is desirable to perform quantitative analyses of biomarkers, as achieved by using quantitative real-time PCR (qRT-PCR), in contrast to qualitative or semi-quantitative immunhistochemical analyses (IHC). Quantitative analysis allows for detection of small differences between different tissue samples. However, qRT-PCR does not discriminate between different cell types within a particular tissue sample, whereas IHC allows for visual assessment of the type and location of cells that express a biomarker of interest.

- IHC for Ki67 is a robust marker of ongoing proliferation, and has been reliably and reproducibly used to evaluate cell type-specific evaluation. Quantitative assessment of the number of proliferating (Ki67+) epithelial cells is easiest and most reliable in IHC.
- For the assessment of CCK2R expression in human esophageal tissue, qRT-PCR seems to be the best method, as IHC with various antibodies has proven to be unreliable and non-specific.
- Biologically relevant COX-2 expression can be quantitatively assessed using qRT-PCR, but cannot be detected by IHC.
- DCAMKL-1 is a putative marker of intestinal stem cells. In order to analyze expansion of these putative stem cells in the development of Barrett's esophagus, it is important to get an impression of the precise location of these cells. Therefore, both qRT-PCR and IHC will be used to analyze DCAMKL-1 expression.
- Changes in the levels of p53 protein expression can be detected best with IHC, as mRNA levels of p53 do not differ very much despite altered protein expression. The function of p53 is more likely determined by protein stability.

All the above described methods have been performed in previously published studies (by the chief investigator's group and others) as well as in Dr. Timothy Wang's laboratory in a new mouse model of Barrett's esophagus and adenocarcinoma. Therefore, the investigators will be able to compare the achieved data with previously published and unpublished results from the chief investigator's laboratory and other groups.

10.3 Laboratory methods

10.3.1 Laboratories

All tissue biomarker analyses will be performed in the laboratory of Dr. Timothy Wang (Columbia University, New York, NY). Histological review of specimens, including IHC assessment of Ki67, p53, and DCAMKL1 expression, will be performed by Dr.

Antonia Sepulveda (Columbia University, New York, NY). Routine testing of blood and urine specimens will be performed locally.

10.3.2 Collection and handling procedures

- *Collection and analysis of tissue for IHC and qRT-PCR* qRT-PCR. Routine endoscopic biopsies (4 quadrant biopsies every 2 cm along the length of BE) will be taken for clinical purposes and placed in formalin. The formalin-fixed biopsies taken at CUMC will be submitted to the Columbia University Department of Pathology (CUDP). The formalin-fixed biopsies taken at NIHR CIW will be stored there; slides of the biopsies will be sent by courier to CUDP at the end of the study, or as agreed between the investigators and sponsor. The respective Departments of Pathology will store the paraffin-embedded samples as per usual practice. Histologic analysis of biopsies from both study sites will be

done at CUDP. As per clinical practice, these biopsies will be reviewed to assess the presence of dysplasia. Six additional biopsies (from each endoscopy; 2 biopsies each from GE junction, mid-Barrett's segment, and normal esophagus) will be taken for study purposes, placed in Qiagen Allprotect™ tissue reagent, and then stored at –80°C for future qRT-PCR analysis. The freezers for storing these samples are located in the laboratory of Dr. Timothy Wang at Columbia University. Samples taken at Cambridge University will be stored in freezers in Prof. Rebecca Fitzgerald's laboratory at the MRC Cancer Unit, University of Cambridge, until dispatch to Columbia University.

IHC analyses. Additional sections will be cut from paraffin-embedded endoscopic biopsies for study purposes. Slides from NIHR CIW will be sent by courier to Columbia University at the end of the study, or as agreed between the investigators and sponsor. Ki67 expression will be assessed by IHC from esophageal biopsies of Barrett's esophagus. Paraffin-embedded biopsies from the most distal esophageal biopsies containing intestinal metaplasia will be immunostained for Ki67. Ki67 index will be calculated as: (# crypt epithelial cells with positive nuclear staining for Ki67) / (# BE crypt cells counted). Five representative crypts with goblet cells will be evaluated from five separate fields at 100x magnification. ΔKi67 for YF476 and placebo arms will be compared. The pathologist evaluating Ki67 staining will be blinded to both treatment arm and timing of endoscopy (ie pre- or post-treatment).

