Food and Drug Administration
Center for Drug Evaluation and Research

Gastrointestinal Drugs Advisory Committee (GIDAC)

Background Material

Meeting: Gastrointestinal Drugs Advisory Committee to provide recommendations to the Agency on the design and size of premarketing cardiovascular safety development programs necessary to support approval of products in the class of serotonin (5-hydroxytryptamine) receptor 4 (5HT4) agonists for the proposed indications of chronic idiopathic (of unknown cause) constipation (CIC), constipation predominant irritable bowel syndrome (IBS-C), gastroparesis, and gastroesophageal reflux disease that does not respond to a proton pump inhibitor

November 17, 2011

The attached package contains background information prepared by the Food and Drug Administration (FDA) for the panel members of the advisory committee. The FDA background package often contains assessments and/or conclusions and recommendations written by individual FDA reviewers. Such conclusions and recommendations do not necessarily represent the final position of the individual reviewers, nor do they necessarily represent the final position of the Review Division or Office. We have brought the serotonin (5-hydroxytryptamine) receptor 4 ($5\text{-}HT_4$) agonists class intended for use to treat certain gastrointestinal diseases to this Advisory Committee in order to gain the Committee's insights and opinions, and the background package may not include all issues relevant to the final regulatory recommendation and instead is intended to focus on issues identified by the Agency for discussion by the advisory committee. The FDA will not issue a final determination on the issues at hand until input from the advisory committee process has been considered and all reviews have been finalized. The final determination may be affected by issues not discussed at the advisory committee meeting.

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1 Introduction

1.1 Statement of Purpose

The purpose of the Gastrointestinal Drugs Advisory Committee is to obtain recommendations to the Agency on the design and size of premarketing cardiovascular safety development programs necessary to support approval of products in the class of serotonin (5-hydroxytryptamine or 5-HT) receptor 4 (5-HT_4) agonists for the proposed indications of chronic idiopathic (of unknown cause) constipation (CIC), constipation predominant irritable bowel syndrome (IBS-C), gastroparesis, and gastroesophageal reflux disease that does not respond to a proton pump inhibitor.

The 5-HT_4 agonists are gastrointestinal prokinetic/promotility agents that may be effective for the treatment of patients with functional bowel disorders, including constipation, gastroparesis and gastroesophageal reflux disease (GERD) unresponsive to proton pump inhibitors (PPIs). Two 5-HT_4 agonists were withdrawn from the United States (US) market due to cardiac arrhythmias (cisapride [Propulsid]) and ischemic cardiovascular (CV) events (tegaserod [Zelnorm]). Off-target (non 5-HT_4) receptor binding has been implicated as an etiology for adverse CV events (e.g., 5-HT_1 & 5-HT_2 receptor subtypes expressed in the CV system, as well as hERG channels).

Newer 5-HT_4 agonists are being pursued commercially and a number of such drugs have been evaluated under Investigational New Drug (IND) applications at the FDA. The sponsors assert that the selective binding of these prokinetic agents to the 5-HT_4 receptor may avoid activity at off-target pathways thought to be associated with increased CV risk. ARYx Therapeutics, INC, the pharmaceutical firm developing naronapride (ATI-7505), has performed several nonclinical and phase 1 & 2 clinical studies to evaluate cardiovascular safety. At this stage of drug development it is important for pharmaceutical sponsors to understand the amount of clinical safety data necessary to be included in a New Drug Application (NDA) supporting approval. This is particularly challenging given the history of cardiovascular safety issues with cisapride and tegaserod.

1.2 Mechanism of Action and Selectivity of Serotonin Agonists

Serotonin is a ubiquitous signaling molecule in the body. The largest amount of 5-HT (~90%) is found in enteroendocrine cells of the gastrointestinal mucosa.

There are 7 main serotonin receptor subtypes found throughout the body and these are presented in Table 1.

Table 1: 5-HT Receptor Distribution

Subtype	Location
5-HT1	Blood vessels, CNS
5-HT2	Blood Vessels, CNS, GI Tract Platelets, PNS, Smooth Muscle
5-HT3	CNS, GI Tract, PNS
5-HT4	CNS, GI Tract, PNS
5-HT5	CNS
5-HT6	CNS
5-HT7	Blood Vessels , CNS , GI Tract

CNS = central nervous system; PNS = peripheral nervous system;
GI= Gastrointestinal

5-HT_4 receptors are G protein-coupled receptors (GPCRs) that activate the stimulatory G-protein, *Gs*, leading to increased intracellular cAMP and activation of protein kinase A. There are several 5-HT_4 receptor splice variants within and between species.

In the gastrointestinal (GI) tract, 5-HT_4 receptors are located on the terminals of myenteric neurons and on GI smooth muscle cells. Their activation stimulates smooth muscle contraction and the peristaltic reflex.

Several 5-HT_4 receptor agonists have been shown to induce intestinal secretion and propulsion in animal tissue and facilitate the peristaltic reflex in human tissue. In addition, 5-HT_4 receptor agonists enhance GI and colonic transit. For example, cisapride has been shown to accelerate gastric emptying, small bowel and colonic transit, and tegaserod accelerates gastric emptying, shortens small bowel transit and accelerates colonic transit.

The prokinetic/promotility effects of 5-HT_4 agonists may have benefit in treating functional bowel disorders, including constipation, gastroparesis and gastroesophageal reflux disease (GERD) unresponsive to proton pump inhibitors (PPIs).

1.3 Cardiovascular Safety Evaluations in non-Cardiac Drugs

FDA is seeking expert advice on the premarketing evaluation that would be required to assess a CV safety risk for newer 5-HT_4 agonists. Several issues should be taken into consideration: 1.) cardiovascular safety of other members in the class, 2.) nonclinical and early stage clinical evaluations, 3.) proposed population and indication for which the product is being developed (the context of use), including associated morbidities and concomitant medication, 4.) unmet medical need, and 5.) assessment of the efficacy and safety. This Advisory Committee will be discussing issues related to the first four issues because we do not yet have a completed development program for any of the newer drugs. The full potential for efficacy of these newer 5-HT_4 agonists in specific GI diseases is unknown at this time.

1.3.1 Cardiovascular Safety of the 5HT4 agonist class

In the case of 5-HT_4 agonists, there is a historical association with previously approved members of this class and CV adverse events; cisapride and tegaserod. Both drugs presented different cardiovascular safety issues after marketing, which resulted in their removal from the U.S. market.[1,2]

The etiology of adverse CV events related to the use of cisapride is believed to be understood, since cisapride is known to prolong the QT interval on surface electrocardiograms (ECG), which itself is a risk factor for fatal cardiac arrhythmias, most clearly torsade de pointes (TdP), but possibly other ventricular tachyarrhythmias as well. In addition, the concentration of cisapride in the blood increased due to specific drug-drug interactions that interfered with its metabolism. These interactions were not known at the time of approval. Cisapride is metabolized by the cytochrome enzyme pathway (CYP450), which makes it difficult to use in combination with other agents which may also inhibit this pathway. Concomitant use may result in increased levels of cisapride.

Presently, all non-cardiac drugs are required to undergo an assessment of their potential to prolong the QT interval, so future 5-HT_4 agonists can be individually assessed for this known risk[3]. This clinical study is referred to as a "thorough QT" (tQT) study. Also, *in vitro* and *in vivo* drug-drug interaction studies are routinely performed to evaluate the potential for increased exposures that may increase the risk of toxicity.

More recently, in 2007, tegaserod was removed from the U.S. market when a meta-analysis of 29 studies revealed a higher risk of serious cardiovascular adverse events (e.g., angina, heart attacks, and strokes) associated with tegaserod use. The etiology of CV ischemic events related to the use of tegaserod was less well understood, partly due to the absence of thorough QT studies at the time, and the low incidence of adverse CV events. Additionally, studies assessing potential effects of tegaserod on platelet aggregation have not been consistent.

Therefore, from a mechanistic standpoint, because it remains unclear how tegaserod would increase the risk of ischemic CV events, some sponsors of investigational new drugs (INDs) have performed additional non-clinical studies to evaluate the CV risk potential of their drugs. These include *in vitro* artery contractility studies, platelet aggregation studies and other CV toxicology studies in animals that are not typically required for nonclinical evaluation of new drugs [ICH Guidances S7A and S7B][4,5]. The utility of these nonclinical studies to rule out

[1] Diane K Wysowski PhD, Ann Corken RPh, MPH, Hugo Gallo-Torres MD, PhD. Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and food and drug administration regulatory actions. *The American Journal of Gastroenterology* (2001) 96, 1698–1703.
[2] FDA Announces Discontinued Marketing of GI Drug, Zelnorm, for Safety Reasons; March 30, 2007 http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/2007/ucm108879.htm
[3] Guidance for Industry: E14 Clinical Evaluation of QT/QTc interval Prolongation and Proarrhythmic Potential for Non-Antiarrhythmic Drugs; October 2005; http://www.fda.gov/downloads/RegulatoryInformation/Guidances/ucm129357.pdf
[4] Guidance for Industry: S7A Safety Pharmacology Studies for Human Pharmaceuticals; July 2001; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074959.pdf
[5] Guidance for Industry: S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals; October 2005; http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM074963.pdf

potential causes of increased CV risk in humans is not absolute, however if there is a reasonable suspicion that 5-HT_4 agonists may be associated with increased CV risks, then additional studies in humans may be warranted.

1.3.2 Non-clinical and clinical pharmacology evaluations

In addition to standard nonclinical testing, the ICH S7A Guidance provides specific recommendations for evaluating effects on the cardiovascular system (e.g., cardiac output, ventricular contractility, vascular resistance, and effects of endogenous and/or exogenous substances on the cardiovascular responses). The ICH S7B Guidance further describes studies for the nonclinical evaluation of the potential for QT interval prolongation for drugs.

For the purposes of hazard assessment related to cardiovascular safety, sponsors may choose to perform the more targeted CV assessments described in the ICH S7A and S7B guidance documents.

The results of nonclinical studies for each of the 5 drugs described in this background document are presented in their respective sections. Negative outcomes of nonclinical studies do not rule-out potential safety concerns in humans. However, positive identification of safety signals in nonclinical studies may necessitate closer assessments in human studies prior to approval.

A clinical pharmacology program in drug development characterizes absorption, distribution, metabolism and excretion (ADME) of a drug following administration to facilitate understanding of exposure-response relationship. It also assesses intrinsic (e.g., organ impairment) and extrinsic factors (e.g., concomitant drugs) that may alter the ADME to inform proper dosing. Evaluation of drug-drug interactions is crucial in this regard, as they may result in significantly higher exposure to the parent compound and/or its metabolites that can alter the safety profile of the drug. Additional clinical pharmacology studies for safety assessment may include thorough QT and platelet aggregation studies.

