Tailored treatment of functional dyspepsia with nortriptyline: a multi-center double-blind placebo-controlled trial

PROTOCOL TITLE: 'Tailored treatment of functional dyspepsia with nortriptyline: a multi-center double-blind placebo-controlled trial (TENDER)'

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
AMC	**Academic Medical Center (Amsterdam)**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CTCM	**Clinical Trial Center Maastricht**
CYP	**Cytochrome P450**
DSMB	**Data Safety Monitoring Board**
ECG	**Electrocardiography**
EMA	**European medicines agency**
EPS	**Epigastric pain syndrome**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
FD	**Functional dyspepsia**
FDA	**Food and Drug Administration**
GCP	**Good Clinical Practice**
GDP	**Good Distribution Practice**
GMP	**Good Manufacturing Practice**
IB	**Investigator's Brochure**
IBS	**Irritable bowel syndrome**
IC	**Informed Consent**
IGJ i.o.	**Dutch: Inspectie Gezondheidszorg en Jeugd in oprichting**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch ethische toetsing commissie (METC)**
MCQ	**Medical consumption questionnaire**
NDI	**Nepean dyspepsia index**
PCQ	**Productivity cost questionnaire**
PDS	**Postprandial distress syndrome**
PPI	**Proton pump inhibitor**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics (in Dutch: officiële productinfomatie IB1-tekst)**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
TCA	**Tricyclic antidepressant**
Wbp	**Personal Data Protection Act (in Dutch: Wet Bescherming Persoonsgevens)**

WMO	**Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Functional dyspepsia (FD) is a common functional gastrointestinal disorder characterized by upper abdominal discomfort/pain and/or symptoms of meal-related fullness/satiety. There is currently no definitive therapy that is beneficial for all FD patients. Accumulating evidence suggests efficacy of tricyclic antidepressants (TCAs) in FD. However, no firm conclusion can be drawn currently due to the relatively small amount of studies and large heterogeneity between studies. In addition, TCAs are often associated with side effects, which occur early after initiation of therapy preceding the therapeutic effect and often result in discontinuation of the therapy. These side effects are related to drug metabolism, which depend on polymorphisms of the cytochrome P (CYP) enzyme system. It is therefore hypothesized that pre-treatment assessment of CYP genotype and subsequent exclusion of abnormal metabolizers limits the occurrence of side-effects and as such improves compliance and efficacy.

Hypothesis: We hypothesize that treatment of FD patients identified as CYP2D6 extensive metabolizers with the second generation TCA nortriptyline results in adequate relief of symptoms.

Objectives:
Primary objective:
1. To assess the efficacy of an escalating dose regimen of nortriptyline as compared to placebo in FD patients without evidence of significant psychiatric disease, that have been identified as extensive metabolizers based on their CYP2D6 genotype.

Secondary objectives:
1. To evaluate the effect of treatment on quality of life as compared to placebo.
2. To evaluate the cost-effectiveness of treatment as compared to placebo.
3. To evaluate the effect of treatment after discontinuation as compared to placebo.
4. To evaluate side-effects of treatment as compared to placebo.

Study design: A randomised, double blind, placebo-controlled clinical trial with two parallel study arms.

Study population: 154 FD patients (according to Rome IV criteria), 18 - 65 years old.
Intervention: Group A will receive 12 weeks of daily treatment with placebo capsules, group B will receive 12 weeks of daily treatment with nortriptyline capsules in an escalating dose regimen (CYP2D6 extensive metabolizers only).

Main study parameters/endpoints:
Currently, no official guidelines from the regulatory authorities are available for trials in FD. Therefore, electronic patient-reported outcome measures are developed with input of patient experts in order to measure dyspepsia symptoms including epigastric pain and side effects on a daily basis throughout the treatment period, according to current recommendations from the Rome Foundation.

Primary parameter:

- Response to therapy, as defined by a 30% reduction from baseline (i.e. the run-in period) in the weekly average of daily symptom scores, during at least 50% of weeks 3-12 of treatment. This is in line with the FDA guidelines on IBS treatment studies. Recorded symptoms include the five core symptoms of FD: epigastric pain, epigastric burning, postprandial fullness, early satiety and upper abdominal bloating.

Secondary parameters:

- Self-reported adequate relief. Adequate relief is defined as a 'yes' response in at least 50% of weeks 3-12 of the treatment.
- Quality of life, assessed with the use of the EQ-5D-5L (change from baseline).
- Dyspepsia-specific quality of life, assessed with the use of the Nepean Dyspepsia Index (change from baseline).
- Cost-utility, as determined by calculations incorporating total treatment costs and changes in EQ-5D-5L (QALYs gained) and MCQ/PCQ results (savings from reduced medical resource use and increased work productivity respectively).
- Use of rescue medication.
- Number and severity of side effects.
- Responder rates following discontinuation of treatment at 6 months follow-up, as defined by a "Yes" to the query regarding adequate relief from baseline symptoms.
- Worst-case-analysis: imputing a non-response day for each day on which the electronic diary entry was missing (due to non-reporting of the patient) in patients assigned to nortriptyline; in patients assigned to placebo, a response day will be imputed for each day the electronic diary entry was missing.
- (Negative) mood; assessed with the use of the PHQ-9 and GAD-7 (change from baseline).

All endpoint analyses will be based on the intention to treat population. The proportion of responders in each treatment group will be compared using logistic regression to provide covariate adjustments for centers, gender, age etc. in the analyses.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Subjects may be confronted with certain inconveniences and minor risks. Study participants have to visit the hospital 3 times (or have appointments by (video) call), including the first visit in which eligible subjects will be screened before participation. The screening will take up to 1 hour and will consist of a simple questionnaire, a general physical exam performed by the physician-investigator and a standard pregnancy test (in women of fertile age, <55 year only). In addition, a small blood sample will be taken for CYP genotyping during the screening visit using a finger prick(performed at this visit as results can take up to one week). If deemed suitable by the investigator, subjects that have been identified as CYP2D6 extensive metabolizer will enter the two week run-in period. During this period, patients are asked to report their daily symptom scores with the use of an electronic diary (mobile phone application). If after the run-in period patients meet the inclusion criteria, they will enter the treatment period. After one week of treatment, a dried blood spot will be performed to assess nortriptyline drug levels. In addition, patients are asked to report daily symptom scores using the same mobile phone application as during the run-in period, and complete several questionnaires electronically at multiple time-points. This will take several hours in total. Patients that are randomised to nortriptyline treatment may

experience relief of FD symptoms. If randomised to placebo, patients may still report some relief as placebo response is often high is this patient group. Side effects can occur with nortriptyline treatment. These generally disappear with continuation of treatment. The most frequent side effects of nortriptyline include dry mouth, headache, nausea, palpitations, weight gain and blurred vision (due to accommodative dysfunction). ECG changes can occur, but are most often harmless. An ECG will be performed prior to the start of treatment in selected patients to ensure that study medication (nortriptyline) can be started safely (see section 8.3.1, medical screening: ECG).

1. INTRODUCTION AND RATIONALE

Dyspeptic symptoms, including upper abdominal pain and symptoms of meal-related fullness occur often in the general population in Western countries with an estimated incidence of up to 40%.(1) Over 70% of patients presenting with dyspepsia do not have an identifiable cause of their symptoms such as peptic ulcer disease or Helicobacter gastritis and are therefore referred to as having functional dyspepsia (FD).(2) As no biomarkers for FD are known, the diagnosis is made on the basis of symptoms. The current standard for the diagnosis of FD are the Rome criteria, developed by the Rome Committee. According to the most recent criteria (Rome IV), issued in May 2016, FD is defined as having bothersome postprandial fullness, early satiety or epigastric pain/burning, in the absence of evidence for structural disease.(3)

The global prevalence of the condition is estimated to be 5-11%, based on Rome III criteria(4). However, large regional differences have been reported, with the prevalence being highest in Western populations, an estimated 10-20%.(5) Unfortunately, there is currently no definitive therapy that is beneficial for all FD patients. This is mainly due to the fact that the disorder is quite heterogeneous and the pathogenesis remains unclear. Several mechanisms have been suggested to play a role in the pathogenesis including visceral hypersensitivity, impaired gastric accommodation, delayed gastric emptying and low-grade duodenal inflammation (increased permeability and mucosal eosinophilia). According to the Rome criteria, FD is subdivided into epigastric pain syndrome (EPS) and postprandial distress syndrome (PDS).(3) This subdivision is based on the assumption that different pathophysiological mechanisms underlie different dyspeptic symptoms (predominantly sensory abnormalities in EPS vs. motoric disturbances of gastric physiology in PDS). The rationale for assigning patients into these two syndrome subtypes in clinical practice is that the classification may help guide therapy; anti-secretory and analgesic therapy for EPS and prokinetics for PDS. However, there is significant overlap between EPS and PDS. In addition, very recently, Vanheel et al. reported on a comprehensive analysis of 560 patients with FD and provided novel data on gastric physiological disturbances in PDS and EPS compared with an overlap group.(6) Interestingly, no differences between the Rome subgroups in the prevalence of gastric hypersensitivity, impaired gastric accommodation, and delayed gastric emptying were found. Furthermore, an association of gastric hypersensitivity was found with the severity of both PDS symptoms, EPS symptoms, and the cumulative symptom score, supporting the hypothesis that this mechanism plays an important role in the pathogenesis of FD symptoms, regardless of the FD subtype.

Treatment entities for FD include dietary modifications, anti-emetics, gastric acid inhibitors, prokinetics, psychological interventions and analgesics. Options cover a wide range and are applied based on the predominant symptom and patient preference. Generally, all patients with dyspepsia are treated with antisecretory drugs. However, in the absence of gastrointestinal reflux disease and/or peptic ulcer disease, literature points to a number needed to treat of 14.6 using proton pump inhibitors (PPI) in dyspepsia.(7) Overall, the effectiveness of PPI treatment in FD appears modest with a therapeutic gain of approximately 7–10%.(8) Therefore, the implementation of an effective alternative (and inexpensive) medicinal therapy has the potential to reduce unnecessary PPI use and has added potential with regards to cost-saving.

Accumulating evidence suggests efficacy for low-dose tricyclic antidepressants (TCAs) in FD. TCAs were approved in the 1960s and are widely used 'off-label' as non-narcotic analgesics.(9) TCAs are believed to act via noradrenergic and serotonergic descending inhibitory perceptive pathways and have extensively been used as neuromodulators in patients with chronic pain.(10) Earlier studies however were of small size and have not been compelling enough to routinely advocate antidepressants for FD,(11, 12) although the recent ACG guideline on FD recommends a TCA trial in symptoms unresponsive to PPI treatment.(13) Widespread use of TCAs, an otherwise relatively inexpensive drug group, is further hampered by the fact that these are traditionally considered antidepressants. There is therefore considerable reluctance from both patients and heath care providers to initiate a therapy with TCAs, especially in the absence of evident psychiatric comorbidity. In addition, TCAs are often associated with side effects, including constipation, drowsiness, dry mouth (due to anticholinergic and antihistaminergic effects), which appear to be dose dependent and occur early after initiation of therapy, preceding the therapeutic effect. This often results in discontinuation of an otherwise potentially effective therapy. On the other hand, previous studies have shown that the clinical response is independent of the total dose of TCAs, supporting the concept of titration of low dosages in FD (10-50 mg/day) – as opposed to the higher dosages traditionally used for psychiatric indications (150-300 mg/day). Furthermore, side effects appear to be dependable on drug metabolism. Fortunately, CYP genotyping can predict drug metabolism.(14) Dose adjustments can subsequently be made, which could possibly reduce side effects.