- *Collection and processing of blood samples for laboratory safety tests:*

20 mL of blood will be collected. 10 mL (one purple top, one gold top tube) will be used for routine measurements. The other 10 mL (one purple top, one gold top tube) will be processed for separate storage of serum, plasma, and Buffy coats at –80°C.

  - Safety tests:
    - haematology: Hb, RBC, haematocrit, WBC and differential, and platelets;
    - biochemistry: creatinine, total bilirubin, BUN, LDH, total protein, albumin, alkaline phosphatase, AST, ALT, glucose, potassium, sodium, calcium, magnesium, chloride, amylase and lipase.

Blood specimens will be collected when the patient is fasting. If the blood collection occurs on the day of the endoscopy, the blood will be collected prior to the endoscopy.

- *Collection of urine for laboratory safety tests*

A routine urine sample will be collected for standard urinalysis: dipstick test: protein, blood, ketones, glucose, specific gravity, pH; microscopy if the result of the dipstick test for protein or blood is abnormal; and pregnancy test (pre-menopausal women only). •

Collection and processing of blood samples for serum gastrin and stored serum

Venous blood for serum gastrin assay and stored serum (2 × 2.5 mL) will be collected into polypropylene tubes and will be allowed to clot at room temperature for at least 20 min.

After clotting, the tubes will be centrifuged at about 1500 G for 10 min at 4°C, and the serum transferred to smaller tubes in equal aliquots then stored at –80°C before shipment to the HMR Analytical Laboratory, Cumberland Avenue, Park Royal, London, NW10 7EW.

Shipment of serum samples for gastrin assay and storage will be agreed between the investigator and sponsor. Serum will be stored by the HMR laboratory for assay of trial-related markers only, which will be decided on at a later date.

- *Collection and processing of plasma for CgA assays*

4 mL venous blood will be collected into an EDTA tube and centrifuged at about 1500 G for 10 minutes at 4°C. 2 equal aliquots of plasma will be transferred to polypropylene tubes, and stored at –80°C, before shipment to the HMR Analytical Laboratory, Cumberland Avenue, Park Royal, London, NW10 7EW. Shipment of plasma samples for CgA assay will be agreed between the investigator and sponsor. All sample handling materials will be provided by the HMR Analytical Laboratory.

- *Gastrin and CgA assays*

Serum gastrin and plasma CgA will be measured by the HMR Analytical Laboratory using commercially available assays. The analytical laboratory may not analyse samples collected from patients with HIV.

- *Collection and processing of blood samples for YF476 PK*

YF476 is light sensitive, so blood samples (4 mL) will be taken into foil-wrapped or amber lithium heparin tubes and immediately placed on ice. Samples will be centrifuged at about 1500 G for 10 minutes at 4°C. Plasma will be transferred to foilwrapped or amber polypropylene storage tubes. Plasma samples will be stored at –80°C within 90 minutes after collection, and stored in darkness until dispatch. Samples will be sent to the HMR laboratory, Cumberland Avenue, London, NW10 7EW for reconciliation before being sent to Analytical Services, St George's Hospital for analysis of YF476 by the method of Redrup *et al* 2002.[18]

- All specimens will be labelled with a code assigned to the study patient.
- A separate tracking sheet will be maintained for each study patient. All specimens will be logged on these sheets, including date and time of collection.
- Samples will be kept in storage for the duration of the study. Unused samples will be kept in storage for an additional three years prior to disposal.

The analytical laboratory may not analyse samples collected from patients with HIV.

11 Reporting adverse events

Definition: an adverse event (AE) is any untoward medical occurrence in a study participant. An AE does not necessarily have a causal relationship with the treatment or study participant. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with participation in a study, whether or not related to that participation. This includes any deaths that occur while a participant is on a study.