1.3.3 Proposed population and indication of drug

The 5-HT_4 agonists are being developed to treat patients with a variety of GI motility disorders: chronic idiopathic (of unknown cause) constipation (CIC), constipation predominant irritable bowel syndrome (IBS-C), gastroparesis, and gastroesophageal reflux disease that does not respond to a proton pump inhibitor. Treatment of the population with constipation in general could expose a very large population of patients to a potential safety risk, which makes the benefit/risk assessment unfavorable if a cardiovascular risk does in fact exist. On the other hand, for a population of patients with a more serious motility disorder such as IBS-C or in patients with gastroparesis (a high morbidity) such a safety risk may result in a more acceptable benefit/risk ratio. Although the benefit-risk discussion is not the focus of this advisory committee meeting, one should consider the context of the disease for which these drugs are being developed.

1.3.4 Unmet Medical Need

With the exception of tegaserod, available only for emergency use, there are no other selective 5-HT_4 promotility drugs currently approved in the United States. In general, there are few approved therapies to treat motility disorders.

For patients with irritable bowel-constipation predominant (IBS-C), lubiprostone (Amitiza) is the only currently FDA approved medication.

For patients with chronic idiopathic constipation (CIC), there are only two approved therapies—lactulose (osmotic laxative) and lubiprostone (a secretory laxative). Miralax is available as an over-the-counter treatment and is currently labeled for seven day courses only. Miralax was formerly approved for chronic constipation, but underwent an OTC switch. Given the prevalence of CIC (~ 2-27% of U.S. adults), there exist limited therapeutic options for these patients and patients with other motility disorders.

Finally, there is only one approved drug for diabetic gastroparesis, metoclopramide, which has been associated with a number of neurologic side effects, including movement disorders. Domperidone is available only through an emergency IND program in the U.S.[6,7]

1.3.5 Efficacy and Safety Evaluations

With regard to drug class and safety issues, not all drugs in a class necessarily have the same safety profile. Attempts to distinguish differences can be made by conducting nonclinical studies and early clinical trials. However, while these types of studies give us some assurance that it is safe to proceed to phase 3 clinical trials, routine clinical drug development plans typically provide for exposure of relatively small numbers of patients to the drug prior to FDA approval and subsequent marketing. Important rare safety events may only be detected after approval when a substantially larger number of patients have been treated with the drug.[8]

The studies required to support the effectiveness of 5-HT_4 agonists for their intended indications (e.g., CIC, IBS) are typically designed in a manner that would not answer the question of whether or not an increased CV risk is associated with the drug, because the length of the study period is too short and the sample size too small to allow such detection. There are two ways to obtain such data regarding CV risk. One approach would be to increase the enrollment to a sufficient number of patients in the intended population and follow them for a sufficient amount of time in order to observe a sufficient number of expected CV events. The CV events are then analyzed by treatment group. This design might require significant

[6] The site for information for physicians wishing to start their patients on Domperidone: http://www.fda.gov/Drugs/DrugSafety/InformationbyDrugClass/ucm073070.htm
[7] Guidance to physicians wishing to apply for an IND for Domperidone: http://inside.fda.gov:9003/CDER/OfficeofTrainingandCommunication/DivisionofDrugInformation/ucm031669.htm
[8] ICH E1A Guidance "The Extent of Population Exposure to Assess Clinical Safety: For Drugs Intended for Long-term Treatment of Non-Life-Threatening Conditions " available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM073083.pdf (accessed June 18, 2011)

resources (time, money, patients) that may be impractical prior to marketing a drug. An alternative approach would be to study the drug in a smaller number of patients in the intended population who also have a greater baseline risk of having CV events, such as older patients or those with a history of CV risk factors (e.g., diabetes) or known CV disease. Such an *enriched* population could provide enough CV events to determine if the drug actually increases CV risk over an appropriate control or placebo.

1.4 Statistical Considerations

For the assessment of cardiovascular safety, dedicated, randomized cardiovascular (CV) outcome trials are considered to be the gold standard in determining the hazard ratio (HR) of an investigational treatment relative to a well understood control. Such trials are typically designed as event-driven trials with the objective of ruling out an excess risk measured by an upper bound of a 95% confidence interval for the hazard ratio.

Several elements play a role in the number of events needed to reach a defined level of statistical power and the number of patients needed to observe the events: definition of cardiovascular events that form the composite safety endpoint; the study population's underlying risk of cardiovascular events; the degree of excess risk to rule out for the hazard ratio margin; the assumed value of the true underlying hazard ratio.

Section 7 displays sample size calculations for various choices of the hazard ratio margin and for various background rates in more detail.

In general, a trial in a non-enriched population with a relatively low event rate requires more patient years than a trial in an enriched population in order to observe the same number of events and achieve the same statistical power for a pre-specified hazard ratio margin.

1.5 Summary

ARYx Therapeutics, Inc. has performed numerous nonclinical cardiac studies, a clinical thorough QT-interval study, and platelet aggregation studies during the development of naronapride. In light of the cardiovascular adverse events reported in post-marketing for cisapride and tegaserod, the Division of Gastroenterology and Inborn Errors Products (DGIEP) has discussed the early clinical development program with ARYx Therapeutics, Inc. Currently, the sponsor is in a position to embark on a phase 3 program and is interested in understanding the amount of clinical data that is necessary to determine the cardiovascular safety of the drug prior to marketing. This advice will be useful for the other sponsors of products in the class, e.g. Theravance Inc.

This background package briefly reviews the data which was known at the time of approval and relevant information since approval for cisapride and tegaserod in order to place the quantity and quality of information for newer products in the class in context with prior regulatory history of this class. In addition, data from ongoing development of new 5-HT_4

agonist drugs is presented. It should be noted, that the regulatory history of this class spans more than 15 years (cisapride was approved in 1993). During that time the Center for Drug Evaluation and Research (CDER) worked with the European Medicines Agency (EMA) to develop ICH E14 guidance for the evaluation of cardiovascular safety, specifically, the QT assessment, during drug development for non-cardiac drugs. More recently CDER has been engaged in the discussion of the evaluation of cardiovascular safety in non-cardiac drugs used in the treatment of diabetes and weight loss. Each of these discussions has viewed the issue in the context of the underlying disease.

Before deciding whether trials should be designed to answer the question of whether or not there is a CV risk associated with a drug, one must first consider whether CV risk assessment in patients is warranted. Because dedicated CV safety studies are impractical to perform on all drugs and for all indications, the evidence implicating a drug or drug class must be carefully weighed prior to requiring studies before they are approved.

Finally, even if CV safety trials are considered to be necessary prior to approval, one must determine what level of CV risk should be excluded. The level of CV risk that would be acceptable to a patient or physician, as discussed previously above, may depend on the nature and severity of the underlying disease. In this case, we will ask the panel to consider what level of CV risk patients might accept across the potential indications of 5-HT_4 agonists (CIC, gastroparesis, IBS-C, GERD, etc.)

The decision to approve a new drug is based on an assessment of the demonstrated efficacy of a drug for a given indication and patient population, weighed against the potential harms that the drug may present to these patients. For example, in the case of life-threatening diseases, a drug with serious risks may be acceptable if there is a reasonable expectation of a survival benefit. However, for non-life threatening diseases or conditions which are not considered to be serious, the risk/benefit balance is not as straightforward. In cases where the underlying disease is manageable with other drugs or lifestyle modifications the risk of a serious rare event may not be acceptable. Therefore it is important to consider the diseases for which the drug is intended for use.

We anticipate that future AC meetings will be held when the phase 3 data and new drug applications (NDAs) for members of this drug class are submitted to the FDA for evaluation. We are asking the Committee now for advice on the extent of cardiovascular safety assessment that should be available for these products in order to make a reasonable risk/benefit assessment to appropriately guide approval decisions. For cardiovascular toxicity, clinical trials may be the best way to estimate the risk; however, the sample size and duration of the study required may preclude the development of necessary new drugs.

1.6 Points for Consideration by the Advisory Committee

The FDA requests that the Advisory Committee consider a number of potential discussion topics during its review of these (and the Sponsors') briefing documents. These topics are intended to frame the major review issues for more specific questions to be posed to the Committee at the meeting.

1. Can nonclinical, clinical pharmacology, and clinical data such as that presented for the newer 5-HT_4 agonists adequately address (i.e., alleviate the need for a dedicated safety study) the cardiovascular safety concerns (e.g., prolonged QT interval, myocardial infarction, ischemic events) associated with the previously approved 5-HT_4 agonists? If yes, specify on which data you are relying.

2. Considering the potential for cardiovascular adverse events, would your willingness to prescribe a 5-HT_4 agonist depend on the indication (CIC, IBS-C, gastroparesis, other functional motility disorders)?
    a. If yes, which ones and why?
    b. What degree of increased risk in each population would be acceptable?

3. For each indication (see below), does the Committee recommend that a dedicated CV safety trial be conducted to demonstrate the safety of 5-HT_4 agonists? If yes, do you recommend that the trial be conducted prior to or post-approval?
    a. CIC
    b. IBS-C
    c. Gastroparesis
    d. Other functional motility diseases

4. If the Committee does not recommend a dedicated CV safety trial, what elements to assess CV safety should be included in a standard phase 3 efficacy trial to assure accurate ascertainment of CV adverse events?

Administrative Note:

This background package is organized by drug in separate sections under the general headings of non-clinical, clinical pharmacology and clinical. For cisapride and tegaserod, highlights of important information relevant to potential cardiovascular safety issues are provided. This document also provides a separate section on biostatistical considerations for designing clinical trials to address cardiovascular safety.

ARYx Therapeutics, Inc., the sponsor of naronapride, has permitted DGIEP to present their data on their behalf, in light of various questions which they posed to DGIEP regarding their phase 3 clinical development program. DGIEP invited pharmaceutical firms to present at this advisory committee who either had marketed 5-HT_4 agonists or are actively developing 5-HT_4 agonists for the treatment of gastrointestinal diseases. Of these, Theravance, Johnson & Johnson Pharmaceutical Research & Development, LLC (J&JPRD) accepted the invitation to present.

This background package is based upon publically available information or when not publically available, the information was included with the permission from the sponsors.

2 Naronapride (ATI-7505)

Naronapride (ATI-7505), a potent and selective 5-HT_4 receptor agonist, is a prokinetic agent proposed for the treatment of chronic idiopathic constipation (CIC). The Sponsor plans to target other GI indications as outlined in Figure 1 below.

Figure 1. Sponsor's Planned Targeted Indications for Naronapride

GERD	FUNCTIONAL DYSPEPSIA
• $17B spent worldwide each year • Est. 10% of population experiences symptoms daily • Estimated 20-25% of patients (6.0-7.5M in US) do not obtain adequate relief from stomach acid-reducing treatments	• Est. 35-44M people (US) with symptoms • Major subtype is postprandial distress syndrome (PDS) defined by: o Postprandial fullness o Early satiety, or o Upper abdominal bloating
GASTROPARESIS	**LOWER GI INDICATIONS**
• Est. 5M people (US) with symptoms • High prevalence in diabetic patients • No existing therapies adequately meet this patient need	• Est. 36M people (US) with chronic constipation • Est. 5.5M adults (US) suffer from IBS with constipation • Est. 28M adults (US) suffer from IBS with intermittent constipation

Source: Sponsor website: www.aryx.com/pdf/factsheet_naronapride.pdf, *last accessed 10/16/2011*

2.1 Regulatory History

The initial Investigational New Drug (IND) application for naronapride was submitted on March 12, 2004.