Through results of this study, we aim to provide strong supportive evidence for TCAs (more specifically, nortriptyline). Such results are currently lacking to allow more widespread use of this drug as an effective therapy for FD. Treatment of FD is currently considered a knowledge gap in the NHG guidelines and is also prioritized by the NVMDL. Convincing results allow more efficient use of current pharmacotherapies and potentially results in better treatment outcomes for patients, with fewer side effects and less healthcare utilization. Using a genotype-based approach, we aim to further optimize the treatment. A pre-treatment assessment of CYP2D6 genotype is used to exclude abnormal metabolizers (i.e. CYP2D6 poor, intermediate and ultrarapid metabolizers), as these patients are potentially at risk of side-effects and/or treatment failure. By including only CYP2D6 extensive metabolizers, we aim to reduce associated side effects, which often result in discontinuation of TCA therapy. Overall, this may also result in significant healthcare cost-reduction, as costs originating from ineffective treatments of FD and additional diagnostic testing could be decreased.

2. OBJECTIVES

Aim: To investigate the efficacy of nortriptyline in FD patients with a predicted CYP2D6 extensive metabolizer phenotype. Nortriptyline is a second generation TCA with significantly fewer anticholinergic side effects compared to the first generation TCA amitriptyline. In addition, its metabolism only involves one enzymatic step, which makes clinical response based on polymorphisms more predictable. Prior to initiation of the drug therapy, genotyping of the cytochrome P450 enzyme CYP2D6 - the primary enzyme involved in nortriptyline metabolism - will be performed. CYP2D6 poor, intermediate and ultrarapid metabolizers are excluded from the current study.

Primary objective:
To assess the efficacy of an escalating dose regimen of nortriptyline as compared to placebo in FD patients without evidence of significant psychiatric disease, that have been identified as extensive metabolizers based on their CYP2D6 genotype.
Hypothesis: We hypothesize that treatment of FD patients identified as CYP2D6 extensive metabolizers with the second generation TCA nortriptyline results in adequate relief of symptoms. This will lead to a higher total percentage of responders in the nortriptyline arm, compared to placebo.

Secondary objectives:
1. To evaluate the effect of treatment on quality of life as compared to placebo.
Hypothesis: We hypothesize that treatment of FD with nortriptyline will lead to an increased quality of life.

2. To evaluate the cost-effectiveness of treatment as compared to placebo.
Hypothesis: We hypothesize that treatment of FD with nortriptyline will lead to a reduction of direct costs (i.e. medical consumption related to FD) and indirect costs (e.g. costs related to absenteeism).

3. To evaluate the effect of treatment after discontinuation, as compared to placebo.
Hypothesis: We hypothesize that some rebound of symptoms will occur after discontinuation of treatment. However, we expect that abdominal pain scores will remain lower than at baseline. Overall medical consumption will be less than at baseline and quality of life and work productivity will remain higher than baseline.

4. To evaluate side-effects of treatment as compared to placebo.
Hypothesis
We hypothesize that the application of a low-dose regimen of nortriptyline in CYP2D6 extensive metabolizers results in little side effects, which will not occur significantly more often than in placebo.

3. STUDY DESIGN

This study is a randomised double-blind placebo-controlled clinical trial in FD patients. Patients that have been identified as CYP2D6 extensive metabolizers will be randomised into one of two arms:
1. Placebo;
2. Nortriptyline in an escalating dose regimen;

In order to include a representative population of patients, subjects will be recruited from both primary care as well as secondary/tertiary care. Patients in the primary care setting will be recruited by their general practitioner and secondary/tertiary care patients will be recruited by their gastroenterologist in the GI-outpatient clinic. The treating physicians will briefly inform eligible FD patients about the study. If the patient is interested, detailed oral and written study information will be given by the researcher. In addition, an announcement will be put on the website of the 'Prikkelbare Darmsyndroom Belangenvereniging', social media, local newspapers (of areas of participating centers) and in GP practices in the Maastricht area (see E3. Poster TENDER for advertisement info). Patients that are interested to participate in the study can contact the researcher directly. A (digital) screening visit will then be planned, at the earliest, seven days after patients have received detailed study information to allow sufficient time for consideration.

An overview of the study design is provided below. Total study duration will be approximately 3 years (1 year preparation, 2 years inclusion). A total of 154 patients will be included.

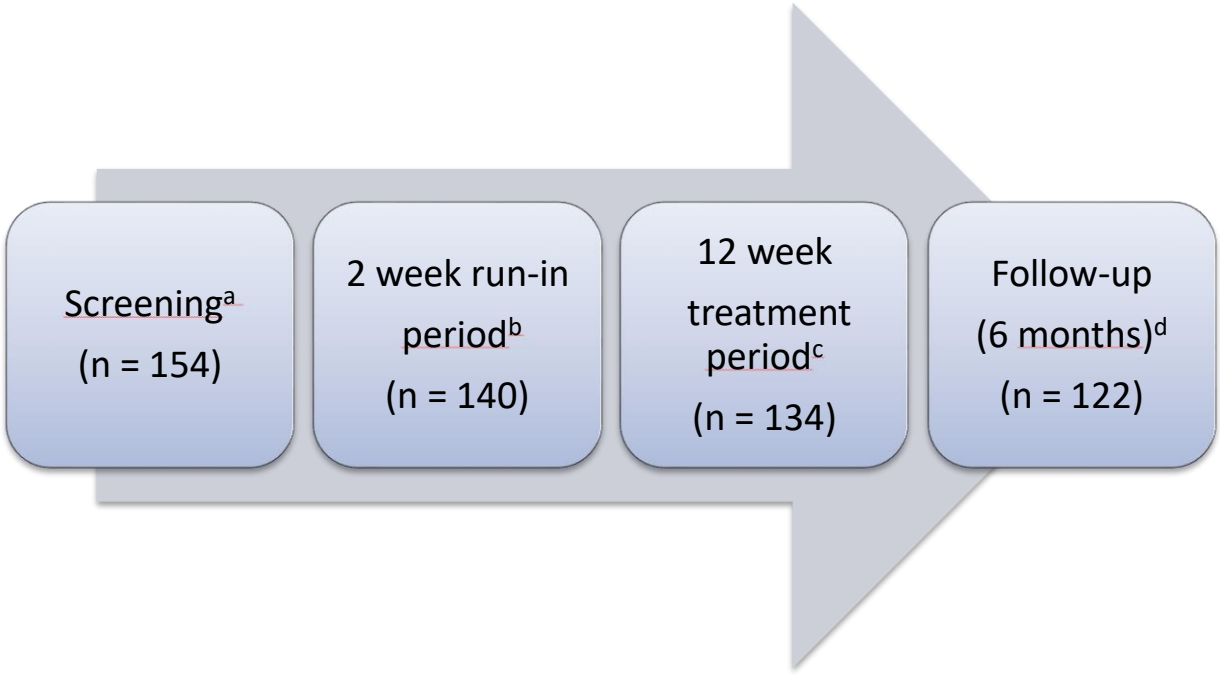

Figure 1. Study design.

a) Patients aged 18-65 years old with a diagnosis of functional dyspepsia according to the Rome IV criteria are considered eligible for the current study. In the presence of alarm symptoms, patients are required to have had a negative upper endoscopy and negative helicobacter pylori stool or breath test 2 years prior to inclusion. In addition, all patients are required to have insufficient effect of first-line treatment with proton

pump inhibitors (twice daily) and prokinetics (as advised by the current NHG guidelines). After signing informed consent and checking in- and exclusion criteria, a medical screening will be performed to assess the eligibility of the patient to participate in this study. This includes a short physical examination (weight, length) and a pregnancy test. In addition, an ECG will be performed when patients have cardiac comorbidity or are using medication that prolongs QT time (see section 8.3.1 Medical screening: ECG). Patients still using prokinetics will be asked to discontinue treatment. A wash-out period of 2 weeks before the run-in period is required. Patients that cannot discontinue prokinetic therapy will be excluded. Patients on proton pump inhibitors should continue these without altering dosage, given that rebound symptoms can occur with discontinuation (see section 5.2 Use of co-intervention). Finally, subjects are asked to complete the GAD-7 and PHQ-9 in order to screen for anxiety and depression using electronic questionnaires (all questionnaires are discussed in section 8.3.5). A cut-off value of 10 is used for both the GAD-7 and PHQ-9, as this corresponds to moderate anxiety and depression, respectively. In the case of a score of 10 or higher on either questionnaire, a detailed interview by the investigator is required. The investigator than decides (if needed, in consultation with the coordinating investigator and/or principal investigator) whether the scores indeed correspond to symptoms of anxiety or depression. If this is the case, the patient will be excluded from the study and the general practitioner and/or gastroenterologist will be informed, as additional/alternative treatment may be required. However, if scores of 10 or higher on the PHQ-9 or GAD-7 appear to be unrelated to depression or anxiety, the patient can still participate in the study.
Patients that are considered eligible will have a small bloodsample taken for CYP genotyping with the use of a finger prick. Results of this test will generally take 5-7 days. Only patients with a predicted CYP2D6 extensive metabolizer phenotype will be included in the current study; poor, intermediate and ultrarapid metabolizers will be excluded. Patients will be informed by telephone – or otherwise agreed upon according to the patient's preference – with regard to CYP phenotype and eligibility for participation. If a patient wishes to already continue with the run-in period, he or she can continue by filling in the symptom diary. However, these patients will still be excluded if predicted CYP 2D6 phenotype is poor, intermediate or ultrarapid metabolizer.

b) Potential study participants are enrolled in a 2 week run-in period. Symptoms will be recorded daily using a smartphone application. These symptoms include the five core symptoms of FD, as defined in the recently developed Functional Dyspepsia Symptom Diary of the PRO consortium's FD working group: epigastric pain, epigastric burning, postprandial fullness, early satiety and upper abdominal bloating. Subjects are required to have reported a minimal average score of 3 for one out of four core symptoms (bloating excluded) during the run-in period (on an 11-point NRS scale). After two weeks, the average symptom score will be calculated. Patients with an average symptom score below 3 will be excluded from the study and will be informed by telephone. Patients with a score of 3 or higher will be invited for the randomisation visit (in person or by telephone). This approach is in line with FDA guidelines on treatment trials in irritable bowel syndrome, a similar functional gastrointestinal disorder.
Patients with a symptom score of 3 or higher will be randomly assigned in equal proportions to one of two groups (placebo or nortriptyline). Randomisation will be

performed by the coordinating investigator (randomisation, blinding and treatment allocation discussed in section 8.2). The patients, the primary physician of the patient, the coordinating physician-investigator and study nurse will all be blinded to the treatment assigned.
The randomisation visit will be planned in the morning if patients choose to do the optional gastric emptying (breath) test (see section 8.3.2 Run-in period: gastric emptying test). Patients will then be asked to be fasting from 10pm the day before their visit. Finally, patients will be asked to complete the Nepean Dyspepsia Index (NDI; dyspepsia-specific quality of life questionnaire), EQ-5D-5L (quality of life questionnaire), MCQ and PCQ (questionnaires on healthcare consumption and work productivity) at the end of the run-in period using electronic questionnaires, i.e. baseline assessments.