A list of adverse events that have occurred or might occur can be found in Section 4.3, and in the Investigator Brochure.

11.1 Adverse events (AEs)

- **Reportable adverse events**

  All adverse events that occur after the informed consent is signed will be recorded on the adverse event CRF whether or not related to study medication.

- **AE data elements** ○ AE reported date ○ AE Verbatim Term
  ○ SOC (System Organ Class from MedDRA)
  ○ Common Terminology for Adverse Events v4.0 (CTCAE) AE term ○ Event onset date and event ended date ○ Severity grade
  ○ Attribution to study medication (relatedness)
  ○ Whether or not the event was reported as a Serious Adverse Event (SAE) ○ Whether or not the patient dropped due to the event ○ Action taken with the study medication ○ Outcome of the event ○ Comments

- **Severity of AEs**

  Adverse events will be identified using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE provides descriptive terminology and a grading scale for each adverse event listed. A copy of the CTCAE can be found at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_4 0

  AEs will be assessed according to the CTCAE grade associated with the AE term. AEs that do not have a corresponding CTCAE term will be assessed according to the general guidelines for grading used in the CTCAE v4.0. as stated below.

- **CTCAE v4.0 general severity guidelines**

  (from http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.02_2009-09-15_QuickReference_8.5x11.pdf)

Grade	Severity	Description
1	Mild	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
4	Lifethreatening	Life-threatening consequences; urgent intervention indicated.
5	Fatal	Death related to AE.

- **Activities of Daily Living (ADL)**

  *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

  **Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

- **Assessment of relationship of AE to treatment**

  The possibility that the adverse event is related to study medication will be classified as one of the following: not related, unlikely, possible, probable, definite.

- **Follow-up of AEs**

  All AEs, including abnormalities that in the opinion of the investigator are clinically significant, will be followed according to good medical practices and documented as such.

11.2 Serious adverse events (SAEs)

11.2.1 Definition

ICH Guideline E2A and Fed. Reg. 62, Oct. 7, 1997 define serious adverse events as those events, occurring at any dose, which meet any of the following criteria:

- Results in death.
- Is life threatening (Note: the term life-threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity.
- Is a congenital abnormality/birth defect.
- Events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner.

11.2.2 Reporting SAEs

- The investigators will report SAEs on the SAE form.
- The Sponsor representative and the Data Safety Monitoring Board (DSMB) representative will be contacted within 24 hours of knowledge of the event.
- The following information will be included when informing the Sponsor and the DSMB representative:

  ○ when the SAE occurred ○ when the SAE report will be available

  ○ Name of reporter ○ Call back phone number (if primary contact is by phone) ○ Protocol number

- Description of the SAE, including attribution to drug

- The investigators will FAX (or email a scan of) written SAE reports to the Sponsor and DSMB within 48 hours of learning of the event using the paper SAE form.
- The Sponsor will determine which SAEs require submission to the competent authorities.
- The Lead Organization and all Participating Organizations will comply with applicable regulatory requirements related to reporting SAEs to the IRB and/or REC.
- Follow-up of SAE: site staff will send follow-up reports as requested when additional information is available. Additional information will be entered on the

  SAE form in the appropriate format. Follow-up information will be sent to the Sponsor and DSMB as soon as available. SAEs will be followed by the study staff until resolved.

12 Data management and quality assurance

Patient data will be collected using protocol-specific case report forms (CRF). These will be designed and produced by HMR. The final version will be approved by the sponsor. All data will be entered legibly in black or blue ink with a ball-point pen. If an error is made, the error will be crossed through with a single line in such a way that the original entry can still be read. The correct entry will then be clearly inserted and the alterations will be initialled and dated by the person making the alteration. Overwriting or use of correction fluid is not permitted.

To preserve confidentiality, the CRFs will not bear the subject's name. The subject's initials, date of birth, and subject number will be used for identification.

It will be the responsibility of the investigator to ensure the accuracy of all data entered in the CRFs.