On September 20, 2010, the Division of Gastroenterology and Inborn Errors Products (DGIEP) received a request for a Special Protocol Assessment (SPA) for the planned phase 3 studies of naronapride for chronic idiopathic constipation (CIC). The purpose of a SPA is to enable a sponsor to reach a binding agreement with the FDA on a protocol's design, planned analysis or other specific issues.

The Division has been unable to provide adequate recommendations regarding the best path forward to address the CV safety concerns associated with the 5-HT_4 agonist drug class. Therefore, the current Advisory Committee meeting is being held to seek advice and recommendations from the GIDAC on the design and size of premarketing cardiovascular

safety development programs necessary to support approval of products in the class of 5-HT_4 agonists.

2.2 Nonclinical

Summary

Naronapride (ATI-7505) was shown to be a relatively selective 5-HT_4 receptor agonist with very low affinities for 5-HT_3 and 5-HT_{2B} receptors. ATI-7505 and its main metabolite, ATI-7500 caused very weak inhibition of hERG K^+ channels with about 2,581- and 21,482-fold lower potencies than cisapride, respectively. In isolated Guinea pig hearts, ATI-7505 had no significant effect on the QT-interval. In rabbit Purkinje fibers, it caused a slight prolongation of action potential duration at 90% repolarization (APD_{90}), while cisapride caused a significant prolongation of APD_{90} in this preparation. In anesthetized male dogs, ATI-7505 caused a slight prolongation of QTc (QT interval corrected) at 1.0 and 2.0 mg/kg IV doses, while 0.1 and 0.3 mg/kg doses had no effects. In anesthetized male Guinea pigs, it had no significant effects on the QTc at IV doses up to 1.0 mg/kg; however, the 3 mg/kg dose caused a significant prolongation of the QTc. Cisapride at a dose of 1 mg/kg caused a significant prolongation of QTc in anesthetized Guinea pigs. Thus, ATI-7505 showed weak cardiovascular adverse effects in the *in vitro* and *in vivo* cardiovascular safety pharmacology studies.

Serotonin Receptor Selectivity Studies

In *in vitro* receptor binding studies, naronapride (ATI-7505) was found to be selective for 5-HT_4 receptors, with very low affinities for the other serotonin receptor subtypes (e.g. 5-HT_3, 5-HT_{2B}). It has higher binding affinity than cisapride for the serotonin 5-HT_4 receptors, and had very weak affinities for 5-HT_3 and 5-HT_{2B} (with Ki values of >6,757 nM and 804 nM, respectively) receptors. Cisapride had Ki values of 1,340 nM and 74 nM for 5-HT_3 and 5-HT_{2B} receptors, respectively. The Ki values for ATI-7505, ATI-7500 and cisapride for 5-HT_4 and other receptors are shown in the Table 2.

Table 2: Selectivity for Serotonin and Dopamine Receptors

Compound	5-HT_4 Receptor (G.pig Striatum)	5-HT_3 Receptor (HEK-293 Cells)	5-HT_{2B} Receptor (CHO Cells)	Dopamine D_{2L} Receptor (CHO Cells)	Dopamine D_{2S} Receptor (CHO Cells)
ATI-7505	1.39 nM	>6,757 nM	804 nM	205 nM	68 nM
ATI-7500	>500 nM	>6,757 nM	6,810 nM	N/A	13,860 nM
Cisapride	150 nM	1,340 nM	74 nM	N/A	N/A
Norcisapride	N/A	150 nM	N/A	N/A	N/A

N/A = data not available

The binding specificity of ATI-7505 and its metabolite, ATI-7500 was also studied using *in vitro* competition radioligand binding assays. A total of 64 targets, corresponding to diverse spectrum of cellular receptors, ion channels and transporters were used in the study. ATI-7505 showed very low binding affinities for dopamine D_{2L} and dopamine D_3 receptors. In addition,

ATI-7505 showed low binding affinities for L-type (benzodiazepine, dihydropyridine and phenylalkylamine) calcium, potassium (KA) and sodium (site 2) channels as compared to cisapride. The % inhibition of binding for different calcium channels, and potassium and sodium channels is shown in Table 3.

Table 3: Binding Affinities for Different Ion Channels

Target	% Inhibition at 10 μM		
	ATI-7505	ATI-7500	Cisapride
Calcium channel L-type, benzodiazepine	15	9	91
Calcium channel L-type, dihydropyridine	13	-11	66
Calcium channel L-type, phenylalkylamine	38	4	94
Potassium channel KA	5	0	35
Sodium channel site 2	17	2	110

Source: Sponsor's data

In Vitro Cardiac Electrophysiology Studies

Effects on the hERG potassium channel currents in HEK293 cells:

The inhibitory effects of ATI-7505 and ATI-7500 on the activity of human I_{Kr} channels were examined in HEK-293 cells using the patch clamp technique. ATI-7505 and its metabolite, ATI-7500, were weak inhibitors of hERG potassium channels in HEK-293 cells with IC_{50} values of 24,521 and 204,080 nM, respectively. Cisapride had an IC_{50} value of 9.5 nM in this study, showing that cisapride was approximately 2,581- and 21,482-fold more potent than ATI-7505 and ATI-7500, respectively in inhibiting the hERG channel. The IC_{50} values for hERG potassium channel inhibition for ATI-7505, ATI-7500, cisapride and norcisapride are shown in Table 4.

Table 4: Comparison of Inhibition of hERG Channels

Compound	IC_{50} (nM)
ATI-7505	24,521 ± 1,932
ATI-7500	204,080 ± 13,970
Cisapride	9.5 ± 0.5
Norcisapride	> 10,000

The vehicle, DMSO had minimal effect on tail I_{kr} (96.6 ± 0.5%)

Source: Sponsor's data.

Effects on non-IKr channels:

In a whole cell patch-clamp electrophysiology study in guinea pig ventricular myocytes, the effects of ATI-7505 and its active metabolite ATI-7500 on non-I_{kr} (I_{Na}, $I_{Ca,L}$, I_{KS} and I_{Kl}) cardiac currents were examined. ATI-7505 and ATI-7500 had no significant effects on early I_{Na} and late I_{Na} currents at concentrations up to 30,000 (IC_{50}, >30,000 nM). However, cisapride inhibited the early I_{Na} current with an IC_{50} value of 11,400 nM. Naronapride had no significant effect on $I_{Ca,L}$ at a concentration of 1,000 nM, while ATI-7500 caused an inhibition of this ion channel (IC_{50}, >10,000 nM). Both naronapride and ATI-7500 had weak inhibitory effects on I_{KS},

and none of them had any effect on I_{KI}. The effects of ATI-7505 and ATI-7500 on different ionic currents are summarized in Table 5.

Table 5: Ionic Currents in Guinea Pig Myocytes

Ionic Current	Compound Tested	% Change from Vehicle Control					IC_{50} (nM)
		100 (nM)	1000 nM	3000 (nM)	10,000 (nM)	30,000 (nM)	
Early I_{Na}	ATI-7505	--	+0.8	-1.6	-2.3	-12.1	>30,000
	ATI-7500	--	-5.2	-0.6	-4.7	-1.5	>30,000
	Cisapride	--	-9.1	-20.1	-44.0	-75.7	11,400
	Flecainide	--	-9.0	-50.4	-83.2	-94.1	3,300
Late I_{Na}	ATI-7505	-30.3	-6.5	--	--	--	>1,000
	ATI-7500	-4.4	+2.9	--	--	--	>1,000
	ATX-II	+871.6	+685.5	--	--	--	n/a
I_{Ca}, L	ATI-7505	+1.4	-8.0	--	--	--	>1,000
	ATI-7500	-10.9	-23.0	--	-32.6	--	>10,000
	Verapamil	--	-34.0	--	-54.4	--	>1,000 – >10,000
I_{KS}	ATI-7505	--	-2.5	-4.6	-7.7	-11.9	>30,000
	ATI-7500	--	-1.3	+0.4	-11.2	-19.0	>10,000
	Chromanol 293B	--	--	-25.9	-58.8	-84.2	7,400
I_{KI}	ATI-7505	--	+2.3	+2.3	+0.4	-3.7	>30,000
	ATI-7500	--	+2.1	+7.8	+1.9	-1.1	>30,000
	Cisapride	--	-1.1	-2.8	-2.8	-5.7	>30,000
	Barium	--	--	-21.6	-42.6	-76.2	11,400

Source: Reproduced from Sponsor's data.

Electrophysiological effects in guinea pig hearts:

The electrophysiological effects of ATI-7505, ATI-7500, cisapride and norcisapride were examined in isolated perfused guinea pig hearts. The effects of the agents on QT-interval, monophasic action potential duration (MAPD90), atrial recording time (SA-interval), QRS interval, atrioventricular nodal contraction time (AH-interval) and His purkinje conduction time (HV-interval) were examined. ATI-7505 caused a slight prolongation of QT intervals (1.1% and 6.2% at 1,000 and 10,000 nM, respectively). Cisapride, at the same concentrations caused 9.6% and 11.1% prolongation of the QT intervals, respectively. Cisapride also caused a prolongation of QRS, AH and HV intervals. Cisapride had a NOEL of 10 nM for the combined set of 6 parameters, while ATI-7505 had a combined NOEL of 1,000 nM. ATI-7500 had no significant effect on any of the parameters. The effects of ATI-7505, ATI-7500, cisapride and norcisapride on ECG parameters in guinea pig heart are shown in Table 6.

Table 6: Electrophysiologic Effects on Guinea Pig Hearts

Electrophysiological Parameters	1,000 nM (mean % increase relative to baseline)				10,000 nM (mean % increase relative to baseline)			
	Cisapride	Norcisapride	ATI-7505	ATI-7500	Cisapride	Norcisapride	ATI-7505	ATI-7500
QT Interval	9.6%	1.7%	1.1%	0.0%	11.1%	8.1%	6.2%	0.0%
$MAPD_{90}$	10.7%	0.2%	0.0%	0.5%	15.9%	6.5%	6.1%	1.1%
SA Interval	23.8%	7.3%	13.1%	3.1%	95.8%	29.1%	17.5%	2.3%
QRS Interval	7.5%	0.0%	0.0%	0.5%	110.6%	0.0%	0.0%	0.0%
AH Interval	19.0%	2.9%	1.0%	2.3%	120.7%	17.3%	7.2%	3.1%
HV Interval	8.9%	8.3%	20.0%	5.3%	230.3%	21.7%	45.0%	6.7%

MAPD, nonophasic action potential; AH interval, atrial wave to His bundle interval; SA interval, stimulus artifact to atrial wave interval; HV interval, His bundle to ventricular complex interval.

Source: Sponsor's data

Effects on the action potential in rabbit Purkinje fibers:

The effects of ATI-7505, ATI-7500 and cisapride on action potential duration (at 50% and 90% repolarization) in electrically paced (1.0 and 2.0 Hz) rabbit Purkinje fibers were examined. Naronapride, at concentrations of 100 and 1000 nM, increased APD_{90} by 18% and 19%, respectively (not statistically significant) at 0.2 Hz stimulation. Cisapride, 100 nM prolonged APD_{90} by 29% and 22% at 1.0 and 0.2 Hz, respectively. ATI-7500 had no effects on the action potential duration at any rate of pacing.