c) The treatment period will be 12 weeks. Patients in both treatment arms will also receive standard care, including diet and lifestyle coaching. In addition, a dried blood spot sample will be taken at week 1 of treatment to assess 'steady-state' drug levels (results blinded). Patients will receive instructions to perform the fingerprick and dried blood spot sample at home. Patients that prefer the sample to be taken by medical personnel can plan an additional visit in week 1. The dried blood spot will be assessed at the Laboratory of Isala Zwolle. Patients and investigators will not be informed about the results of the nortriptyline plasma level assessments until the end of the study.
During the treatment period patients will again be asked to report symptoms daily using a smartphone application, including the same symptoms as during the run-in period. In addition, the digital diary will include a query on the occurrence of side effects. An 11-point numerical scale will be used to score severity of side effects. Answers will not be visible for the researchers during the study period however. Patients will therefore be advised to contact one of the researchers in the case of suspected side effects. If the presence of side effects is confirmed, the patient will be invited for additional blood sampling (conventional venepuncture), after which the sample will be analysed for nortriptyline plasma level (see section 8.33 Venepuncture). Finally, patients are asked to complete electronic questionnaires in week 0, 4, 8 and 12 (see section 8.3.5).

d) After a follow-up of 3 & 6 months after treatment is completed, patients will again be asked to complete several additional questionnaires sent by email. In addition, patients will be asked to report whether they experienced adequate relief of symptoms (as compared to baseline) during the follow-up.

4. STUDY POPULATION

4.1 Population (base)

FD patients aged 18-65 years will be recruited in both the primary and secondary/tertiary healthcare setting for a representative population of patients. The Rome IV criteria are used for a diagnosis of FD.(3) These criteria include:
One or more of the following;
a. Bothersome postprandial fullness
b. Bothersome early satiation
c. Bothersome epigastric pain
d. Bothersome epigastric burning
AND
2. No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms

In addition, patients must fulfil criteria for postprandial distress syndrome or epigastric pain syndrome to be diagnosed with FD (FD subtypes, listed below).

Postprandial Distress Syndrome (PDS)
Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.
Must include one or both of the following at least 3 days per week:
1. Bothersome postprandial fullness (i.e., severe enough to impact on usual activities)
AND/OR
2. Bothersome early satiation (i.e., severe enough to prevent finishing a regular-size meal)

Epigastric Pain Syndrome (EPS)
Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis.
Must include at least 1 of the following symptoms at least 1 day a week:
1. Bothersome epigastric pain (i.e., severe enough to impact on usual activities)
AND/OR
2. Bothersome epigastric burning (i.e., severe enough to impact on usual activities)

Note that a patient can fulfil criteria of both PDS and EPS.

4.2 Inclusion criteria (run-in period)

In order to be eligible to participate in this study, a subject must meet all of the following criteria:
- Age 18-65 years;
- A diagnosis of FD according to the Rome IV criteria(3);
- Predicted CYP2D6 extensive metabolizer phenotype on the basis of CYP genotyping
- Insufficient effect of first line treatment with proton pump inhibitors (twice daily) or prokinetics;
- In the presence of alarm symptoms, patients are required to have undergone an upper gastrointestinal endoscopy (without evidence of organic disease), and have tested negative for Helicobacter pylori 2 years prior to inclusion;
- Women in their fertile age (<55 years old) must use contraception or be postmenopausal for at least two years.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Predicted CYP2D6 poor, intermediate or ultrarapid metabolizer phenotype on the basis of CYP genotyping*
- Evidence of current anxiety and/or depression disorder as defined by a score ≥ 10 on the GAD-7 and/or PHQ-9 questionnaire, supported by a detailed interview by the investigator (i.e. the investigator is required to confirm suspicion of anxiety or depressive disorder);
- Current use or any previous use of psychotropic medication in the last 3 months prior to inclusion;
- Inability to discontinue prokinetics**, NSAIDs or opioids;
- Using drugs of abuse;
- Using more than 2 or 3 units of alcohol per day (females and males respectively)
- Previous major abdominal surgery or radiotherapy interfering with gastrointestinal function:
    a. Uncomplicated appendectomy, cholecystectomy and hysterectomy allowed unless within the past 6 months;
    b. Other surgery upon judgment of the principle investigator;
- History of gastric ulcer;
- History of liver disease, cholangitis, achlorhydria, gallstones or other diseases of the gallbladder/biliary system;
- History of epilepsy
- History of glaucoma
- Pregnancy or lactation.

* Patients who are predicted poor or intermediate metabolizers will have the option to continue participating in an open-label extension of the current trial in which all study procedures apart from the randomization will be followed. Patient will receive a standard dose of 10 mg nortriptyline during the 12-week treatment period without an increase in dosage (See addendum A).

** Patients still using prokinetics at the time of inclusion will be asked to discontinue treatment. A wash-out period of 2 weeks before the run-in period is required. Patients that cannot discontinue prokinetic therapy will be excluded. Patients on proton pump inhibitors should continue these without altering dosage, given that rebound symptoms can occur with discontinuation.

4.4 Inclusion study period

Potential study participants are enrolled in a 2 week run-in period, during which symptoms will be recorded daily using a smartphone application. Subjects are required to have reported a minimal average score of 3 for one out of four core symptoms (bloating excluded)during the run-in period (on an 11-point NRS scale). Subjects with lower scores on these symptoms will be excluded from the study. This approach is in line with FDA guidelines on treatment trials in irritable bowel syndrome, a similar functional gastrointestinal disorder. In addition to the above, no changes in in- and exclusion criteria for the run-in period may have occurred for subjects to participate in the study.

4.5 Sample size calculation

The primary outcome measure of this study is the reduction in average symptom score, compared in the nortriptyline standard dose regimen group to the placebo group (see section 8.1.1 Main study parameter/endpoint). According to an earlier trial by Talley et al, amitriptyline results in significant improvement of symptoms in 67% of patients, compared to 39% of patients treated with placebo.(11) A study by Camilleri et al demonstrated a strong correlation between adequate relief (as used as primary endpoint in the trial of Talley et al) and symptom reduction (continuous variable, primary outcome measure of this study) in IBS, a functional GI disorder similar to FD.(15) Based on this correlation, we performed a power calculation using estimation of sample size for comparing two binomial proportions, which can be found at the following web address: http://clincalc.com/stats/samplesize.aspx.
We applied the following values; significance level (α two-sided) = 0.05; power = 0.80. To detect a 28% difference in response rate as determined by the primary outcome (nortriptyline classical dose regimen vs. placebo), 49 subjects per arm are needed (67% vs 39% response rate estimate based on results from Talley et al).(11) Therefore, based on these calculations, the inclusion of 49 completers for each of the two treatment arms (in total 98) will result in sufficient power to assess differences between placebo and nortriptyline, assuming a minimal difference of 28% in effect, regardless of the treatment formulations.
Due to possible inhomogeneity's in our population because of the heterogeneity of FD and the multicentre aspect of our study as well as a cluster-level analysis, we applied a variance inflation factor of 1.23 (mean cluster size 12, coefficient of variation of cluster size 0.6 and intra-cluster correlation coefficient of 0.015) according to the formula by Eldrigde et al.(17) This formulation was designed specifically for cluster-randomised trials however. In the current study, we will be using the minimization method (discussed in randomisation section 8.2). Since there are no formulations available for minimization-randomised trials, it is assumed that an inflation of the sample size with 23% corrects for the above-mentioned heterogeneity. Therefore, we would need 122 completers in this study. In addition, in order to account for exclusions/drop-outs due to a) not meeting inclusion criteria, either during screening (e.g. abnormal CYP2D6 phenotype; ~10%) or the run-in period (i.e. insufficient symptom score; ~5%) and b) drop-outs during the treatment period (~5%), we will include an additional 20%, resulting in 77 subjects per arm and a total of 154 patients to be included.

5. TREATMENT OF SUBJECTS

In this study, the efficacy of Nortrilen (nortriptyline) in FD is compared with a placebo.

5.1 Investigational product/treatment

Patients will be randomised to one of the following two treatment arms: nortriptyline escalating dose regimen, or placebo capsules. All capsules will look identical due to overencapsulation. For more information on the medicinal product and dose regimens, see chapter 6.

5.2 Use of co-intervention

Patients in both treatment arms will also receive standard care such as reassurance, dietary advice and lifestyle coaching. No new treatment for FD can be started throughout the study period. Patients on PPIs are required to continue these without altering dosage in order to avoid rebound effects, an approach that is similar to the large multi-center study of Talley et al.(11) The use of prokinetics during the study period is prohibited. Prokinetic therapy should be discontinued 2 weeks prior to inclusion (before the run-in period). This is an acceptable approach as only patients that have had insufficient effect of prokinetics are included. In addition, discontinuing prokinetics before the start of treatment reduces the risk of QT interval prolongation, which is associated with both prokinetics and TCAs. The start of laxatives during the study period is allowed, as patients receiving nortriptyline treatment may experience constipation as a side effect. Patients that are on a gluten or lactose free diet or FODMAP diet should have been on this for at least two months already and continue with this throughout the study. Lifestyle and dietary measures for treating FD should be stabilized prior to study entry (i.e. prior to the run-in period) and will be maintained during the course of the clinical trial. Women in fertile ages should use contraception continuously. The use of drugs with analgesic action is restricted (unless adequately justified as escape medication, see section below). NSAIDs and opioids are prohibited.

5.1 Escape medication

During the entire study period (i.e. run-in-period, treatment period and follow-up period), subjects are permitted to use paracetamol if necessary. The number of tablets, dose and the reason of medication use have to be reported in the electronic diary. Opioids, NSAIDs and anti-emetics such as ondansetron, metoclopramide and domperidon are not allowed.

6. INVESTIGATIONAL PRODUCT

In this paragraph, investigational products Nortrilen (nortriptyline) and placebo will be discussed.

6.1.1 Name and description of investigational product(s)

Nortrilen (nortriptyline) is a second generation tricyclic antidepressant. The drug is currently registered for use in depression. It is frequently used off-label to treat neuropathic pain. In fact, a recent study demonstrated that nortriptyline was more often prescribed off-label for neuropathic pain than depression in primary care in Canada (75% off-label prescriptions, of which 60% for pain disorders).(18)

6.1.2 Summary of findings from non-clinical studies

For a summary of findings from non-clinical studies we would like to refer to the document Summary of Product Characteristics (SPC) of Nortrilen, page 5, see appendix D2.2 (SmPC Nortrilen).