The data will be securely stored by the investigator. After the CRFs have been signed off by the principal investigator, the original NCR copies will be sent to the Data Management department at HMR, where they will be securely stored on behalf of the sponsor.

Data will be double-entered into a clinical database management system (ClinPlus Version 3.3), which is based on SAS® Version 9.2. Edit checks and generation of queries will be done in ClinPlus. Tabulations and listings will be produced using validated, trial-specific SAS® programs.

Data will be checked by the HMR Quality Assurance (QA) Department. In addition, the HMR QA Department will audit the trial report; that audit will include checks to ensure that statistical output is correctly reproduced in the report.

Source documents. Before the start of the study, the sponsor and investigator(s) will sign an agreement listing the source documents to be used in this trial. The CRF will be source for the following datapoints: heart rate and blood pressure.

12.1 Data and safety monitoring plan

The Data Safety Monitoring Board (DSMB) will review all SAE forms as well as all AEs. If additional information is required, the DSMB will request that information from the PI. The DSMB will review reported AEs at regular meetings and provide written reports to the PI. The reports will contain written information regarding findings for the trial as a whole related to cumulative toxicities observed and any relevant recommendations related to continuing, changing, or terminating the trial.

Members of the DSMB are as follows:
Benjamin Lebwohl (Columbia University, Division of Digestive & Liver Diseases) Lorna Dove (Columbia University, Division of Digestive & Liver Diseases)
Zhezhen Jin (Columbia University, Department of Biostatistics)
Hugo Ford (Cambridge University, Cancer Services)

12.2 Sponsor or regulatory monitoring

Trio Medicines, Ltd. (or their designee) or the relevant regulatory authorities may monitor/audit various aspects of the study. These monitors will be given access to facilities, databases, supplies and records to review and verify data pertinent to the study.

12.3 Record retention

The sponsor and both principal investigators will keep the following in a trial master file.

- All the essential documents required by GCP
- All data generated during the study

All documents will be stored such that they are readily available for inspection at the request of the sponsor or a regulatory authority. Any transfer of ownership of the investigator's data or documents will be documented, and the sponsor will be informed.

For at least 15 years after the end of the study, and at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the study medication:

- HMR will ensure that the sponsor's master file and original copies of CRFs will be archived in a secure place; and
- the investigators will ensure that the investigator's master file, and original source documents (other than CRFs) will be archived in a secure place.

12.4 Clinical Trial Agreement

The sponsor and the investigators will sign an agreement outlining each party's obligations with respect to the trial.

Each party will allow the other 30 days to comment before any results are submitted for publication, or 10 days prior to an abstract presentation. Authorship should reflect work done by the investigators and personnel of the sponsor, in accordance with generally recognized principles of scientific collaboration.

13 Statistical methods

13.1 Analysis Sets

The safety analysis set will include all patients who are randomized and receive at least one dose of study medication. In this population, treatment will be assigned based upon the treatment patients actually receive regardless of the treatment to which they were randomized.

The pharmacokinetic analysis set will include all patients who receive at least one dose of study medication, and yield at least one plasma concentration for YF476.

The Intent-to-Treat (ITT) analysis set will include all randomized patients who receive at least one dose of study medication and have at least one post-baseline assessment. In this population, treatment will be assigned based upon the treatment to which patients were randomized regardless of which treatment they actually received. A per-protocol analysis will also be completed.

13.2 General Statistical Considerations

The null hypothesis is that there is no difference between YF476 and placebo in the treatment of patients with Barrett's esophagus, the alternative hypothesis is that there is a difference between the treatments. Statistical significance will be defined as $p \leq 0.05$.

Statistical analysis will be done by HMR. The statistical package SAS (version 9.2) will be used to produce all summary tables and data listings.

For the data analysis, missing or dropout data will not be imputed; analyses will be performed only on observed data. In this study, there will be no significance level adjustment for multiplicity.