Table 7: Action Potential Duration in Rabbit Purkinje Fibers

ATI-7505, 90% Repolarization						
	Control	Vehicle	ATI-7505			Sotalol (10μM)
			10 nM	100 nM	1000 nM	
1 Hz 20 min	226 ±11	234 ± 9	243 ± 9	248 ± 9	253 ± 8	283 ± 12
1 Hz 30 min	228 ± 8	237 ± 9	246 ± 9	251 ± 8	262 ± 12	302 ± 15
0.2 Hz	254 ± 12	264 ± 11	290 ± 20	311 ± 29	315 ± 16	469 ± 43
ATI-7500, 90% Repolarization				**ATI-7500**		
1 Hz 20 min	267 ± 24	269 ± 26	279 ± 25	276 ± 24	283 ± 32	312 ± 40
1 Hz 30 min	264 ± 23	283 ± 31	281 ± 26	281 ± 20	287 ± 32	328 ± 49
0.2 Hz	316 ± 26	341 ± 34	351 ± 34	331 ± 45	369 ± 44	440 ± 54
Cisapride, 90% Repolarization				**Cisapride**		
	Control	Vehicle	10 nM	100 nM		Sotalol (10μM)
1 Hz 20 min	257 ± 17	260 ± 16	271 ± 18	315 ± 23		367 ± 41
1 Hz 30 min	259 ± 17	260 ± 15	276 ± 18	335 ± 26		391 ± 55
0.2 Hz	326 ± 46	333 ± 50	345 ± 46	406 ± 53		502 ± 93
ATI-7505, 50% Repolarization				**ATI-7505**		
	Control	Vehicle	10 nM	100 nM	1000 nM	Sotalol (10μM)
1 Hz 20 min	176 ± 8	187 ± 6	198 ± 7	205 ± 7	210 ± 6	253 ± 9
1 Hz 30 min	172 ± 5	191 ± 6	201 ± 6	209 ± 6	220 ± 11	252 ± 12
0.2 Hz	197 ± 9	212 ± 8	240 ± 20	262 ± 30	263 ± 14	411 ± 47
ATI-7500, 50% Repolarization						
1 Hz 20 min	194 ± 20	196 ± 26	215 ± 21	202 ± 24	213 ± 25	238 ± 41
1 Hz 30 min	194 ± 19	211 ± 24	215 ± 22	199 ± 23	222 ± 26	257 ± 39
0.2 Hz	217 ± 20	221 ± 20	232 ± 25	254 ± 43	263 ± 42	377 ± 53

Source: Sponsor's data. Values are expressed in msec. Sotalol included as a positive control

In Vivo Cardiac Safety Pharmacology Studies

The effects of single intravenous doses of ATI-7505 (0.1, 0.3, 1.0 and 2.0 mg/kg) on the cardiovascular functions were assessed in anesthetized male beagle dogs. ATI-7505 at 0.1 and 0.3 mg/kg had no significant effects on blood pressure, heart rate, +dp/dt or left ventricular pressure. At 1.0 and 2.0 mg/kg doses, there was a transient decrease in mean arterial pressure (19-22%) and dp/dt (17-28%). At 1 and 2 mg/kg doses, a slight increase in QT intervals was observed at 5-10 minutes after dosing (QTc change, 5.7%). The effects of 1.0 and 2.0 mg/kg doses of ATI-7505 on QT and QTc are summarized in Table 8.

Table 8: Effects on QT intervals in Dogs

Treatment	QT (sec)	QTc (sec)	QTc (% Change)
Vehicle (5% Dextrose)			
0 min post-dose	0.230±0.009	0.266±0.007	--
5 min post-dose	0.225±0.007	0.263±0.005	-1.1
10 min post-dose	0.237±0.007	0.275±0.005	3.4
30 min post-dose	0.233±0.003	0.271±0.002	1.9
ATI-7505 (1.0 mg/kg)			
0 min post-dose	0.225±0.008	0.264±0.006	--
5 min post-dose	0.239±0.012	0.279±0.011	5.7
10 min post-dose	0.233±0.010	0.271±0.008	2.7
30 min post-dose	0.219±0.007	0.258±0.006	-2.3
ATI-7505 (2.0 mg/kg)			
0 min post-dose	0.226±0.011	0.265±0.009	--
5 min post-dose	0.241±0.014	0.280±0.012	5.7
10 min post-dose	0.242±0.014	0.280±0.012	5.7
30 min post-dose	0.231±0.015	0.270±0.013	1.9

Data presented as Mean ± SEM of 4 experiments; reproduced from Sponsor

ATI-7505, ATI-7500 and cisapride were studied for their effects on QT interval in urethane anesthetized male guinea pigs. ATI-7505 (0.3, 1 and 3 mg/kg), ATI-7500 (3 mg/kg), cisapride (0.3 and 1 mg/kg) and the vehicle were administered intravenously, and the EGC was recorded for up to 20 minutes after dosing. ATI-7505, at 0.3 and 1 mg/kg had no significant effect on QTc (Bazett's formula). At 3 mg/kg, ATI-7505 caused a significant and transient (3-5 minutes) prolongation of QTc (22 ms). Cisapride (1 mg/kg) caused a significant prolongation of QTc (14.3 to 24.3 ms) that persisted throughout the 20 minutes study period. ATI-7500 had no significant effect on QTc at a dose of 3 mg/kg. The effects of ATI-7505, ATI-7500 and cisapride on changes in QTc at different periods are shown in Table 9.

Table 9: Effects on QT Intervals in Guinea Pigs

QTc Values						
Treatment	**Baseline (0 min)**	**Δ, 1 min.**	**Δ, 3 min.**	**Δ, 5 min.**	**Δ, 10 min.**	**Δ, 20 min.**
Vehicle	254.3	+3.0	+2.0	+2.7	+0.7	-1.7
Cisapride						
0.3 mg/kg	276.3	+0.3	+1.3	-0.7	-0.7	-1.7
1.0 mg/kg	250.7	+14.3	+24.3	+21.3	+17.7	+16.3
ATI-7500						
3.0 mg/kg	258.7	+4.3	+4.3	+3.7	-0.3	-1.3
ATI-7505						
0.3 mg/kg	250.7	+5.0	+4.3	+2.7	-0.7	-4.3
1.0 mg/kg	231.0	+6.0	+3.0	+1.7	+0.7	+2.0
3.0 mg/kg	263.0	+4.3	+22.0	+8.7	-1.7	-6.7
Dofetilide						
30 µg/kg	232.3	+9.0	+12.0	+25.0	+21.3	+19.3

Source: Sponsor's data. Values are expressed as msec. Dofetilide is a positive control.

2.3 Clinical Pharmacology

2.3.1 Metabolism of Naronapride

Upon oral administration, naronapride is extensively metabolized. ATI-7500, ATI-7400 and ATI-7100 are the major metabolites in the plasma with the systemic exposure (AUC) approximately 17-, 8-, and 2.6-fold higher than that of naronapride, respectively. The AUC of quinuclidinol, a byproduct of metabolism, is 72-fold higher than that of naronapride.

2.3.2 Drug-drug Interaction Studies

The hydrolysis of naronapride to ATI-7500 is mediated by esterases, which is further metabolized to ATI-7400 and then ATI-7100 through non-CYP pathways. CYP enzyme mediates the metabolism of naronapride to norcisapride as a minor pathway. The concentration of metabolites formed by CYP enzymes is lower than the detection limit in plasma although it is detectable in urine. Based on these observations, the potential for drugs that are CYP450 enzyme inhibitors to affect naronapride PK is low. The potential factors that may affect esterase activity are not well understood.

In vitro studies suggest that naronapride is a substrate of p-glycoprotein (P-gp), an efflux transporter. As such, the potential for P-gp inhibitors to affect naronapride PK cannot be ruled out at this time.

2.3.3 Thorough QT Study

The effect of naronapride on QT prolongation was evaluated in a randomized, double-blind, multiple-dose, parallel group study. In this study, 242 healthy subjects received either multiple doses of naronapride 40 mg, naronapride 200 mg, placebo q6h, or a single oral dose of moxifloxacin 400 mg.

Based on CDER's review, the largest upper bound of the 2-sided 90%CI for the mean difference between naronapride and placebo was below 10 ms. The assay sensitivity of the study was adequately established with moxifloxacin as a positive control. As such, no

significant QT prolonging effect of naronapride (40 mg q6h and 200 mg q6h) was detected in the thorough QT study.

Table 10: The Point Estimates and the 90% CIs Corresponding to the Largest Upper Bounds for Naronapride and the Largest Lower Bound for Moxifloxacin (Analysis by IRT-QT team)

Treatment	Time (hour)	ΔΔQTcI (ms)	90% CI (ms)
ATI-7505 40 mg	4.00	3.1	(-1.2 , 7.3)
ATI-7505 200 mg	0.00	5.5	(1.5 , 9.5)
Moxifloxacin 400 mg*	1.75	11.4	(7.1 , 15.8)

* Multiple endpoint adjustment is not applied. The largest lower bound after Bonferroni adjustment for 3 time points is 6 ms.

The mean Cmax of the supratherapeutic dose (200 mg) was about 10-fold higher than that for the then therapeutic dose (40 mg). The margin for Cmax is estimated to be about 3-5 fold for the currently proposed highest dosing regimen of 80 mg twice daily. The highest tolerated dose was 250 mg following q6h dosing.

Table 11: Mean Cmax (ng/ml; range) at Steady-State following Q6h Dosing

Study	tQT	tQT	CLN-710
Dose	40 mg	200 mg	80 mg
Naronapride	5.88 (0.89-18.7)	66.2 (17.9-154)	11.9 ± 5.9
ATI-7500	52.9 (12.9-51.2)	518.1 (227-890)	129 ± 83

2.3.4 Platelet aggregation study

Effect of naronapride on platelet aggregation was studied *in vitro* using a standard Light Transmission Aggregometry (LTA) based on stimulation of platelet aggregation by an agonist, collagen.

Naronapride was incubated with platelet rich plasma from healthy volunteers at 10, 30, and 100 ng/ml with or without an esterase inhibitor to prevent hydrolysis of naronapride; platelet aggregation was induced by adding collagen. Under these experimental conditions, naronapride did not induce or inhibit platelet aggregation. Nevertheless, the formation of metabolites in the absence of an esterase inhibitor was not quantified. As such, the effects of the major metabolites on platelet aggregation were not addressed in this study.

2.4 Clinical

2.4.1 Completed Studies

In the phase 1 and phase 2 studies completed to date, 982 patients have been exposed to naronapride. Of those exposed, 53.3% have reported adverse events (compared with 42.4% of placebo patients). No deaths and no ischemic CV events have been reported.

2.4.2 Ongoing Studies

A phase 2 study of visceral hypersensitivity in GERD patients is being conducted by a research investigator. Current safety results are not available.