6.1.3 Summary of findings from clinical studies

Accumulating evidence suggests efficacy for low-dose tricyclic antidepressants (TCAs) in FD. TCAs were approved in the 1960s and are widely used ‘off-label’ as non-narcotic analgesics. TCAs are believed to act via noradrenergic and serotonergic descending inhibitory perceptive pathways and have extensively been used as neuromodulators in patients with chronic pain. A recent multicenter study by Talley et al. demonstrated that the TCA amitriptyline (50 mg) may be beneficial in FD.(11) However, the therapeutic gain was modest (response rate 53% vs 40% placebo; the response rate was higher in a pain-predominant group, 67 vs 39%). A recent meta-analysis concluded that TCAs were indeed effective in the treatment of FD with a number needed to treat of 6 and a number needed to harm of 7.(12) However, these authors noted that no firm conclusion could be drawn due to the relatively small amount of studies and large heterogeneity between studies. Of note is that none of the trials conducted in FD patients included patients from primary care. Overall, these results have not been compelling enough to routinely advocate antidepressants for FD. Widespread use of TCAs, an otherwise relatively inexpensive drug group, is further hampered by the fact that these are traditionally considered antidepressants. Therefore there is considerable reluctance for from both patients and heath care providers to initiate a therapy with TCAs, especially in the absence of evident psychiatric comorbidity. In addition, TCAs are often associated with side effects, including constipation, drowsiness, dry mouth (due to anticholinergic and antihistaminergic effects), which appear to be dose dependent and occur early after initiation of therapy preceding the therapeutic effect. This often results in discontinuation of an otherwise potentially effective therapy. On the other hand, previous studies have shown that the clinical response is independent of the total dose of TCAs, supporting the concept of titration of low dosages in FD (10-50 mg/day) – as opposed to the higher dosages traditionally used for psychiatric indications (150-300 mg/day).

6.1.4 Summary of known and potential risks and benefits

Subjects may or may not benefit directly from participating in this study. There is evidence that TCAs cause relief of FD symptoms. We have reasons to believe that personalized therapy with nortriptyline (i.e. CYP genotyping and subsequent exclusion of abnormal

CYP2D6 metabolizers) will have even higher efficacy and fewer side effects than empirical treatment. Therefore, it is expected that at least part of patients randomised to nortriptyline treatment will have some sort of benefit from participating in this study. Subjects randomised to placebo may experience small burden due to not receiving any treatment (although dietary and lifestyle advice continue as usual and placebo effects are not negligible in this patient group). Although the occurrence of side effects is expected to be decreased with the current approach (low dose nortriptyline in CYP2D6 extensive metabolizers only), side effects can occur. The most frequent side effects of nortriptyline include dry mouth, headache, nausea, palpitations, weight gain and blurred vision (due to accommodative dysfunction). These often disappear with treatment continuation. ECG changes can occur, but are most often harmless. An ECG will be performed prior to the start of treatment in selected patients to ensure that study medication (nortriptyline) can be started safely (see section 8.3.1, medical screening: ECG).
For a structured risk analysis see Chapter 13.

6.1.5 Description and justification of route of administration and dosage

Subjects will self-administer one capsule of study medication orally every day of the 12 week treatment period. Capsules are preferably taken in the evening due to sleepiness as a possible side effect of nortriptyline treatment.
On the basis of CYP genotype, individuals can be categorized as poor (5-10% of the population), intermediate (2- 11%), extensive (77-92%) or ultrarapid (1-5%) metabolizers.(16) Poor, intermediate and ultrarapid metabolizers have a higher chance of drug toxicity (the latter on the basis of possible higher levels of toxic metabolites), and will therefore be excluded in the present study.

The following dose regimen is used in this study:
Week 1-2: 10mg per day
Week 3-4: 25mg per day
Week 5-12: 50gm per day

Treatment of psychiatric conditions generally requires administration of doses around 150-300 mg/day. In contrast, treatment of chronic pain including functional GI disorders generally requires lower doses of around 10-50 mg/day. For the treatment of major depression, there is a linear relationship between drug plasma concentrations and therapeutic response in the majority of patients.(20) This provides the rationale within the psychiatry practice for monitoring drug levels and adjusting dosages to prevent toxicity and to monitor compliance. As far as functional gastrointestinal conditions are concerned, the presence of detectable drug levels in irritable bowel syndrome, which is a functional GI disorder comparable to FD, (indicating adherence to therapy) was associated with good clinical response.(21) However, higher dosages or high blood levels were not predictive of a better clinical response, which supports the contention that lower TCA dosages appear sufficient to treat functional GI disorders.(22) On the other hand, even using these low dosages result in a significant number of patients discontinuing therapy, hence our current proposal for a genotype-based pre-selection.

6.1.6 Dosages, dosage modifications and method of administration

See section 6.1.5

6.1.7 Preparation and labelling of Investigational Medicinal Product

Preparation and labelling of the nortriptyline products will be done according to the Good Manufacturing Practice (GMP) guidelines at the GMP certified laboratory of TioFarma B.V. Labels comply with the GCP guidelines. Capsules will be packed in bottles, with three separate bottles for each dose stage (i.e. three bottles, 2 bottles with 14 capsules for week 1-2 and 3-4 and 1 bottle with 56 capsules for week 5-12).

6.1.8 Drug accountability

Nortriptyline capsules (RVG03285/RVG03286/RVG11407) are purchased by TioFarma B.V. The pharmaceutical-technical quality of capsules is guaranteed by the marketing authorization, Lundbeck B.V.
TioFarma is provided with the concealment list by the Clinical Trial Center Maastricht (CTCM). TioFarma will supply the pharmacy of the AMC with medication (according to GDP guidelines). The Hospital Pharmacy of the AMC will hold responsibility for further storage before usage and distribution (according to GDP guidelines). Once a patient is randomised online with TENALEA, the Hospital Pharmacy of the AMC will receive a notification mentioning subject number and kitnumber, after which the study medication will be shipped to the patient in accordance with GDP guidelines. The capsules will be “discarded” after the expiry date.

6.2.1 Name and description of investigational product(s)

Placebo capsules do not contain an active pharmaceutical ingredient, but are Coni-Snap® size 4 white hard gelatin capsules filled with Microcrystalline cellulose. As a comparator product, placebo capsules that look identical to the over-encapsulated nortriptyline have been produced. Coni-Snap® size 4 white hard gelatin capsules will be filled with Microcrystalline cellulose. Microcrystalline cellulose is an inert substance, made of refined wood pulp and looks like white free flowing powder. It is used as an excipient in many drugs but is not degraded nor absorbed. As such, microcrystalline cellulose is safe and harmless for usage.

6.2.2 Summary of findings from non-clinical studies

Not applicable.

6.2.3 Summary of findings from clinical studies

Not applicable.

6.2.4 Summary of known and potential risks and benefits

Not applicable.

6.2.5 Description and justification of route of administration and dosage

Subjects will self-administer one capsule of study medication orally every day of the 12 week treatment period. Capsules are preferably taken in the evening due to sleepiness as a possible side effect of nortriptyline treatment (although not applicable to placebo, the same

instructions will be given here). One placebo capsule contains 69,3mg of microcrystalline cellulose. This is a very common and harmless dosage.

6.2.6 Dosages, dosage modifications and method of administration

See 6.2.5.

6.2.7 Preparation and labelling of Investigational Medicinal Product

TioFarma B.V. will prepare the placebo capsules according to the Good Manufacturing Practice guidelines in the GMP-certified laboratory of TioFarma. TioFarma will also handle the blinding and labelling of all the medicinal products (they have received the concealment list from the CTCM). Capsules will be packed in bottles, with three separate bottles to correspond with each dose stage of nortriptyline (i.e. three bottles, 2 bottles with 14 capsules for week 1-2 and 3-4 and 1 bottle with 56 capsules for week 5-12).

6.2.8 Drug accountability

TioFarma has produced placebo capsules. TioFarma will supply the Hospital Pharmacy of the Academic Medical Center (AMC) of Amsterdam with medication. The Hospital Pharmacy of the AMC will hold responsibility for further storage before usage and distribution (according to GDP guidelines). Once a patient is randomised online with TENALEA, the Hospital Pharmacy of the AMC will receive a notification mentioning subject number and kitnumber, after which the study medication will be shipped to the patient in accordance with GDP guidelines. The capsules will be “discarded” after the expiry date.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Response to therapy, as defined by a 30% reduction from baseline (i.e. the run-in period) in the weekly average of daily symptom scores, during at least 50% of weeks 3-12 of treatment. This is in line with the FDA guidelines on IBS treatment studies. Recorded symptoms include the five core symptoms of FD as defined in the recently developed Functional Dyspepsia Symptom Diary of the PRO consortium's FD working group: epigastric pain, epigastric burning, postprandial fullness, early satiety and upper abdominal bloating.(23) Note that week 1 and 2 are excluded in order to allow for establishment of steady-state drug levels.

8.1.2 Secondary study parameters/endpoints

1. Self-reported weekly global adequate relief of symptoms. This is defined as a "yes" response in at least 50% of weeks 3-12 of the treatment. Responses are collected electronically. Note that week 1 and 2 are excluded in order to allow for establishment of steady-state drug levels.
2. Quality of life, assessed with the use of the EQ-5D-5L (change from baseline).
3. Dyspepsia-specific quality of life, assessed with the use of the Nepean Dyspepsia Index (change from baseline).
4. Cost-utility, as determined by calculations incorporating total treatment costs and changes in EQ-5D-5L (QALYs gained) and MCQ/PCQ results (savings from reduced medical resource use and increased work productivity respectively).
5. Use of rescue medication.
6. Number and severity of side effects.
7. Responder rates following discontinuation of treatment at 6 months follow-up, as defined by a "Yes" to the query regarding adequate relief from baseline symptoms.
8. Worst-case-analysis: imputing a non-response day for each day on which the electronic diary entry was missing (due to non-reporting of the patient) in patients assigned to nortriptyline; in patients assigned to placebo, a response day will be imputed for each day the electronic diary entry was missing.9. (Negative) mood; assessed with the use of the PHQ-9 and GAD-7 (change from baseline).

8.2 Randomisation, blinding and treatment allocation

The Clinical Trial Center Maastricht (CTCM) will perform a computer-scheduled randomization. The randomization will be based on the minimization method. Minimization is a largely nonrandom method of treatment allocation, aiming to balance treatment arms with respect to predefined patient factors.(24) This trial will be balanced in the two treatment arms for the following factors: center, age (18-30; 31-50; 51-65), gender and subtype (epigastric pain syndrome, postprandial distress syndrome or overlap syndrome).

Two concealment lists will be generated by the CTCM and provided to TioFarma, one for each run of packaging and labelling of the study medication. The concealment list provides information on which kits are packed with nortriptyline and placebo capsules, and will not be given to the investigators. All medication labels will be blinded for the treatment allocation and will only have the "kit number" on it, not which capsules are inside.

When a patient can be included after completing the run-in period, the coordinating investigator will receive a notification in Ldot. The coordinating investigator will then confirm eligibility, and will then randomize the patient in an online environment provided by the CTCM (TENALEA). Once the patient is randomised into one of the treatment arms, both the coordinating investigator and the hospital pharmacy of the Academic Medical Center will receive an automatic email via the system of TENALEA. This email will contain information on the patient that has been included and the assigned kit number. In contrast to the email to the investigator, the email to the pharmacy also includes information on the medication in the assigned kit (deblinded). Study medication is then shipped directly to the patient from the hospital pharmacy, according to GDP guidelines. In this manner, neither the coordinating investigator nor the patient will have any knowledge on treatment allocation. In case of emergencies during office hours, Tiofarma will be able to break to the randomisation code. For emergencies out of office hours, drs. L. Meulen, has been provided with the concealment list and will be able to de-blind.