Quantitative data will be tabulated with descriptive summary statistics: arithmetic mean, standard deviation, median, minimum and maximum values, and number of observations. Geometric mean and geometric CV% will be provided for C_{max} and C_{trough}. For categorical data, frequency tables will be provided.

A detailed Statistical Analysis Plan (SAP) describing the methodology to be used in the final analysis will be prepared by the HMR Statistics and Data Management Department after completion of the final protocol. Any deviations from the planned statistical analyses in the protocol will be fully described in the SAP.

13.3 Study Population Data

The safety population will be summarized for demographic characteristics, and will be presented. Continuous demographic variables (age, weight, height, and BMI) will be summarised with descriptive statistics. Categorical demographic variables (gender, race, and ethnicity) will be summarised with frequency counts and corresponding percentages.

13.4 Efficacy Analyses

All efficacy analyses, summaries and listings will be generated using the efficacy analysis set.

Primary efficacy variables are:

- Change in Ki67 expression (ΔKi67) Secondary efficacy variables are:
- Change in CCK2R mRNA levels (measured by qRT-PCR)
- Change in DCAMKL1 expression (measured by IHC)
- Change in DCAMKL1 mRNA levels (measured by qRT-PCR)
- Change in COX-2 mRNA levels (measured by qRT-PCR)
- Change in p53 expression (measured by IHC)
- Change in serum gastrin and plasma CgA between baseline and Visit 5 For all efficacy variables listed above, individual values will be presented by patient and summarised by visit.

The primary endpoint (Change from baseline (Visit 2) in Ki67 expression (ΔKi67)) will be summarised using descriptive statistics. The primary analysis for the primary endpoint will use two sample t-tests comparing between treatment groups. Nominal p-value and 95% confidence intervals for treatment difference will be reported. To evaluate the robustness of the primary analysis, the following sensitivity analysis will also be performed:

Baseline Adjusted Analysis: this sensitivity analysis will use an analysis of covariance model with the primary endpoint as the dependent variable, treatment group as a factor and baseline (Visit 2) as a covariate. Adjusted least square means and corresponding 95% confidence interval will be reported.

Serum gastrin and chromogranin A (CgA) will be measured at baseline (Visit 2) and at Weeks 6 and 12. Analyses will be performed to determine whether there is a correlation between baseline gastrin and subsequent ΔKi67. YF476 assays will be performed at Weeks 6 and 12.

Analysis of the secondary endpoints will be similar to the primary analysis. No sensitivity analysis is planned for these endpoints.

13.5 Pharmacokinetic Analyses

The statistical analysis of pharmacokinetic parameters will be done by the HMR Statistics and Data Management Department.

All pharmacokinetic analyses, summaries, and listings will be generated using the pharmacokinetic analysis set.

The pharmacokinetic parameters will be listed in tables of individual values and aggregated in summary tables. Descriptive statistics (sample size, mean, standard deviation, coefficient of variation [%CV], standard error of the mean, minimum, maximum, and median) will be calculated at each evaluation timepoint for plasma concentrations of YF476.

Individual, mean and median plasma concentration-time data will be presented graphically.

The relationship between PK concentrations and plasma CgA concentration or serum gastrin concentration will be examined using a graphical approach.

13.6 Safety Analyses

Summaries and listings of safety data will be generated using the safety analysis set.

13.6.1 Adverse Event Analyses

Adverse events (AEs) will be coded using the version of the Medical Dictionary for Regulatory Activities (MedDRA) that is current when the database is locked. All AEs including serious AEs will be mapped to system organ class and preferred term, and will be listed in the data listing.

A treatment-emergent adverse event (TEAE) is defined as an AE that emerges during treatment, having been absent pre-treatment, or that worsens relative to the pre-treatment state.

The number and percentage of patients reporting TEAEs will be tabulated for the safety analysis set by MedDRA preferred term and system organ class, and further by relationship to study drug administered and by severity.

Listings of deaths, SAEs, and AEs that lead to discontinuation of a patient will be presented.