2.4.3 Proposed Studies

The proposed study is a phase 3, randomized, double-blind, placebo-controlled, parallel group, multicenter study. Approximately 600 patients are planned to be enrolled. Patients will be excluded from the study if they have the presence or suspected presence of unstable coronary artery disease, myocardial infarction, stroke, or transient ischemic attack within six months of screening. After completing 12 weeks of active treatment, all patients will be eligible for an open-label, long-term safety study.

Cardiac and Ischemic Event Monitoring

The proposed phase 3 study is not designed to evaluate whether naronapride has an increased cardiac risk compared to placebo. However, the Sponsor has proposed to incorporate the following into the proposed study to aid in cardiac and ischemic event monitoring:

- A dedicated case report form will be used to characterize any CV events that occur and an effort will be made to ensure that patients reporting an event are followed clinically and that all hospital records are obtained to further characterize the event.
- An independent endpoint assessment and adjudication committee composed of cardiologists, neurologists and other physicians experienced in the adjudication of cardiovascular endpoints will blindly review all records for each event and determine the final classification of the event. For the purposes of safety reporting, only adjudicated events will be analyzed statistically.
- The following cardiovascular events will be considered, as well as more transient ischemic events such as angina and transient ischemic attacks:
    - Non-fatal myocardial infarction
    - Non-fatal ischemic stroke
    - Cardiovascular death, including sudden death
    - Acute coronary syndrome
    - Angina pectoris not leading to hospitalization
    - Transient ischemic attack
    - Hospitalization for coronary revascularization

In addition to the above, standard, resting 12-lead electrocardiograms will be recorded and reviewed at screening, randomization, weeks 4 and 8 of treatment, and termination visits.

3 Cisapride (Propulsid)

Cisapride is a prokinetic agent that was approved to treat symptomatic nocturnal heartburn due to GERD. It was also used off-label for a number of conditions, including diabetic gastroparesis and peptic ulcer disease. Cisapride was also widely used to treat infants with gastroesophageal reflux.

3.1 Regulatory History

Cisapride was marketed in the United States from 1993-1999. During that time, the FDA received postmarketing safety reports of patients experiencing fatal and non-fatal cardiovascular events. Specifically, the FDA received reports of cardiac arrhythmias, some of which resulted in death. A total of 341 events were reported. See Table 12. Of these events, 80 (23%) were fatal. These events were believed to be associated with the blockade of IKr(hERG) channels and exacerbated by CYP450 drug-drug interactions that increased exposures.

Factors that suggested an association with cisapride included a temporal relationship between use of cisapride and the arrhythmia, the absence of identified risk factors and other explanations for arrhythmia in some patients, and cases of positive dechallenge and rechallenge. In most individuals, the arrhythmia occurred in the presence of risk factors (other drugs and/or medical conditions).[1]

Table 12: CV Events Associated with Cisapride Use

CV Event	Number of Patients
QT prolongation	117
Torsades de pointes	107
Polymorphic ventricular tachycardia	16
Ventricular fibrillation	18
Ventricular tachycardia	27
Cardiac arrest	25
Serious (unspecified) arrhythmia	16
Sudden death	15
Total Events	341

Wysowski, *et al.* 2001[1]

Johnson and Johnson voluntarily discontinued the marketing of cisapride worldwide in July 2000. Marketing licenses have been withdrawn voluntarily in all countries except Belgium, France, Portugal and The Netherlands. In order to ensure that patients in need and without

[1] Wysowski D, Corken A, Gallo-Torres H, Talarico L, Rodriguez E. Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and food and drug administration regulatory actions. *The Am J of Gastroenterol* 2001; 96, 1698–1703.

alternative therapy have access to cisapride, the company is making cisapride available to patients through the Limited Access Program under an investigational new drug (IND) mechanism. See Section 3.4.2.

3.2 Nonclinical

Summary

In addition to its affinities for 5-HT_4 receptors, cisapride also binds to other 5-HT receptor subtypes, e.g. 5-HT_2 receptors. Cisapride is a potent inhibitor of hERG K^+ channels with IC_{50} values in the nM range. In isolated rabbit cardiomyocytes, cisapride concentration-dependently blocked (IC_{50} = 9 nmol/L) the rapid component of delayed rectifying potassium current (I_{Kr}), and in isolated rabbit Purkinje fibers, cisapride caused a significant increase in the action potential duration. In both conscious and anesthetized dogs, cisapride caused an increase in the heart rate, and in anesthetized guinea pigs, it caused a dose-dependent prolongation of the monophasic action potential duration recorded from the left ventricle.

Serotonin Receptor Selectivity Studies:

Cisapride is a prokinetic agent, and it stimulates gastrointestinal tract motility by facilitating post-ganglionic cholinergic neurotransmission in the myenteric plexus and increasing acetylcholine release in the stomach, small intestine and colon. However, studies in isolated tissue preparations from different parts of the GI tract have shown that the cisapride-induced increase in GI motility is mediated by cholinergic nerves through release of acetylcholine, and this response is blocked by atropine, a cholinergic antagonist, but not by antihistamines, adrenergic blocking agents, dopaminergic (D1 and D2) blocking agents, serotoninergic blocking agents or GABA receptors antagonists. The stimulatory effects of cisapride on guinea pig ileum were not blocked by spasmolytic agents, such as papaverine, secoverine, trimebutine and calcium entry blockers, suggesting that the stimulatory effect is mediated directly by the cholinergic neurons.

Cisapride (0.1 to 1 μM) antagonized serotonin actions both *in vitro* and *in vivo*, and blocked 5-HT_2 receptors at the rat ileum at a concentration of 5×10^{-5} M. Cisapride also showed a slight to moderate binding to 5-HT_2 receptors in the basolateral membranes from the rabbit ileal epithelial cells. It blocked contractile response to 5-HT on colliculi neurons of mouse embryos which has 5-HT_4 receptors as this effect was blocked by ICS-205-930, a 5-HT_4 receptor antagonist. In addition, cisapride antagonized 5-HT-induced contractions of guinea pig ileum through 5-HT_4 receptors, since this effect was also blocked by ICS-205-930.

This suggests that the prokinetic effect of cisapride is mediated by the activation of 5-HT_4 receptors.

In Vitro Cardiac Electrophysiology Studies

Effects on the hERG potassium channel currents in HEK293 cells:
Cisapride is a potent inhibitor of hERG channels with IC_{50} values in the nM range[2, 3].

Effects on the action potentials of guinea pig auricular muscle:
In an electrophysiological study in auricular muscle of the guinea pig, no effects were observed at concentrations up to 0.5 µg/mL.

Effects on the action potentials in dog purkinje fibers:
In *in vitro* electrophysiological studies in dog purkinje fibers and trabecular muscles, cisapride, at concentrations up to 0.5 µg/mL, did not show any effect.

Effects on rabbit cardiomyocytes and purkinje fibers:
In isolated rabbit cardiomyocytes, cisapride concentration-dependently blocked (IC_{50} = 9 nmol/L) the rapid component of delayed rectifying potassium current (I_{Kr}), and in isolated rabbit Purkinje fibers, cisapride caused a significant increase in the action potential duration[4].

In Vivo Cardiac Safety Pharmacology Studies:

In conscious dogs, cisapride at an intravenous dose of 0.63 mg/kg, had no cardiovascular adverse effects. At an intravenous dose of 1.25 mg/kg, it caused an increase in heart rate, increase in left ventricular dp/dt, a slight positive inotropic effect on the myocardium, and a decrease in PQ interval. An oral dose of 250 mg/kg produced similar effects in dogs.

In anesthetized dogs, cisapride at a dose of 0.12 mg/kg, produced vasodilation and an increase in the heart rate. An increase in left ventricular dp/dt max and aortic blood flow velocity was observed at 0.05 to 0.25 mg/kg.

In anesthetized guinea pigs, cisapride dose- dependently prolonged the duration of monophasic action potential recorded from the left ventricle[3].

In a rabbit model of acquired long QT syndrome, infusion of cisapride (0.3 µmol/kg/min for 10 min) was associated with a significant lengthening of QTU interval[3].

[2] Mohammad S, Zhou Z, Gong Q, January CT. Blockage of the HERG human cardiac K+ channel by the gastrointestinal prokinetic agent cisapride. *Am J Physiol* 1997; 273 (5 Pt 2):H2534-8.
[3] Potet F, Bouyssou T, Escando D, Baro I. Gastrointestinal prokinetic drugs have different affinity for the human cardiac human ether-a-gogo K+ channel. *J Pharmcol Exp Ther*. 2001; 299(3):1007-1012.
[4] Carlsson L, Amos GJ, Andersson B, Drews L, Duker G, Wastedt G. Electrophysiological characterization of the prokinetic agents cisapride and mosapride in vivo and in vitri: implications for proarrhythmic potential. *J Pharmcol Exp Ther.* 1997; 282(1):220-227.

3.3 Clinical Pharmacology

3.3.1 Metabolism of Cisapride

Following oral administration, the absolute bioavailability of oral cisapride is about 35-40% and once absorbed cisapride is extensively metabolized. Unchanged drug accounts for less than 10% of urinary and fecal recovery following oral administration. Norcisapride, formed by N-dealkylation, is the principal active metabolite in plasma, feces and urine[5]. At the time of approval, the metabolic pathway was not elucidated. After approval, CYP3A4 was identified as a principal metabolizing enzyme for cisapride and a significant inhibitory effect of ketoconazole, a potent CYP3A4 inhibitor on cisapride metabolism, was observed *in vivo* and *in vitro*.

3.3.2 Drug-drug Interaction Studies

During the development program, several *in vivo* drug interaction studies were conducted mainly for the effects of cisapride on other concomitant drugs including anticoagulants. Cisapride significantly prolonged the coagulation time in patients treated with acenocoumarol but no significant interactions were found between cisapride and warfarin. Cisapride also shortened the Tmax but did not influence wither Cmax or AUC of phenprocoumon in healthy volunteers[6]. In patients receiving oral anticoagulants, monitoring of coagulation time within the first few days after the start and discontinuation of cisapride is recommended[5].

In addition, studies were conducted to evaluate the potential interaction with drugs commonly used in the target patient population. Concomitant antacids (Maloxx) and ranitidine did not significantly affect the absorption of cisapride while coadministration of cimetidine increased mean Cmax and $AUC_{(0\text{-}24)}$ of cisapride by 45%[5,6]. Because the metabolic pathway of cisapride was not known, mechanism-based drug interaction studies were not conducted prior to the original approval. Of note, the FDA guidance on the in-vitro drug interaction studies was first published in 1997.

After original approval of cisapride in January 1995, the sponsor submitted an in vitro study result on the cytochrome P-450 forms of human liver microsomes involved in the metabolism of cisapride and on possible inhibitory effects of ketoconazole, itraconazole, hydroxyl-itraconazole, fluconazole and erythromycin on its biotransformation. The in vitro inhibitory potency for cisapride metabolism was in following rank: ketoconazole > hydroxyl-itraconazole > itraconazole > fluconazole[7].