8.3 Study procedures

8.3.1 Medical screening

If an FD patient is willing to participate in the study after a minimum time for consideration of 7 days, an informed consent will be signed during the screening visit (i.e. hospital visit 1)*. At t = -2 weeks, a medical screening will be performed to assess the eligibility of the patient to participate in this study. In- and exclusion criteria will be checked and a short physical examination will be performed (weight, length). Subjects are asked to complete two questionnaires at the screening visit; the PHQ-9 and the GAD-7. This is necessary in order to screen for depression and anxiety, respectively, which are both exclusion criteria. A cut-off value of 10 is used for both the GAD-7 and PHQ-9, as this corresponds to moderate anxiety and depression, respectively.
In the case of a score of 10 or higher on either questionnaire, a detailed interview by the investigator is required. The investigator than decides (if needed, in consultation with the coordinating investigator and/or principal investigator) whether the scores indeed correspond to symptoms of anxiety or depression. If this is the case, the patient will be excluded from the study and the general practitioner and/or gastroenterologist will be informed, as additional/alternative treatment may be required. However, if scores of 10 or higher on the PHQ-9 or GAD-7 appear to be unrelated to depression or anxiety, the patient can still participate in the study.
All questionnaires are discussed in detail below in section 8.3.5. Like all questionnaires, the GAD-7 and PHQ-9 will be completed electronically and can be accessed through a link that is sent to the patients' email address.

* Informed consent can also be obtained without an on-site appointment (digital screening visit). After the researcher provided verbal information over the phone, participants will sign the informed consent form during a video call and send this to the researcher. Upon receiving the informed consent form, the researcher will also sign the informed consent form. After obtaining the informed consent the screening will be performed via a video call.

Pregnancy test

Women in fertile ages that are suitable for inclusion will undergo a simple (urine) pregnancy test to exclude pregnancy. When completed, the pregnancy tests and urine samples will be destroyed immediately at the end of the screening visit. No personal information will be visible on the samples.

Finger prick blood sample
A small blood sample will be taken to assess CYP genotype. Samples will be collected at the end of each screening visit (when patients have fulfilled all other criteria). Sampling at this point will also be seen as a practicing opportunity for patients. Instructions will be given on how to perform the finger prick at home (see below), and patients will be motivated to perform at least part of the sampling themselves during the screening visit.
Sample analysis is performed at the laboratory of MUMC+. Once CYP genotype has been established, results are translated into a matching phenotype (poor, intermediate, extensive or ultrarapid metabolizer). Test results generally take 5-7 days. If a patient wishes to already continue with the run-in period, he or she can continue by filling in the symptom diary. However, these patients will still be excluded if predicted CYP 2D6 phenotype is poor, intermediate or ultrarapid metabolizer.
As all screening visits will be performed by the coordinating investigator, he will take care of transport of samples (transport by car only). If multiple screenings visits have been planned on the same day at different study sites, samples will not be left in the car but will be carried safely by the coordinating investigator. No personal information will be visible on blood samples; patient information will be coded (see 12.1 Handling and storage of data and documents). Blood samples are highly stable at room temperature due to the incorporated DNA stabilizer in the sampling cups. Therefore, no additional measures are required for transport. Samples will be delivered directly at the laboratory upon arrival in Maastricht. After analysis, all samples are destroyed immediately.
Test results will be communicated with patients by telephone (after 5-7 days).
After confirmation of eligibility (CYP2D6 extensive metabolizer phenotype), patients should be enrolled in the run-in period within 2 weeks. This will be communicated with patients when planning the screening visit, in order to prevent exceedance of the time-window. If enrolment within 2-week time-window is not possible, in- and exclusion criteria may have to be checked again in order to ensure that the patient is still eligible.

ECG (on indication only)
Patients will undergo an ECG if they are using medication that can prolong QT interval (e.g. sotalol), or if they are known with cardiac comorbidity (e.g. history of myocardial infarction, significant arrhythmias or cardiomyopathy), as nortriptyline can induce ECG changes (i.e. increase QT interval). If the corrected QT interval exceeds 450 or 460ms in males and females respectively, the subject will be excluded from the study.

8.3.2 Run-in period (t = -2 weeks → t = 0)

Potential study participants are enrolled in a 2 week run-in period. During this period, the five core symptoms of FD will be recorded daily using a mobile phone app. A minimal average score of 3 for one out of four core symptoms (bloating excluded) is required for inclusion (on an 11-point NRS scale). In addition, a minimum amount of 8 of 14 days is to be completed by subjects, in order to be considered eligible. Subjects whom complete less days during the run-in, will automatically receive a symptom score below 3. Subjects with an average score

below 3 will be excluded from the study, and will be referred back to their clinician. This approach is in line with FDA guidelines on treatment trials in irritable bowel syndrome, a similar functional gastrointestinal disorder. Patients that are eligible for the study period are asked to complete the Nepean Dyspepsia Index (NDI; dyspepsia-specific quality of life questionnaire), EQ-5D-5L (quality of life questionnaire), MCQ and PCQ (questionnaires on healthcare consumption and work productivity) at the end of the run-in period.

Gastric emptying test (optional)
During the run-in period, patients can choose to undergo the optional gastric emptying test. We will use a (non-invasive) validated breath test, which is part of routine care in FD.(25) This test includes the consumption of a standardized meal containing ^{13}C labelled octanoic acid. This substance is considered harmless. ^{13}C is a stable carbon isotope, meaning that it is not radioactive. Once the labelled substance enters the intestine it starts to be degraded. After this, ^{13}C is exhaled through carbon dioxide. ^{13}C labelled carbon dioxide levels are subsequently measured to assess gastric emptying. Measurements are performed over a period of 4 hours, in order to obtain adequate information on gastric emptying. Patients are required to be fasting before the test (i.e. when planned in the morning, patients should be fasting from 10pm the day before). The test will take place at the end of the run-in period, when the patient is eligible (depending on the pain scores, described above). This way, the test can be combined with the randomisation visit (i.e. hospital visit 2).

8.3.3 Treatment period (t = 0 → t = 12 weeks)

Following inclusion, patients will be randomly assigned in equal proportions to one of two groups (placebo, nortriptyline escalating dose regimen). Patients will have to start with the treatment period within 4 weeks after the run-in period. This will be communicated with patients prior to the run-in period, in order to prevent exceedance of the time-window. If enrolment within this 4-week time-window is not possible, patients will have to undergo the run-in period again.
The treatment period will be 12 weeks. Patients in each treatment arm will also receive standard care, including diet and lifestyle coaching. Patients will be asked to report symptoms daily using a mobile phone app. The same app will ask patients about the occurrence of side effects and the use of escape medication (daily). Patients will be contacted by telephone by a research nurse every two weeks to further inform about side effects and treatment evaluation. Finally, patients will be asked to complete the EQ-5D-5L, MCQ and PCQ in week 4, 8 and 12, and PHQ-9, GAD-7 and NDI at the end of the treatment period. The final visit will take place after the treatment period, to evaluate treatment (i.e. hospital visit 3 or an appointment by telephone).

Dried blood spot
A dried blood spot sample will be taken to assess nortriptyline drug levels after one week of treatment (patient, physician and coordinating investigator blinded), before the first dose escalation from 10mg to 25mg. Patients will receive instructions along with a finger prick kit to perform the dried blood spot sampling at home. If deemed necessary, the patient can practice the dried blood spot sampling during visit 2 (the randomisation visit). If patients prefer the test to be performed by medical personnel, an additional visit will be planned.

The blood sample is drawn with the use of an automated lancet from the finger onto specially manufactured absorbent filter paper (complete kits are supplied by the Laboratory of Isala Zwolle). The blood is allowed to saturate the paper and is air dried for several hours. Specimens are stored in low gas-permeability plastic bags with desiccant added to reduce humidity. Patients are not required to be fasting prior to sampling. Time between medication use and sampling should ideally be between 12-14 hours. This means that when study medication is taken at 8pm, dried blood spot sampling should be performed between 8-10am the next day. It should be noted however that plasma levels of nortriptyline have been demonstrated to be very stable between 12 and 24 hours after medication intake.(26) Sampling in this window will therefore also be acceptable. Patients will be asked to write down time of medication intake and sampling on the application form, but not personal information; samples and application forms will be coded (see 12.1 Handling and storage of data and documents). Thereafter, patients will send the samples (with the application form) to the University of Maastricht with the use of the included return envelope. Upon arrival, samples will be stored at -20 degrees Celsius at the Laboratory of the Gastroenterology Department. The moment at which a batch has reached a size of 50 samples, it will be sent to the Laboratory of Isala Zwolle, where samples will be analysed. After analysis, samples are destroyed immediately.
The coordinating investigator will not be informed about the results of the nortriptyline plasma level assessments until the end of the study. The laboratory of Isala will therefore keep a list of coded results. After analysis, dried blood spot samples are destroyed immediately.

Venepuncture (on indication only)
An additional blood sample will be acquired when a patient has significant side effects (defined as a score of 5 or higher on an 11-point numerical scale). These blood samples will be acquired and assessed for nortriptyline plasma level at the center of inclusion. Samples will be drawn by either the local study nurse or the coordinating investigator. After samples have been drawn, they will immediately be delivered at the local laboratory. No personal data will be visible on blood samples and application forms, all data will be coded (see 12.1 Handling and storage of data and documents). Local laboratories will be informed to communicate results of plasma level assessments with the independent researcher, A.E.P. van Rooy (gastroenterology department MUMC+). She will also be the one to contact the patient, with the exception of patients with a (potentially) toxic plasma level (see below). Results of the plasma level assessments will not become visible in the patient's hospital file. In the unlikely event of plasma levels exceeding 150 ng/ml, the independent researcher will inform the coordinating investigator. He will then exclude the patient and inform him/her about (possible) toxicity. The patient will be advised to discontinue treatment and visit the general practitioner for additional assessment (i.e. ECG). If the plasma level is lower than 150ng/ml and dose escalation is not planned within 48 hours of plasma level assessment, the independent researcher will inform the patient that he/she can continue treatment as planned, if the patient still wishes to, despite of the presence of side effects. If dose escalation is planned within 48 hours, and plasma level is below 100ng/ml, the same applies. However, if dose escalation is planned within 48 hours but the plasma level is between 100ng/ml and 150ng/ml, the patient may be at risk of toxicity upon dose escalation. In this case, the patient is advised to discontinue treatment and is excluded from the study.

8.3.4 Follow-up (t = 9 months, 6 months after treatment)

After a follow-up of 3 & 6 months after treatment, patients will again be asked to complete NDI, EQ-5D-5L, MCQ, PCQ, GAD-7 and PHQ-9 electronically (sent by email). In addition, patients will be asked to answer the “adequate relief” query (see below) each month until 6 months follow-up.

8.3.5 Questionnaires

All questionnaires will be filled out in a secure, electronic environment that can be accessed via Internet by telephone or computer. A hyperlink to access the questionnaires is sent to the patients’ email address at each time point discussed above. The electronic questionnaires are completed in Castor, which uses a NEN7510 and ISO 27001:2005 certified server called “True”.