13.6.2 Analyses of Clinical Laboratory Tests

Standard haematology, biochemistry and urinalysis at each planned assessment, and change from baseline at each planned post-baseline assessment, will be summarized.

13.6.3 Electrocardiogram Analyses

The ECG variables (PR, RR, QRS, QT, QTcB, and QTcF) and heart rate (HR) at each planned assessment will be summarised. The QT intervals will be corrected for heart rate by the Bazett's and Fridericia's formula ($QTcB = QT/[RR]^{1/2}$ and $QTcF = QT/[RR]^{1/3}$). The incidence of outliers in absolute QT, QTcF and QTcB intervals (>450, >480, and >500 msec), and the change from time-matched baseline in QT, QTcF and QTcB intervals (>30 and >60 msec) will be summarised.

13.7 Determination of sample size

Based on our preliminary data, we estimate a mean baseline Ki67 index of 45–50%, with a common SD of 14–18%. Assuming a two-sided type I error rate of 0.05, a sample size of 20 (10 per arm) has 98% power to detect a 30% absolute difference in ΔKi67 between the two arms if the common SD is 16%, and 97% power for a 25% difference with a 14% common SD (see Table 1).

Table 1. Power calculations based on 20 patients (10 per arm)

	$\Delta Ki67_{YF} - \Delta Ki67_{pl}$		
SD	**0.25**	**0.30**	**0.35**
0.14	97%	99%	>99%
0.16	91%	98%	>99%

0.18	84%	94%	98%

14 Ethical and regulatory considerations

This trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements.

14.1 Columbia University Medical Center, New York, US

14.1.1 Form FDA 1572

Prior to initiating this study, the Chief Investigator and the Principal Investigator at the Participating Organization(s) will provide a signed Form FDA 1572 stating that the study will be conducted in compliance with regulations for clinical investigations and listing the investigators that will participate in the protocol.

14.1.2 Other required documents

- Signed and dated current (within two years) CV or short biography for all investigators listed on the Form FDA 1572 for the Lead Organization and all Participating Organizations.
- Current medical licenses for all investigators listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.
- Laboratory certification (eg, CLIA, CAP) and laboratory normal ranges for all labs listed on Form FDA 1572 for the Lead Organization and all Participating Organizations.
- IRB Membership list/letter from IRB for the Lead Organization and all Participating Organizations.
- Documentation of training in “Protection of Human Research Subjects” for all investigators listed on the FDA Form 1572 for the Lead Organization and all Participating Organizations.
- Documentation of Federal-wide Assurance (FWA) number for the Lead Organization and all Participating Organizations.
- Signed receipt of Investigator Brochure
- Delegation of Responsibility form
- FDA Form 3455 Financial Disclosure Form
- Evidence of submission of this protocol to the FDA

14.1.3 Institutional Review Board (IRB) approval

Prior to initiating the study and receiving study medication, the Investigators at the Lead Organization and the Participating Organization(s) will obtain written approval to conduct

the study from the appropriate IRB. Should changes to the study become necessary, protocol amendments will be submitted to the respective institutional IRBs.

14.2 National Institute for Health Research Clinical Investigation Ward, Cambridge, UK

The trial protocol will be reviewed by a recognised REC, the Scientific Advisory Board (SAB) at Addenbrooke's Clinical Research Centre, and by the MHRA. The trial will not proceed in the UK unless the sponsor obtains from the MHRA a clinical trial authorisation (CTA), and the main REC and SAB approves the trial. The trial will not proceed until the local National Health Service Trust has approved it.

The trial will be done in compliance with EU Directives 2001/20/EC and 2005/28/EC, The Medicines for Human Use (Clinical Trials) Regulations 2004 and current amendments, the Declaration of Helsinki (South Africa Revision, 1996), GMP, the standard operating procedures issued by NRES for RECs in the UK, and Good Clinical Practice.

The sponsor will ensure that the MHRA and the REC are informed promptly of suspected serious adverse reaction (SUSARs), and that any new reports of SUSARs from other ongoing trials of the IMP under investigation in this trial are notified to the MHRA, and to the REC, if applicable. The sponsor will provide the investigator, the REC and the MHRA with annual safety reports of each IMP under investigation, and listings of all SSAR reports.