Subsequently, drug interactions between cisapride and concomitant CYP3A4 inhibitors such as ketoconazole, clarithromycin, fluconazole were also evaluated in human. In healthy volunteers, co-administration of oral ketoconazole, clarithromycin, and fluconazole markedly

[5] Propulsid® (cisapride) Package Insert
[6] NDA 20-210 Clinical Pharmacology Biopharmaceutics Review, 7/29/1993 (http://www.accessdata.fda.gov/drugsatfda_docs/nda/pre96/020210_S000_CisprideTOC.cfm)
[7] Bohets et al. (2000) Identification of the cytochrome P450 enzymes involved in the metabolism of cisapride: in vitro studies of potential co-medication interactions. *British Journal of Pharmacology* (2000) 129, 1655-1667

inhibits the metabolism of cisapride, resulting in a mean 8-fold, 3-fold and greater than 2-fold increase in AUC of cisapride, respectively[8,9,7]

Co-administration of grapefruit juice increases the bioavailability of cisapride by an average of 50%. Patients were advised to refrain from consuming grapefruit juice for the duration of cisapride therapy[5].

A boxed warning to contraindicate concomitant use of cisapride with CYP3A4 inhibitors (ketoconazole, fluconazole, clarithromycin) and with drugs that prolong the QT interval was added to the Package Insert in October 1995 and November 1997, respectively[10].

3.3.3 Thorough QT Study

In post-marketing surveillance, in some cases where serious ventricular arrhythmias, QT prolongation, and torsades de pointes occurred when cisapride was taken in conjunction with one of the cytochrome P450 3A4 inhibitors, elevated blood cisapride levels were noted at the time of the QT prolongation. In addition, a study in healthy volunteers conducted after approval suggested that coadministration of cisapride and ketoconazole could result in prolongation of the QT interval on the ECG[11].

No thorough QT study of current standard was conducted before or after approval of cisapride.

However, effects of cisapride on the QT prolongation were evaluated in two studies after initial approval. In a study conducted in 1996, the effects of cisapride on the ECG were evaluated after administration of 10 mg q.i.d., 20 mg b.i.d. or 20 q.i.d. for six days. The study report noted no clinically meaningful effect of cisapride at studied doses on QTc prolongation and no correlation between plasma cisapride concentrations and QTc values. However, the study was conducted without a placebo or a positive control to validate the results[12].

In another study conducted in 2000, a dose-dependent increase in the QT interval was noted[13]. This was a placebo-controlled study after a single ascending dose of cisapride of 10, 20, 40, 80, and 130 mg in healthy subjects. In this study, although without a positive control, there was a dose-dependent increase in the QT interval observed, especially at doses higher than 40 mg. A statistically significant increase in QTcB (Bazett's formula) from baseline was noted from 1 hour to approximately 6 to 12 hours after dosing. The peak changes in QTcB from baseline at 1.5 hours post-dose were 37.2, 30.1 and 44.9 msec for 40, 80 and 130 mg cisaspride, respectively ($p<0.05$). It was 9.8 msec for placebo at 6 hours post-dose. The data

[8] Propulsid® (cisapride) Package Insert
[9] Van Haarst et al. (1998) The influence of cisapride and clarithromycin on QT intervals in healthy volunteers. *Clinical Pharmacology and Therapeutics*. 64, 542–546
[10] Wysowski et al. (2001) Postmarketing reports of QT prolongation and ventricular arrhythmia in association with cisapride and food and drug administration regulatory actions. *The Am J of Gastroenterol*, 96:1698-1703
11 Propulsid® (cisapride) Package Insert
[12] Evaluation of the effect of cisapride on electrocardiographic function in normal, healthy volunteers. Clinical Research Report, March 1998
[13] Double-blind trial to evaluate cardiovascular safety and the pharmacokinetics of cisapride in healthy subjects administered as escalating single doses up to 200 mg. Clinical Research Report. August 2003

were analyzed using different approaches to correct QT interval for change in heart rate. A similar trend of dose-dependent QT prolongation was observed regardless of the correction method. Using Fridericia's formula, no statistically significant change was reported at 10 mg and 20 mg dose levels in QTcF. However, statistically significant increases in QTcF were reported after the 40, 80, and 130 mg doses. The peak changes were 23.6, 20.5 and 34.2 msec at 1.5 hours, 1.5 hours and 3 hours post-dose, respectively. The statistically significant peak increase of 6.8 msec in QTcF with placebo was noted at 6 hours post-dose. A statistically significant relationship between cisapride plasma concentration and changes in corrected QT intervals was also reported. The study report was submitted to the Agency in 2003 after the market withdrawal.

3.3.4 Platelet aggregation study

The sponsor noted that cisapride, at concentrations up to 1×10^{-5} M in vitro, did not significantly affect the human platelet aggregation induced by ADP, adrenaline or collagen in citrated plasma. The study was conducted in 1986 before the approval.

3.4 Clinical

3.4.1 Completed Studies

Since cisapride was withdrawn from the market, two of the four Limited Access Program (LAP) protocols have been completed. Only certain relevant studies will be discussed in this document.

3.4.2 Ongoing Studies

Two Limited Access Program Protocols for the use of oral cisapride are currently ongoing.

Protocol CIS-USA-155 is in place to provide cisapride access to adult patients with GERD, gastroparesis, pseudo-obstruction or severe chronic constipation disorders who have failed standard therapy.

Protocol CIS-USA-156 is in place to provide cisapride access to pediatric patients with refractory symptoms of GERD, or acute, life-threatening symptoms secondary to GERD; severe chronic constipation; or pseudo-obstruction.

Table 13: Cardiovascular Safety of the LAP

Protocol	Number of Cardiovascular Deaths	Number of reported episodes of TdP	Total number of patients enrolled in the study
CIS-USA-154	3	0	1320
CIS-USA-156	0	0	295
CIS-USA-155	0	0	8
CIS-USA-157	0	0	4

The CIS-USA-155 protocol is no longer active (closed as of April 1, 2003).
The CIS-USA-157 protocol is no longer active (closed as of April 1, 2004).

3.4.3 Proposed studies

No proposals for new studies have been received by the FDA.

4 Tegaserod (Zelnorm)

Tegaserod was approved for women with constipation predominant Irritable Bowel Syndrome (IBS-C) in 2002 and for patients less than 65 years old with CIC in 2004. Tegaserod is considered a non-selective 5-HT_4 agonist that also has 5-HT_1 partial agonism.

4.1 Regulatory History

On February 22, 2007, Novartis informed the FDA that a recent retrospective analysis of pooled tegaserod clinical trials revealed an imbalance in coronary ischemic events between tegaserod and placebo. The Swiss regulatory authority requested that Novartis perform this retrospective analysis to evaluate all ischemic events due to postmarketing reports of ischemic colitis. On March 9, 2007, Novartis submitted the full safety report entitled, “Coronary ischaemic events: Initial Analysis.” This report contained a pooled analysis of ischemic events from 29 placebo-controlled clinical trials of tegaserod. The events seen in this retrospective meta-analysis in patients taking tegaserod included four myocardial infarctions (one fatal), three strokes, and six episodes of unstable angina. This was compared to one event of transient ischemic attack in a patient taking placebo. The rate of CV events in patients taking tegaserod was 13/11,614. The rate of CV events in patients taking placebo was 1/7,031. Because of this imbalance in ischemic cardiovascular events, on March 30, 2007, the FDA asked Novartis to suspend the marketing and sale of tegaserod in the U.S.

It should be noted that during the initial review of the tegaserod registration trials, cardiovascular adverse events were not noted to be a safety issue. The major safety issue noted was lower abdominal pain leading to abdominal and pelvic surgeries, particularly cholecystectomy. Prior to approval, there were three deaths reported in the 5,632 patients exposed to tegaserod. These deaths included two suicides and one suspected pulmonary embolism (autopsy not performed). None of the deaths were suspected to be related to the use of tegaserod.

After the marketing suspension, tegaserod was made available to patients through a Novartis sponsored treatment IND (tIND). Investigators who participated in this open-label, non-randomized tIND program enroll adults who met certain restrictive eligibility criteria. Enrolled patients received tegaserod 6 mg twice daily and were to be seen twice monthly. One goal of the tIND was to provide additional data on the safety of tegaserod to support possible resubmission of tegaserod for regulatory approval. On 25 May 2008 the tIND program was closed. Tegaserod is now available by IND for emergency situations only.

4.2 Nonclinical

Summary

Tegaserod is a 5-HT_4 partial agonist with moderate to high affinity for 5-HT_1 receptor subtypes. Tegaserod is a weak inhibitor of hERG potassium currents, but did not induce QT prolongation in *in vivo* studies in dogs. Neither action potentials in isolated guinea pig ventricular papillary muscle nor QT intervals in isolated perfused rabbit heart were affected by tegaserod at clinically relevant concentrations (i.e., human plasma concentrations). Tegaserod did not induce contractions in isolated coronary artery preparations from pigs, non-human primates, and humans, but produced a small and variable contractile response in canine coronary arteries.

Serotonin Receptor Selectivity Studies

Tegaserod (HTF 919) is a 5-HT_4 partial agonist with moderate to high affinities for 5-HT_1 receptors. The affinity of HTF 919 for 5-HT_4 receptors was determined in calf and human caudate. The results indicated that HTF 919 had a high affinity for 5-HT_4 receptors in both calf and human caudate with pK_D of 7.84±01 (calf) and 7.71±0.07 (human). The affinity of cisapride for 5-HT_4 receptors is similar to that of HTF 919 in both species, with pK_D of cisapride of 7.37 (calf) and 7.22 (human). The ligand binding studies were also conducted in the pig and rat cortex, pig choroid plexus and calf caudate for 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_2 receptors. HTF 919 has moderate to high affinity for 5-HT_{1A}, 5-HT_{1C}, 5-HT_{1D} but not for 5-HT_2 receptors. The *in vitro* binding properties of HTF 919 and cisapride to 5-HT_4 receptors are summarized in Table 14.

Table 14: Binding Affinities for Different Serotonin Receptors ([HTF 919])

	5-HT_4	5-HT_{1A}	5-HT_{1C}	5-HT_{1D}	5-HT_2
	K_D (nM)	K_D (nM)	K_D (nM)	K_D (nM)	K_D (nM)
HTF 919	20	324	68	20	2512
Cisapride	60	1906	501	5495	7.24

In vitro Cardiac Electrophysiology Studies

Effects on the hERG potassium channel currents in HEK293 cells:

An *in vitro* study was conducted to assess the effects of HTF 919 on cloned hERG channels expressed in HEK293 cell. The results indicated that HTF 919 inhibited the hERG channel, with IC_{50} of 13,000 nM. In contrast, cisapride produced almost complete inhibition at 2,000 nM, with IC_{50} of 44 nM.

Effects on the action potentials of guinea pig ventricular papillary muscle:

HTF 919 had no significant effects on action potential duration, amplitude and maximum rate of depolarization, and diastolic membrane potential recorded from isolated guinea pig ventricular papillary muscle at 10, 100 and 1000 nM.