Baseline characteristics questionnaire – See F1.1_Algemene_vragenlijst_TENDER
At t = 0, subjects will fill out this questionnaire regarding baseline characteristics, demographics and life-style.

Generalized Anxiety Disorder-7 (GAD-7) **- See F1.2 _GAD7**
During the screening visit, subjects will be asked to complete the GAD-7 in order to screen for anxiety (exclusion criterion). The GAD-7 is a validated 9-item questionnaire which has been demonstrated to be an efficient tool for screening for generalized anxiety disorder and assessing its severity in clinical practice and research.(27) A cut-of value of 10 is used for screening purposes (which is subsequently complemented with a detailed interview by the researcher). The GAD-7 will be repeated at the end of the treatment period (t = 12 weeks) and at follow-up (3 & 6 months after treatment).

Patient Health Questionnaire-9 (PHQ-9) - See F1.3_PHQ9
During the screening visit, subjects will be asked to complete the PHQ-9 in order to screen for depression (exclusion criterion). The PHQ-9 is a validated multipurpose instrument for screening, diagnosing, monitoring and measuring the severity of depression.(28) It incorporates DSM-IV depression diagnostic criteria with other leading major depressive symptoms into a brief self-report tool. A cut-of value of 10 is used for screening purposes (which is subsequently complemented with a detailed interview by the researcher) The PHQ-9 will be repeated at the end of the treatment period (t = 12 weeks) and at follow-up (3 & 6 months after treatment).

Nepean Dyspepsia Index (NDI) - See F1.4_NDI
The Nepean dyspepsia index includes a questionnaire on dyspeptic symptoms (informing about the frequency, severity and bothersomeness of these symptoms) with additional psychometric properties (i.e. the effect of symptoms on various quality of life aspects). Patients will be asked to complete the NDI at the end of the run-in period, at the end of treatment, and at 3 and 6 months follow-up.

Big Five Inventory (BFI) - See F1.5_BFI

At the end of the run-in period, subjects will be asked to complete the BFI. This questionnaire is intended to identify personality traits which will later be analysed for correlation with treatment outcome.

Quality of Life (EQ-5D-5L) - See F1.6_ EQ-5D-5L
The EQ-5D-5L assesses five dimensions of quality of life: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Previous research indicated that the EQ-5D-5L is a valid and responsive questionnaire to measure quality of life in FD.(29) The EQ-5D-5L will be completed at t = 0, 4, 8 and 12 weeks, and after 3 & 6 months follow-up.

Medical Consumption Questionnaire (MCQ) – See F1.7_MCQ
The MCQ is a questionnaire developed by the Institute for Medical Technology Assessment (Rotterdam) and is designed to measure healthcare consumption.(30) The MCQ will be completed at t = 0, and 12 weeks, and after 3 & 6 months follow-up.

Productivity Cost Questionnaire (PCQ) – See F1.8_PCQ
The PCQ is a questionnaire developed by the Institute for Medical Technology Assessment (Rotterdam) and is designed to measure work productivity and absenteeism.(30) The PCQ will be completed at t = 0, 4, 8 and 12 weeks, and after 3 & 6 months follow-up.

14-day symptom diary (run-in period) – core symptoms – See F2.1_14dagen_dagboek
During the run-in period, subjects will be asked to rate their daily symptoms on an 11-point NRS scale. These symptoms include the five core symptoms of FD, as defined in the recently developed Functional Dyspepsia Symptom Diary of the PRO consortium's FD working group.(23)

84-day symptom diary – core symptoms – See F2.2_12weken_dagboek
Every day during the treatment period, subjects will be asked to rate their symptoms on an 11-point NRS scale. These symptoms include the five core symptoms of FD, as defined in the recently developed Functional Dyspepsia Symptom Diary of the PRO consortium's FD working group.(23) In addition, subjects will be asked to report any adverse event and/or use of escape medication on a daily basis. At the end of each week, patients are asked to report global symptom relief (yes of no).

Adequate relief query
Each month at follow-up, until 6 months after treatment discontinuation, patients will be asked to complete the "adequate relief" query. This is one question concerning patients' symptom relief. The same question is asked during the treatment period via the digital diary, with the difference that the recall period during follow-up is 1 month instead of 1 week.

8.3.6 Missing data from questionnaires/electronic diary

Questionnaires should be completed within a predefined time-window. If not completed within this window, questionnaires for that specific time-point are closed and the answers are considered as missing data.

Time-windows are the following:

1. Questionnaires that should be completed during the entire study period (treatment and follow-up), have to be completed within 3 weeks after the questionnaires have been sent to the patient.
3. The electronic diary should be completed each day during the run-in and treatment period. Questions from each day can be answered from 7pm until 12pm. If the diary entry has not been completed within this time-window, (residual) answers for that day will be considered as missing data.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.5 Replacement of individual subjects after withdrawal

We anticipated dropouts within our sample size calculation.

8.6 Follow-up of subjects withdrawn from treatment

Subjects withdrawn from the study will not be followed up, unless it seems necessary in case of any urgent medical reason.

8.7 Premature termination of the study

We don’t expect that this study will be terminated prematurely. However, if certain urgent medical problems occur during the study (for example SAEs that result in death or are life threatening) the investigator (if necessary together with METC or CCMO) can decide to halt the study to check whether it is safe to proceed or that termination is preferred.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product or trial procedures. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been, based upon appropriate judgement by the investigator. An elective hospital admission will not be considered as a serious adverse event.

SAEs are reported from the moment of signing the informed consent until 4 weeks after discontinuation of treatment. During every hospital visit and telephone call (every two weeks during the treatment period), patients will be asked about the occurrence any (S)AE. After treatment discontinuation, patients will be contacted once by telephone after 4 weeks to ask about their current condition and possible occurrence of any (S)AE.

The coordinating investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
    - Summary of Product Characteristics (SPC) for an authorised medicinal product

The coordinating investigator will report all SUSARs to the sponsor without undue delay after obtaining knowledge of the events. The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;
- SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority. The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.
This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

10. STATISTICAL ANALYSIS

Statistical analyses will be carried out using IBM SPSS statistics 22.0 for Windows (Chicago IL, USA). Demographics, baseline characteristics (including the outcomes of the questionnaires at baseline) will be analysed and summarised using descriptive statistics. Dichotomous variables will be analysed using the Chi-square test. A Yates correction (continuity correction) or Fisher exact test will be performed if necessary. Normality of continuous or ordinal variables will be evaluated by the Kolmogorov-Smirnov test and histograms. The dependent variables will be analysed with the Wilcoxon signed-ranked test if the variables are not normally distributed. If the variables are normally distributed a paired t-test will be performed. The independent variables will be analysed with the Mann-Whitney test if the variables are not normally distributed. If the variables are normally distributed an independent sample t-test will be done. Correlations will be assessed according to Spearman or Pearson and regression analyses will be done. A p-value < 0.05 is considered to be statistically significant. A Bonferroni correction will be applied for multiple testing.

10.1 Primary study parameter(s)

All endpoint analyses will be based on the intention to treat population. The proportion of responders in each treatment group will be compared using multiple logistic regression to provide covariate adjustments for center, FD subtype, gender, age and BMI in the analyses. The differences in responder rates, corresponding 95% confidence intervals (CIs) and two sided P-values will be calculated. An observed case approach to missing data will be applied for responder variables and continuous efficacy variables: if a patient does not have a score (responder/non-responder) for a particular treatment period week the patient will be considered a non-responder for that particular treatment period week. Patients are required to complete at least 4 diary entries (of 7) within one week, to be considered a responder. This approach is conservative and any bias is against treatment. A sensitivity analysis will be conducted using Baseline Observation Carried Forward (BOCF). In addition, a worst-case analysis will be performed; imputing a nonresponse day for each day on which the electronic diary entry was missing (due to non-reporting of the patient) in patients assigned to nortriptyline; in patients assigned to placebo, a response day will be imputed for each day the electronic diary entry was missing.

10.2 Secondary study parameter(s)

Changes from baseline in scores for NDI, MCQ, PCQ, EQ-5D-5L will be analysed longitudinally using a generalized linear model, based on the observed cases during the treatment period; treatment group, inclusion center, period, period by treatment interaction and subject as fixed effects and baseline values as covariates. With regards to side effects, nature of side effects, number of events for each side effect, number of subjects with side effects and incidence will be described. A mixed-Markovian model will be used to analyse incidences of side effects in each treatment group. The association of side effects with subject characteristics, such as body weight, age, sex, and concomitant drug will be analysed using binary logistic regression.

10.3 Cost-utility analysis

A trial based cost-utility analysis will be performed from a societal perspective with a time horizon of 6 months after completion of the 12-week treatment period. The outcome measure

for the cost utility analysis will be the incremental costs per QALY gained (i.e. 6 months). Patients will also fill in the generic quality of life questionnaire EQ-5D-5L at 6 months in order to examine quality of life after patients stopped the treatment. The scores of patients on the EQ-5D-5L will be converted to utility scores by using the Dutch tariff based on the preferences from a general population.(31) Subsequently, quality adjusted life years (QALY's; in this study restricted to 6 months) are calculated by multiplying the utility score relating to a certain health state with the length of time a patient spent in that state. For the longer term (1 year), decision analytic modelling will be used to examine the cost effectiveness of the treatment strategies. Resource use within and outside healthcare will be collected using the MCQ and PCQ, using a reference period of 6 months. In line with the guidelines for cost research, costs of productivity loss (both absenteeism and presenteeism) will be calculated by means of the friction cost method, based on the average standardized wages per hour. The friction cost method takes into account the time needed to replace a sick employee in order to restore the production at the original level.(32) With regards to the cost-effectiveness analysis, data will be analysed according to the intention-to-treat principle. Since the distribution of costs is general skewed, non-parametric bootstrap analysis will be used. This method estimates the sampling distribution of a statistic with replacement from original data.(33) In addition, bootstrap analysis will also be used to quantify the uncertainty surrounding the incremental cost-effectiveness ratio (ICER). Probabilistic sensitivity analysis will be performed to estimate for example the impact of parameters (like different cost-prices, utility) on the incremental cost-effectiveness ratio. Missing data will be imputed by a using a multiple imputation approach. In addition, a budget impact analysis will be performed to quantify the financial consequences of implementing the nortriptyline in either classical and/or adjusted dose regime.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the Declaration of Helsinki (last version October 2013, Brazil), the Medical Research Involving Human Subjects Act (WMO) and the FDA guidelines for the conduct of clinical trials.

11.2 Recruitment and consent

Patients with FD (diagnosed according to the Rome IV criteria, the most widely accepted scientific criteria) will be recruited in both the primary and secondary/tertiary healthcare setting. In addition to recruitment via the treating physician, announcements will be placed on the website of the 'Prikkelbare Darmsyndroom Belangenvereniging', local newspapers (of areas of participating centers) and in GP practices in the Maastricht area.
Recruitment will start in 14 hospitals: Maastricht University Medical Center, Academic Medical Center of Amsterdam, Alrijne Hospital in Leiden, VUmc in Amsterdam, Martini Hospital in Groningen, Tergooi Hospital in Hilversum, Bernhoven in Uden, Rijnstate in Arnhem, Gelderse Vallei in Ede, Jeroen Bosch Hospital in Den Bosch, Medical Center Leeuwarden, Diakonessenhuis in Utrecht, Medisch Spectrum Twente in Enschede, Maasziekenhuis Pantein in Boxmeer, and Maxima Medical Center in Veldhoven. All visits and study procedures will take place in the center the subject was included. Recruitment in the primary care will primarily take place in the south of Limburg. Patients included there will visit the MUMC for their screening, checkups and collection of their medication.