The investigator will promptly inform the sponsor and chief investigator of any SAE that occurs during this trial. The principal investigator will in turn inform the REC, if applicable. The principal investigator will provide the REC with annual progress reports of the trial, if it lasts longer than a year.

The principal investigator will report to the sponsor and chief investigator any protocol deviation that is, in his or her opinion, of clinical significance. The principal investigator will also inform the sponsor and chief investigator in the event of several deviations which, although of no clinical significance, cause inconvenience and/or discomfort to the volunteers. The principal investigator will in turn promptly inform the REC.

The sponsor will notify the MHRA and REC of any serious breach of Good Clinical Practice (for example, the investigator puts subjects' safety at risk, falsifies data, or persistently fails to comply with this protocol or Good Clinical Practice).

Within 90 days after the end of the trial, the sponsor will ensure that the REC and the MHRA are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The sponsor will supply a summary report of the clinical trial to the MHRA and REC within 1 year after the end of the trial.

14.3 Informed consent

All potential study participants will be given a copy of the IRB- or REC-approved information and consent form (ICF) to review. The investigator will explain all aspects of

the study in lay language and answer all questions regarding the study. If the patient decides to participate in the study, he/she will be asked to sign and date the ICF. The study medication will not be released to a patient who has not signed the Informed Consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

Prior to study initiation, the site-specific ICFs will be reviewed and approved by the IRB or REC, as appropriate. Prior to implementation, any changes to the ICF for Columbia University will be approved by the IRB, and any substantial changes to the ICF for Cambridge University will be approved by the REC.

15 Financing, expenses, and/or insurance

The baseline endoscopy and biopsies are considered standard of care for eligible study patients, so the cost of those procedures will not be covered by the trial. At CUMC, the baseline endoscopy and biopsies will be billed to the study patients' insurance. All additional tests and procedures, including the endoscopies and biopsies at the Week 12 visit, will be covered by the trial. The YF476 and placebo capsules will be provided free of charge. If the participant is injured and requires medical treatment as a direct result of taking part in this study, the study sponsor will pay reasonable medical costs (doctor's fees and medical expenses) needed to treat the injury. The treatment must be authorized by the investigator except in the event of an emergency in which case the investigator should be notified as soon as possible.

16 Obligations of the sponsor and investigator

16.1 Publication

Each party will allow the other 30 days to comment before any results are submitted for publication, or 10 days prior to an abstract presentation. Authorship should reflect work done by the investigators and personnel of the sponsor, in accordance with generally recognized principles of scientific collaboration.

16.2 Archiving

The sponsor and both principal investigators will keep the following in a trial master file.

- All the essential documents required by GCP
- All data generated during the study

All documents will be stored such that they are readily available for inspection at the request of the sponsor or a regulatory authority. Any transfer of ownership of the investigator's data or documents will be documented, and the sponsor will be informed.

For at least 15 years after the end of the study, and at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of clinical development of the study medication:

- HMR will ensure that the sponsor's master file and original copies of CRFs will be archived in a secure place; and
- the investigators will ensure that the investigator's master file, and original source documents (other than CRFs) will be archived in a secure place.

16.3 Premature termination of the trial

The sponsor and investigator reserve the right to terminate this trial should serious or severe AEs or any other safety issue occur during the trial. In addition, the sponsor may, at any time for any reason, terminate the trial by halting further recruitment of patients. If the trial is terminated prematurely, the investigator will return all CRFs to the sponsor, and the sponsor or investigator, as appropriate, will provide a written statement of the reasons for termination. The sponsor will ensure that the IRB, REC and competent authorities are notified.

17 References

1. Shaheen NJ. Advances in Barrett's esophagus and esophageal adenocarcinoma. Gastroenterology 2005;128:1554-66.

2. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2008. CA Cancer J Clin 2008;58:71-96.

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