In vitro Rabbit heart Langendorff preparation:

In this study, rabbit hearts were removed and placed in a Langendorff apparatus and ECG was recorded. The perfused isolated hearts were then treated with HTF 919 at 0, 0.5, 1, 5, 10, and 50 μM. QT intervals measured from ECG recordings were 230±4, 226±2, 233±4, 233±7, 233±8, and 259±19 ms at 0, 0.5, 1, 5, 10, and 50 μM, respectively. HTF 919 had no effects on QT interval at concentrations up to 10 μM, and it prolonged QT interval by ~12% at a concentration of 50 μM (~21 μg/ml), which is much higher than the therapeutic plasma level (~6 ng/ml) following an oral dose of 12 mg/day. The main human metabolite (5-methoxyindole-3-carboxylic acid glucuronide) had no effects on QT interval at concentrations up to 50 μM. In contrast, cisapride, used as a positive control, increased QT interval by 15% at concentration of 0.1 μM. (This study was performed after cisapride was identified to cause cardiac electrophysiologic changes.)

Effects on the contractility in the coronary artery:

Tegaserod had no contractile activity at concentrations up to 10 or 30 μM in isolated coronary artery preparations from pigs, non-human primates, and humans. Tegaserod produced a small and variable contractile response in canine coronary arteries at 5-10 μM (Naunyn-Schmiedeberg's Arch Pharmacol, September 8, 2011).

In vivo Cardiovascular Safety Pharmacology Studies

Intravenous administration of HTF 919 at doses of 0.01 and 0.1 mg/kg had no effects on blood pressure, heart rate, left ventricular dp/dt, cardiac output and total peripheral resistance in anesthetized rats. ECGs were not affected at these doses. However, HTF 919 at 1 mg/kg decreased systolic (~9-15%), diastolic (~13-20%) and mean arterial blood pressures (~10-17%) and total peripheral resistance (~16-19%), and produced negative dp/dt (~6-19%).

In anesthetized dogs, intraduodenal administration of HTF 919 had no effects on blood pressure, respiration rate, heart rate, femoral arterial blood flow, and ECG at 0.1, 1, and 10 mg/kg. In conscious dogs, single oral doses of HTF 919 had no clear treatment related effects on heart rate, blood pressure, and ECG at 0.3, 4, and 10 mg/kg. Plasma levels of HTF 919 were determined in this study. Maximum plasma levels of HTF 919 following 0.3, 4, and 10 mg/kg were 16.9, 138, and 401 ng/ml (males) and 3.62, 104, and 277 ng/ml (females), respectively.

HTF 919 had no effects on ECG at 0.1 and 1 mg/kg/day in the 2-week I.V. toxicity study in dogs, at 5, 15, and 50/60 mg/kg/day in the 26-week oral toxicity study in dogs, and at 5, 15, and 60/70 mg/kg/day in the 52-week oral toxicity study in dogs.

4.3 Clinical Pharmacology

4.3.1 Metabolism of Tegaserod

Upon oral administration, approximately two-thirds of the orally administered dose of tegaserod is excreted unchanged in the feces, with the remaining one third excreted in the urine, primarily as the main metabolite. Unchanged tegaserod was not detectable in the urine. Only unchanged tegaserod was detectable in the feces. The absolute bioavailability of oral tegaserod is about 10%.

Tegaserod is metabolized mainly via two pathways. The first is a presystemic acid-catalyzed hydrolysis in the stomach followed by oxidation and conjugation, which produces the main metabolite of tegaserod, 5-methoxy-indole-3-carboxylic acid glucuronide. In humans, systemic exposure to tegaserod was not significantly altered at neutral gastric pH values. The main metabolite has negligible affinity for human 5-HT4 receptors in vitro[22]. The fate of the pentylaminoguianidine (PAG) side chain of tegaserod was investigated in mice following a single oral dose of labeled tegaserod. After 72 hours, 82% and 15% was excreted in feces and in urine, respectively. Recovery of radioactivity in urine suggests the PAG is absorbed systemically to some extent[23]. The information on systemic absorption of PAG in human is not available.

The second metabolic pathway of tegaserod degradation is direct glucuronidation, which leads to generation of three isomeric N-glucuronides[22]. Maximal tegaserod plasma concentrations following the recommended single 6 mg oral dose are in the range of 3-13 nM[23].

4.3.2 Drug-drug Interaction Studies

In vitro, tegaserod is neither a substrate nor an inhibitor of cytochrome P450 enzymes such as CYP2C8, 2C9, 2C19, 2E1 and 3A4. *In vivo* studies showed that there was no effect on the pharmacokinetics of digoxin, oral contraceptives, and warfarin. No clinically relevant drug-drug interactions have been observed in vivo with dextromethorphan, a substrate of CYP2D6, and theophylline, a substrate of CYP1A2. The main metabolite, 5- methoxyindole-3-carboxylic acid glucuronide, did not inhibit the activity of any of the above cytochrome P450 isoenzymes in *in vitro* tests[22].

On the other hand, tegaserod is a substrate of P-gp. The *in vivo* interaction potential between concomitant p-gp inhibitor(s) and tegaserod was not evaluated prior to the approval[23]. An increase in the systemic exposure of tegaserod by 74% by a concomitant P-gp inhibitor quinidine was later reported[24].

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[22] Zelnorm® (tegaserod maleate) Package Insert
[23] NDA 21-200 Clinical Pharmacology Biopharmaceutics Review, 7/24/2002 (http://www.accessdata.fda.gov/drugsatfda_docs/nda/2002/21-200_Zelnorm.cfm)
[24] (European Medicines Agency Evaluation of Medicines 19 May 2006: http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/000621/WC500058849.pdf).

4.3.3 Thorough QT Study

A thorough QT study was not conducted before or after approval. However, at the time of approval the sponsor performed a pooled analysis of ECG data from phase 3 and long-term trials and did not observe differences in QT prolongation between the treatment and placebo groups. In 2002 Morganroth *et al*[25] reported no increase in the change from baseline in the QTc interval with an increase in tegaserod plasma concentration up to 100 times those measured after therapeutic doses (6 mg b.i.d. oral) after administration of tegaserod (0.8, 3.2, 5, 8, 14, and 20 mg; each dose, n=6) and placebo as a constant rate IV infusion of 40 min. The study did not include a positive control. The results in the publication were not verified by the Agency. It should be noted that the ICH E14 guidance was published in 2005.

4.3.4 Platelet aggregation study

There is conflicting information about effects of tegaserod on platelet aggregation in two recent publications[26,27].

Serebruany *et al*.[26] investigated the effects of *in vitro* addition of tegaserod on the function of platelets obtained from healthy volunteers and subjects with irritable bowel syndrome with constipation (IBS-C). Pre-incubation with tegaserod at concentrations mimicking human Cmax values (10 nM), 3.3 times Cmax (33 nM) and 10 times Cmax (100 nM), respectively, resulted in mild but statistically significant increases (mostly at supratherapeutic concentrations) in platelet aggregation induced by ADP, collagen, epinephrine, or serotonin. The effects of tegaserod on platelet aggregation were consistent across various agonists in the study. No differences were observed between the effects on aggregation of platelets obtained from healthy volunteers and subjects with IBS-C.

Table 15: Platelet Aggregation in Healthy Subjects (n=20)[26]

	Tegaserod concentration/Platelet aggregation (%)				
Agonist	Vehicle	10 nM	33 nM	100 nM	Reference range
ADP 5 μM*	64.9 ± 5.4	74.8 ± 13.4†	75.4 ± 11.2†	79.3 ± 11.0†	60–82
ADP 20 μM*	74.7 ± 5.3	77.8 ± 6.7†	81.6 ± 7.8†	87.1 ± 7.6†	68–88
Collagen 1 μg/mL*	75.1 ± 5.2	77.1 ± 6.4	82.5 ± 6.9†	90.3 ± 7.3†	68–84
Collagen 5 μg/mL*	81.3 ± 7.0	84.9 ± 7.7	88.7 ± 8.3†	90.1 ± 8.6†	79–92
TRAP 20 μM	88.3 ± 1.4	88.6 ± 1.9	89.0 ± 1.5	88.9 ± 3.2	78–93
Epinephrine 5 μM*	82.7 ± 6.9	85.6 ± 8.5	90.0 ± 6.6†	94.7 ± 7.2†	72–90
Serotonin 5 μM*	15.3 ± 4.4	21.6 ± 4.8†	22.0 ± 6.2†	26.7 ± 7.5†	10–18

Mean ± standard deviation.
*$P < 0.05$ (overall) Friedman's test.
†$P < 0.05$ (paired) sign test compared with vehicle.
ADP, adenosine diphosphate; TRAP, thrombin receptor activating peptide.

In contrast, Higgins *et al*.[27] reported a failed attempt to reproduce the observed effects of tegaserod on platelet aggregation. They reported that there was no statistically significant

[25] Morganroth et al., Tegaserod, a 5-hydroxytryptamine type 4 receptor partial agonist, is devoid of electrocardiographic effects, *Am J Gastroenterol*. 2002;97(9): 2321-2327
[26] Serebruany et al. Investigations on 5-HT4 receptro expression and effects of tegaserod on human platelet aggregation in vitro. *American Journal of Therapeutics* . 2010;17, 543-552
[27] Higgins et al. (2011) The inability of tegaserod to affect platelet aggregation and coronary artery tone at supratherapeutic concentrations, Naunyn-Schmiedeberg's *Archive of Pharmacology*, Published online: 08 September 2011)

difference in the % platelet aggregation between control and tegaserod at concentrations 10, 33 and 100 nM under similar experiment condition with the previous study. When platelet aggregation was induced with 5 μM ADP and platelet concentration 350, 000/μL, the % platelet aggregation without tegaserod was similar in two reports (64.9% by Serebruany *et al*. vs. 62% by Higgins *et al*.) although a higher variability was observed in the Higgins study. No other platelet agonists except ADP were used by Higgins *et al.* while various agonists were used in studies by Serebruany *et al*.

Table 16: Effect of Tegaserod on Platelet Aggregation in Healthy Subjects (n=10)[27]

		% Platelet Aggregation (mean±SD)				
Platelet concentration	Agonist	Vehicle	Tegaserod (10 nM)	Tegaserod (33 nM)	Tegaserod (100 nM)	*p* value (Friedman's test)
250,000/μL	5 μM ADP	51.5±16.9	54.4±15.4	51.3±16.8	54.1±18.2	0.9777
	1 μM ADP/5 μM 5-HT	34.1±14.8	25.7±12.3	29.7±12.0	28.4±11.8	0.3667
350,000/μL	5 μM ADP	62.0±13.9	60.2±18.0	59.3±16.4	58.6±18.4	0.9197
	1 μM ADP/5 μM 5-HT	41.5±14.3	30.1±13.0	36.9±14.6	36.8±18.5	0.0794

4.4 Clinical

4.4.1 Completed Studies

After tegaserod was withdrawn from the U.S. market, the tegaserod treatment IND provided access to female patients. During this study, 182 patients were exposed to tegaserod for a mean of 76.4 days. No deaths or major CV ischemic events were reported during the study.