Treating physicians will notify eligible FD patients about the current study. If a patient is potentially interested in the study, the treating physician will ask the patient for permission to be contacted by the coordinating researcher (by telephone). Permission to contact the patient by telephone will be logged in the subject screening and enrolment log. If allowed by the patient, the treating physician will provide the coordinating researcher with the patient's telephone number either by telephone or via a designated e-mail address (specifically created for the current study). Further information will be provided verbally by telephone by the investigator and the written information brochure will be sent by regular mail or email. A time period of one week (minimum) is provided to decide whether the subject would like to participate. The subject is asked to contact the investigator by telephone or email if he or she would like to participate. If the patient does not contact the investigator however, the coordinating investigator will contact the patient once (at the earliest 7 days after sending the written information brochure, and only if the patient has given permission to do so during the first conversation). If the patient wishes to participate, an appointment with the physician investigator will be made for the medical screening. All subjects will have to sign an informed consent before screening can commence.

11.3 Benefits and risks assessment

Subjects may or may not benefit directly from participating in this study. There is evidence that TCAs cause relief of FD symptoms.(13) Therefore, it is expected that at least a part of patients randomised to nortriptyline treatment will have some sort of benefit from participating in this study. Subjects randomised to placebo may still experience some relief as placebo response is generally high is this patient group, as demonstrated by the recent trial in FD of Talley et al. (39% response in placebo group).(11)

The risks associated with participation are of such small extent that they are in proportion to the scientific value of the study. FD is a very common gastro-intestinal disorder and TCAs have been shown to be effective in reducing symptoms, but it is currently not widely used in medical practice due to the moderate level of scientific evidence. To improve its efficacy and reduce side effects, the personalized treatment method based on CYP genotype has been developed. Because approach and its efficacy have not been tested in FD patients before, it is essential to conduct this randomised controlled trial. This study can thereby yield useful information for doctors and DF patients and thereby pave the way for a wider use of the relatively cheap but effective TCAs, including nortriptyline.

The risks associated with participation are minor. The most frequent side effects of nortriptyline include dry mouth, headache, nausea, palpitations, weight gain and blurred vision (due to accommodative dysfunction). These often disappear with treatment continuation. ECG changes can occur, but are most often harmless. An ECG will be performed prior to the start of treatment to ensure that study medication (nortriptyline) can be started safely.
It should be noted that information on the frequency of side effects described above is related to the treatment of depression. As already mentioned, the treatment of psychiatric disorders requires higher dosages. Therapeutic drug monitoring is often required in these conditions. In contrast, low doses used in this study do not necessitate monitoring of drug levels. We therefore expect fewer side effects in the current study, as compared to the treatment of depression. For a structured risk analysis see Chapter 13.

Participating in this study will also cost time. Subjects have to visit the hospital 3-4 times (or an appointment by telephone or (video) call), including the first visit in which eligible subjects will be screened before participation. The screening will take up to 1 hour. The randomisation visit will take 4.5 hours, if the patients chooses to do the optional the gastric emptying test during this visit (if not, this visit will take half an hour).

11.4 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study
.

11.5 Incentives

Subjects completing the study will receive 150 euro compensation. Furthermore, we will compensate them for their travel expenses, reimbursing €0.19 per kilometer (with a maximum distance of living address to hospital of 100 km). If a subject visits the hospital for the medical screening, but does not meet the in- and exclusion criteria, travel expenses will be compensated. If a subject completes the run-in period, but cannot be included for the treatment period due to an insufficient symptom score, the subject will be paid 12,50 euro.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Data will be handled confidentially (coded) and the privacy of the subjects will be guaranteed. All subjects will receive an individual study ID-number, coded depending on the inclusion site:
Maastricht University Medical Center: MUMC-001, 002 and so on.
Amsterdam Medical Center: AMC-001, 002 and so on.
VU Medical Center Amsterdam: VUMC-001, 002 and so on.
Maxima Medical Center Veldhoven: MMC-001, 002 and so on.
Alrijne Hospital Leiden: AZL-001, 002 and so on.
Bernhoven Uden: BU-001, 002 and so on.
Gelderse Vallei Ede-Wageningen: ZGV-001, 002 and so on.
Medical Center Leeuwarden: MCL-001, 002 and so on.
Maasziekenhuis Pantein Boxmeer: PAN-001, 002 and so on.
Martini Hospital Groningen: MZG-001, 002 and so on.
Jeroen Bosch Hospital Den Bosch: JBZ-001, 002 and so on.
Rijnstate Hospital Arnhem: RZA-001, 002 and so on.
Tergooi Hospital Hilversum: TZH-001, 002 and so on.
Diakonessenhuis Utrecht: DIAU-001, 002 and so on.
MST Enschede: MST-001, 002 and so on.

Case Report Files (CRFs), questionnaires and biomaterial will all be coded this way, and will not contain personal details of the subjects. The principal investigator will keep the key of the code in a locked cabinet, to which only the principal investigator has access. Together with members of the project team, assigned monitors from the CTCM, IGJ i.o. and members of the medical ethical committee will have access to research data. In addition, ZonMw will have access to the coded research data (not original documents or personal patient information). All primary documents and data shall be kept for 15 years after the end of the experimental phase of the study for possible inspection. The electronic CRF, electronic diary and electronic questionnaires will be built and/or stored by qualified organizations such as MEMIC. Data will only be stored on secure databases: Data from the electronic diary (mobile phone application) will be stored on the NEN-7510 certified ITS-azM database. Data from the additional questionnaires and electronic CRF are acquired with Castor, which uses “True”, a NEN7510 and ISO 27001:2005 certified server.

12.1.1 Source documents

A source document is a document in which collected data is first recorded. For medical information (e.g. medical history, laboratory/imaging results, anamnesis etc.), the electronic patient dossier is often considered the source document. However, in the current study, patients are also recruited via advertisement info (e.g. E3. Poster TENDER version 2.2 dd 23.02.2022). Consequently, a source document in the form of an electronic patient dossier is often lacking. In these cases, the eCRF itself becomes the source document.

12.2 Monitoring and Quality Assurance

A qualified monitor of the CTCM will monitor the conduct of the study. A monitoring plan will be drafted after the first application to the METC.

12.3 Amendments)

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion.

A 'substantial amendment' is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or
- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

Results from this project will be published in international peer-reviewed journals, in the field of Gastroenterology and presented at national and international congresses. To further promote knowledge transfer and implementation, and in particular to reach general practitioners, results (and resulting recommendations) will also be disseminated in national journals. Results and recommendations will be presented to scientific societies responsible for national guidelines relevant for FD (guidelines of the Dutch College of General Practitioners 'NHG richtlijnen'). Two of the project team members, Prof. de Wit and Dr. Muris, have been involved in the development of previous NHG-guidelines for FD. The results will

also be incorporated into continuous medical education for general practitioners and gastroenterologists and published in Dutch Medical Journals. In addition, an implementation plan will be made in cooperation with the Dutch Institute for Rational Use of Medicine (Utrecht). Furthermore, the IBS patient federation (PDS Belangenvereniging) will assist in dissemination of study results among FD patients. Publication will be done in accordance with the CCMO-statement “Publicatie beleid”.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

Nortriptyline, the N-demethylated active metabolite of amitriptyline, is a dibenzocycloheptene-derivative tricyclic antidepressant (TCA).(9) TCAs contain a tricyclic ring system with an alkyl amine substituent on the central ring. TCAs are potent inhibitors of serotonin and norepinephrine reuptake. Nortriptyline is a secondary amine TCA, which means that it is a more potent inhibitor of norepinephrine reuptake than for example amitriptyline (a tertiary amine TCA). TCAs also down-regulate cerebral cortical β-adrenergic receptors and sensitize post-synaptic serotonergic receptors with chronic use. TCAs block histamine-H1 receptors, α1-adrenergic receptors and muscarinic receptors, which accounts for their sedative, hypotensive and anticholinergic effects (e.g. blurred vision, dry mouth, constipation, urinary retention), respectively. Nortriptyline exerts less anticholinergic and sedative side effects compared to the tertiary amine TCAs (e.g. amitriptyline).

As for its use in pain conditions, several possible mechanisms of action have been proposed. Pain suppression via monoaminergic links in diffuse noxious inhibitory control induced by inhibition of presynaptic reuptake of serotonin and noradrenaline was suggested as a mechanism by which antidepressants could relieve neuropathic pain many years ago.(34, 35) Currently, the noradrenergic mechanism appears to be the most important.(10) However, as TCAs have a more potent analgesic effect than of SNRIs it is thought that other mechanisms contribute to the effect of TCAs. Proposed additional mechanisms of action are blockade of NMDA receptors and sodium channels. It therefore appears that the mechanism of action of TCAs in pain conditions such as neuropathic pain is multimodal, with contribution of monoamine reuptake inhibition and blockade of NMDA receptors and sodium channels.(10)

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

TCAs have been used for the treatment of depression since the 1960s. Nortriptyline has been registered for the treatment of depression since November 1964 in the Netherlands (see SmPC).(32) Moreover, nortriptyline is often used for several additional indications (unlabelled usage), including: chronic pain disorders, irritable bowel syndrome, diabetic neuropathy, post-traumatic stress disorder, and for migraine prophylaxis).(9, 18) Clinicians have therefore gained a lot of experience with this drug.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

One of the key pathophysiological factors in functional dyspepsia (FD) appears to be linked to visceral hypersensitivity, in which visceral pain signals are augmented either peripherally or centrally. To study the potential benefit of new therapies in animals, researchers often use visceral hypersensitivity models (e.g. by injecting acetic acid in the submucosal layers of the stomach wall). Indeed, fluoxetine, a serotonin reuptake inhibitor, was demonstrated to reduce visceral hypersensitivity in one study using a visceral hypersensitivity model in rats.(36) Desvenlafaxine, a serotonin and norepinephrine reuptake inhibitor (thus more resembling the properties of TCAs), has equally been shown to reduce visceral hypersensitivity.(37) Furthermore, in a mouse model, mirtazapine (a non-TCA but a compound with similar

properties as this class) ameliorated visceral sensitivity dose-dependently.(38) Indeed, a recent meta-analysis including the effects of various TCAs in FD patients suggested beneficial effects.(12) However, currently only low quality studies exist on this topic.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Tissue selectivity of nortriptyline is limited due to widespread presence of receptors influenced by the compound. This also accounts for the diversity of side effects. For example, anticholinergic effects of nortriptyline are responsible for side effects such as dry mouth, blurred vision (due to accommodative dysfunction) and nausea. On the other hand, antihistaminergic effects of nortriptyline are responsible for the drowsiness associated with administration, the negative effects of which however can easily be kept to a minimum with administration before bedtime. Importantly, the side effects of nortriptyline have been shown to vary considerably between individuals. CYP polymorphisms are considered responsible for this variation in effects, which is why we use CYP genotyping in the current study.

e. Analysis of potential effect

The therapeutic index of nortriptyline has proven to be quite high, with toxic effects only occurring at doses far greater than considered for use in clinical practice.(39) Moderate severe intoxications have been seen with the use of 500mg nortriptyline, and fatal intoxications have been observed with doses around 1000mg. In an overdose situation, the central nervous system (elevated body temperature, seizures) and cardiovascular system (tachycardia, arrhythmias and instable blood pressure) become affected. If not treated properly (see paragraph j), this can be fatal. Suicidal ideations are therefore sometimes considered a relative contra-indication of TCAs. Subjects included in this study are screened for psychiatric co-morbidity, and patients with evidence of an anxiety disorder or depression are excluded. Furthermore, subjects are monitored closely via the daily reporting of side effects, contact by phone every two weeks and nortriptyline plasma level assessment in the case of significant side effects (see section 8.3.3 Treatment period, venapuncture). Due to these safety measures, we consider the risk of (intentional) overdose to be very low.
For more detailed information on the toxicity of nortriptyline we refer to the SmPC.

f. Pharmacokinetic considerations

Nortriptyline is the principal active metabolite of amitriptyline. Parts of metabolism of nortriptyline include hydroxylation, N-oxidation and conjugation with glucuronic acid. CYP2D6 is the primary enzyme involved in nortriptyline metabolism, which we will therefore use for genotyping to predict patients' metabolism of nortriptyline. A multitude of polymorphisms of the CYP2D6 enzyme has been identified. Five major variants and their known enzyme activity are summarised in Table 1. A complete list of known CYP2D6 polymorphisms (including their effect on the enzyme' activity) is provided on URL: *http://www.imm.ki.se/cypalleles/cyp2d6.htm*.(16)

Table 1. Major polymorphic variants of CYP2D6 and their effect on enzyme activity.(16)			
Polymorphism	**Mutation**	**Consequence**	**Allele frequency in Caucasians**
CYP2D6*2xn	Gene duplication/ multiduplication	Increased enzyme activity	1-5%
CYP2D6*4	Defective splicing	Inactive enzyme	12-21%
CYP2D6*5	Gene deletion	No enzyme	2-7%
CYP2D6*10	P34S, S486T	Unstable enzyme	1-2%
CYP2D6*17	T107I, R296C, S486T	Altered affinity for substrates	0% (20-35% in Africans)

Nortriptyline is widely distributed throughout the body and is extensively bound to plasma and tissue protein. Since nortriptyline has a very long half-life (around 26 hours), once daily dose regimens are suitable, with a single dose usually given at night. For more detailed information on pharmacokinetics please see the SmPC of nortriptyline.

g. Study population

Patients between 18-65 years old that are diagnosed with FD (according to the Rome IV criteria) will be included in the present study. Dutch pharmacotherapeutic guidelines consider nortriptyline safe for use in elderly patients, as anticholinergic side effects are less common with nortriptyline as compared to other TCAs.(40) On the other hand, the American Geriatrics Society consider TCAs, including nortriptyline, to be potentially inappropriate medication in patients older than 65 years.(41) We have therefore chosen an age limit of 65 years in the current study. Furthermore, pregnant women or those in fertile ages not using any contraceptives will be excluded. Subjects with evidence of a psychiatric disorder or current use of psychotropic medication will be excluded as well. The latter will be excluded for the confounding effect of psychiatric disorders (i.e. treatment effect of antidepressant in psychiatric disorders).

h. Interaction with other products

Extensive knowledge exists on the topic of interaction of nortriptyline with other products. The SmPC provides a detailed overview. Below we will discuss the most important known interactions.

Nortriptyline cannot be given concurrently with, or within two weeks of cessation of, therapy with monoamine oxidase inhibitors. This is due to possible hyper pyretic crises, severe convulsions and fatalities, which have occurred when similar tricyclic antidepressants were used in such combinations.

Anaesthetics given during tricyclic antidepressant therapy may increase the risk of arrhythmias and hypotension. If surgery is necessary, the drug should be discontinued, if possible, for several days prior to the procedure, or the anaesthetist should be informed if the patient is still receiving therapy.

Tricyclic antidepressants may potentiate the CNS depressant effect of alcohol. The use of alcohol should therefore be limited during the study period. Patients participating in the study will be advised not to consume alcohol in the first two weeks of treatment and not to consume more than one glass per day in the following weeks of treatment.

Because nortriptyline's metabolism involves CYP2D6, concomitant therapy with drugs also metabolised by this enzyme may lead to drug interactions. One example that may be

relevant in the study population is ondansetron (used to reduce nausea and vomiting). Concomitant use may increase the risk of serotonin syndrome (shivering, fever and hemodynamic instability). In addition, the concomitant use of anti-emetics (including ondansetron) increases the risk of QT interval prolongation. Considering the above, the use of anti-emetics during the study is prohibited.
Multiple CYP2D6 inhibitors have currently been identified, including cimetidine and ranitidine.(42) The latter is particularly relevant to our study population. Lower doses than are usually prescribed for either the tricyclic antidepressant or the other drug may be required. Currently, CYP2D6 has no known inductors.

i. Predictability of effect

For the treatment of major depression, there is a linear relationship between drug plasma concentrations and therapeutic response in the majority of patients.(20) This provides the rationale within the psychiatry practice for monitoring drug levels and adjusting dosages to prevent toxicity and to monitor compliance. As far as functional gastrointestinal conditions are concerned, the presence of detectable drug levels in irritable bowel syndrome (indicating adherence to therapy) was associated with good clinical response.(21) However, higher dosages or high blood levels were not predictive of a better clinical response, which supports the contention that lower TCA dosages appear sufficient to treat functional GI disorders.(22)

All study subjects are asked to report any adverse event on a daily basis using a mobile phone app. In addition, all subjects are contacted by telephone every two weeks to further inform about adverse event and to evaluate treatment. This way, patients are monitored closely for any undesired effect.
A study by Thiwan et al investigated side effects of the TCA desipramine in a patient group with various functional GI disorders.(43) Two issues concerning suspected side effects were discussed in this study: 1) most suspected side effects of desipramine were found to be already present at baseline (with no increase in frequency or severity at two weeks treatment), indicating that these symptoms were not true side effects and 2) suspected side effects correlated little with plasma drug levels, but were clearly associated with psychological distress (i.e. anxiety and depression). It thus seems that the predictability of (side-)effects depends on factors other than drug levels, including patient anxiety level. In the current study however, patients with high levels of anxiety (as assessed by the GAD-7) are excluded, possibly limiting the occurrence of these ‘false’ side effects.

Finally, the use of TCAs (although less common with nortriptyline) can result in sedation. Due to this effect, it is advised not to drive a vehicle in the first week after treatment is initiated (https://www.rijveiligmetmedicijnen.nl/). After this, it is at the patients’ discretion to decide whether it is safe to drive.

j. Can effects be managed?

The known side effects of nortriptyline are mild and often only transient. Moreover, dosages used in the current study are relatively low as compared to dosages used in psychiatric disorders. Therefore, the anticipated risk is low.
Extensive knowledge on the topic of overdose exists. For a list of signs and symptoms we refer to the SmPC of nortriptyline. In case of overdose, symptomatic and supportive therapy is recommended. No direct antidote or antagonist for nortriptyline is available. Activated

charcoal may be more effective than emesis or lavage to reduce absorption. Diuresis and dialysis have little effect. Haemoperfusion is unproven.
Ventricular arrhythmias, especially when accompanied by lengthened QRS intervals, may respond to alkalinisation by hyperventilation or administration of sodium bicarbonate. Serum electrolytes should be monitored and managed. Refractory arrhythmias may respond to propranolol, bretylium or lignocaine. Quinidine and procainamide usually should not be used because they may exacerbate arrhythmias and conduction already slowed by the overdose.

Seizures may respond to diazepam. Phenytoin may treat seizures and cardiac rhythm disturbances. Physostigmine may antagonise atrial tachycardia, gut immotility, myoclonic jerks and somnolence. The effects of physostigmine may be short-lived.
Monitoring should continue at least until the QRS duration is normal.

13.2 Synthesis

All subjects are FD patients without psychiatric comorbidity with a CYP2D6 extensive metabolizer phenotype. The use of alcohol is limited during the study in order to prevent interaction. In addition, other medication used by the subjects will be checked before and during the study for possible interactions.(44) Pregnancy tests will be performed to prevent any damage to the unborn child (although current guidelines do not describe pregnancy as an absolute contra-indication for nortriptyline use).(45) Subjects will be asked to report the occurrence of side effects on a daily basis through the electronic diary and contact one of the researchers in the case of significant side effects. If deemed necessary, the patient will be invited for additional blood sampling for nortriptyline plasma level assessment. At the beginning and the end of 12 weeks, subjects will visit the hospital for evaluation or have an appointment by (video) call. Every two weeks, the investigator will inquire about side effects by telephone. Overall, expected side effects are mild and transient.

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ADDENDUM A

Background

CYP2D6 genotyping results in four categories:
- Poor metabolizers
- Intermediate metabolizers
- Extensive (normal) metabolizers
- Ultrarapid metabolizers

As described above, only patients that (by genotype) are predicted to being extensive metabolizers, can be eligible for randomization in this study. Patients that are excluded on the basis of their genotype however, might still benefit from nortriptyline. We therefore aim to offer open label nortriptyline treatment to FD patients recognized as poor or intermediate metabolizer. Ultrarapid metabolizers will not be eligible for this open label extension.

Hypothesis: We hypothesize that treatment with the second generation TCA nortriptyline (open label) results in adequate relief of symptoms in FD in patients with predicted poor or intermediate metabolizing capacity for CYP2D6.

Primary objective:

1. To explore the efficacy of low dose (10 mg) nortriptyline in FD patients without evidence of significant psychiatric disease, that have been identified as poor or intermediate metabolizers based on their CYP2D6 genotype.

Secondary objectives:

In addition to the objectives described in section 2, we aim to explore the relationship between CYP2D6 genotype and nortriptyline plasma levels at week 1 of treatment, when dosages are similar in all nortriptyline regimens (i.e. 10mg)

Open label treatment

Patients that are recognized as poor or abnormal metabolizers will be offered nortriptyline in a dose of 10 mg (open label), as customary in regular clinical care. No more than 10 mg dose will be administered to mitigate the occurrence of potential side effects.

Prescriptions and regular follow-up will be performed by the treating physician. Treatment duration will generally be 12 weeks, which is regarded routine clinical practice. When deemed necessary by the treating physician, treatment duration may be longer (or shorter). During the treatment period, the same study procedures as described in section 8.3 will be applicable (with the exception of randomisation). After treatment, the follow-up period will encompass the same study procedures (i.e. completion of questionnaires). After completion of the follow-up period, patients will receive the same monetary reward as patients in the randomized setting (i.e. 150 euros). Finally, the total number of inclusions will not exceed the pre-calculated sample size described in section 4.5 (i.e. 154 subjects). This open label extension is entirely exploratory and therefore no additional sample size calculation was performed.