4.4.2 Ongoing Studies

No U.S. studies are currently ongoing and access to tegaserod is limited to emergency IND use only.

4.4.3 Proposed studies

No U.S. studies are currently ongoing and access to tegaserod is limited to emergency IND use only.

5 TD-5108 (velusetrag)

TD-5108 (velusetrag) is a 5-HT_4 agonist being studied for the treatment of disorders of reduced gastrointestinal motility and treatment of cognitive disorders.

5.1 Nonclinical

Summary

TD-5108 is a selective 5-HT_4 receptor agonist with minimal binding affinity for 5-HT_1 and 5-HT_2 receptors. TD-5108 is a weak inhibitor of hERG potassium currents, but did not induce QT prolongation in *in vivo* studies in dogs. No electrophysiologic effects were observed in isolated canine Purkinje fibers at clinically relevant concentrations of TD-5108. TD 5108 did not induce contractions in isolated coronary artery preparations from pigs, dogs, and humans.

Serotonin Receptor Selectivity Studies

The Sponsor stated in a summary that TD-5108 is a potent agonist for the 5-HT_4 receptor. The sponsor also compared the *in vitro* binding and selectivity of TD-5108 with other 5-HT_4 agonists, including cisapride and tegaserod, to these receptors. The results are summarized in Table 17.

Table 17: Binding Affinities and Selectivity for different serotonin receptors (TD-5108)

	Human 5-$HT_{4(c)}$ Ki (nM)	Human 5-HT_{3A} Ki (nM)	Human 5-HT_4/5-HT_3 Selectivity
TD-5108	20	63,000	3,000
Cisapride	126	2,000	20
Tegaserod	4	1,585	400

Source: Sponsor's data

TD-5108 had minimal binding affinity to other 5-HT receptor types such as 5-HT_1, and 5-HT_2 at 10 μM.

In vitro Cardiac Electrophysiology Studies

Effects on the hERG potassium channel currents in HEK293 cells:

TD-5108 inhibited the hERG current with IC_{50} of 5.2 μM. No effect was observed at 0.3 μM. A major metabolite, THRX-830449, inhibited hERG current with IC_{25}, IC_{50} and IC_{75} of 0.45, 1.4 and 4.4 μM, respectively. The results suggest that the metabolite, THRX-830449, (IC_{50} = 1.4 μM), is more potent than its parent compound, TD-5108 (IC_{50} = 5.2 μM).

The peak human plasma levels of free TD-5108 and THRX-830449 were 16 and 8 nM, respectively, following an oral dose of 15 mg TD-5108, which are much lower than the hERG IC_{50} values.

Effects on the action potentials of canine Purkinje fibers:

TD-5108 induced action potential triangulation at 3 μM and decreased maximum rate of depolarization and upstroke amplitude at 10 μM or higher. The action potential triangulation refers to the shape change of the action potential caused by a drug (i.e., agents that produce action potential triangulation are known to be proarrhythmic). These effects were not seen at 0.3 μM. Effects on APD_{60} and APD_{90} were inconsistent at higher concentrations (10 and 30 μM), in some instances prolonging action potential duration (APD_{90} at 0.5 Hz and 10 μM) and in other instances shortening the duration (APD_{60} at 1 Hz at 30 μM).

Effects on the contractility in the coronary artery of pigs, dogs, and humans:

TD 5108 had no contractile activity at concentrations up to 10 or 30 μM in isolated coronary artery preparations from pigs, dogs, and humans.

In vivo Cardiovascular Safety Pharmacology Studies

TD-5108 increased heart rate slightly from baseline as compared to vehicle-treated animals up to one hour after oral administration of 25 mg/kg. Systolic and diastolic blood pressures increased slightly from baseline and relative to vehicle-treated animals, 1 to 4 hours after oral administration of 0.5 mg/kg. However, these increases were not observed at higher doses (5 and 25 mg/kg). There were no adverse effects on ECG parameters, including QTc intervals.

TD-5108 had no effects on ECG at doses up to 25 mg/kg/day in the 28-day oral toxicity study in dogs, at doses up to 30 mg/kg/day in the 13-week oral toxicity study in dogs, and at doses up to the high-dose of 6 mg/kg/day in the 39-week oral toxicity study in dogs.

5.2 Clinical Pharmacology

5.2.1 Metabolism of TD-5108

As noted in a publication by Manini *et al*.[28], "The major metabolite (CYP3A4 mediated) detected in the plasma after oral velusetrag is THRX 830449, which is a full agonist and approximately equipotent to velusetrag. Thus, pKi and pEC50 values for THRX-830449 at the human recombinant 5-HT4(c) receptor are 7.6 and 8.2, respectively compared with 7.7 and 8.3 for velusetrag respectively. In healthy humans, at steady-state, the THRX-830449 to velusetrag AUC ratio is ~0.5 following once daily dosing of velusetrag (15 mg). The elimination t1/2 of THRX-830449 after single and multiple dosing are 16 and 35 h respectively."

5.2.2 Drug-drug Interaction Studies

[28] Manini et al, *Neurogastroenterol Motil*. 2010;22, 42–e8

Effect of other drugs on TD-5108: *In vitro* data indicate that TD-5108 is a substrate of CYP3A4 and human P-gp. Drugs that inhibit or induce these enzymes/transporters could affect the *in vivo* PK of TD-5108.

Effect of TD-5108 on other drugs: In vitro studies
TD-5108 did not cause direct inhibition or metabolism-dependent inhibition of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or CYP3A4/5. No inhibitory effect of THRX-830449 (10 µM) is noted on the metabolic activity of major human CYP enzymes. TD-5108 at 100 µM did not significantly inhibit P-gp mediated efflux transport in Caco-2 cells.

Neither drug nor active metabolite induced CYP1A2, 2B6, 2C9, 2C19, or CYP3A4/5 enzyme activities.

5.2.3 Thorough QT Study

Not available for public discussion by the FDA.

5.2.4 Platelet aggregation study

Platelet aggregation response to 5 µM ADP in presence of velusetrag (7 nM and 70 nM), and its active metabolite THRX-830449 (50 nM) was assessed to evaluate whether drug or metabolite can augment platelet aggregation. This was an *in vitro* assessment using human platelet-rich plasma. Thrombopoietin (TPO) was included as a positive control. Light transmittance aggregometry was performed to measure platelet aggregations.

Sponsor's conclusion suggests an absence of a significant effect of velusetrag or its active metabolite on platelet aggregation. Table 18 is produced from a sponsor-provided summary.

Table 18: Maximum Platelet Aggregation Data (TD-5108)

Solution Added (final concentration)	Agonist	Maximum Aggregation	
		Mean	SD
Velusetrag (70 nM)	ADP	51%	25%
Velusetrag (7 nM)	ADP	52%	28%
THRX-830449 (50 nM)	ADP	51%	26%
Vehicle: PBS for TPO	ADP & TPO	85%	9%
Vehicle: PBS	ADP	49%	25%

5.3 Clinical

5.3.1 Completed Studies

Two phase 2 clinical trials of velusetrag are available in the published literature; however there are limited safety data reported.

The first trial evaluated the effects of TD-5108 on gastrointestinal transit and compared its pharmacokinetics (PK) in healthy volunteers and chronic constipation (CC) patients[29]. Sixty healthy volunteers were randomly assigned, double-blind to placebo, 5, 15, 30 or 50 mg TD-5108 (single and 6-day dosing). Primary endpoints were colonic transit and ascending colon emptying (ACE) $T_{1/2}$ after first dose. There were no serious adverse events. Notable adverse events were the predictable GI effects such as diarrhea or altered bowel movements. Authors also report that clinical laboratory test results, vital sign measurements prior to dosing, and periodic electrocardiograms did not indicate clinically significant changes. One patient who received a 30 mg dose experienced palpitations, and one who received a 50 mg dose developed asymptomatic junctional escape rhythm on electrocardiogram.

The second clinical trial was a phase 2, randomized, double-blind, placebo-controlled study of TD-5108 for the treatment of chronic constipation[30]. In this study, patients receiving velusetrag (15, 30 and 50 mg) achieved increases in weekly spontaneous bowel movement (SBM) frequency relative to those receiving placebo. Mean increases were 3.6, 3.3 and 3.5 SBM/week respectively, compared with 1.4 SBM/week for placebo. Increases in the weekly frequency of complete SBM (CSBM) were also reported (mean increases of 2.3, 1.8 and 2.3 for 15, 30 and 50 mg velusetrag respectively, compared with 0.6 for placebo). Common adverse events reported with velusetrag were diarrhea, headache, nausea and vomiting, generally occurring during the initial days of dosing. Authors also note that “no cardiac adverse events were reported by velusetrag-treated patients in this study, and there were no clinically relevant trends on 12-lead ECGs after 2 and 4 weeks of treatment.”

5.3.2 Ongoing Studies

Information not available.

5.3.3 Proposed studies

Information not available.

[29] Mhd Louai Manini, M.D., Michael Camilleri, M.D., Michael Goldberg, M.D., Ph.D.. Effects of TD-5108 on Gastrointestinal Transit and Bowel Function in Health and Pharmacokinetics in Health and Constipation. *Neurogastroenterol Motil*. 2010 January ; 22(1): 42-9.

[30] Goldberg M, Li YP, Johanson JF, Mangel AW,, et al. The efficacy and tolerability of velusetrag, a selective 5-HT4 agonist with high intrinsic activity, in chronic idiopathic constipation – a 4-week, randomized, double-blind, placebo-controlled, dose–response study. *The Aliment Pharmacol Ther.* 2010 Nov;32(9):1102-12.

6 TD-8954

TD-8954 is a 5-HT_4 agonist being studied for the treatment of disorders of reduced gastrointestinal motility and for the treatment of cognitive disorders

6.1 Nonclinical

Summary

TD-8954 is a selective 5-HT_4 receptor agonist with moderate to high intrinsic activity at the human 5-HT_4 receptor. TD-8954 binds to the 5-HT_4 receptor with high affinity (K_i = 0.4 nM). *In vitro* studies demonstrated weak inhibition of hERG channel currents and the absence of contractile activity in coronary arteries of pigs, dogs, and humans. High concentrations of TD-8954 produced prolongation of action potential duration in canine Purkinje fibers. Administration of high dose levels in dogs (15 – 150 times the human dose in Phase 1 studies based on mg/kg) resulted in increased heart rate, increased mean arterial pressure, increased diastolic pressure, and increased QTc.

Serotonin Receptor Selectivity Studies

The Sponsor stated in a summary that TD-8954 has very minimal binding at 1 □M to the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_{2C}, 5-HT_{5A}, 5-HT_6 and 5-HT_7 receptors, and is > 250,000-fold more selective for the human 5-$HT_{4(c)}$ receptors over the 5-HT_{3A} receptors. A comparison of the *in vitro* binding and selectivity properties of TD-8954 and other 5-HT_4 agonists to human recombinant 5-$HT_{4(c)}$ and 5-HT_{3A} receptors is summarized.

Table 19: Binding Affinities and Selectivity for Different Serotonin Receptors (TD-8954)

	Human 5-$HT_{4(c)}$	Human 5-HT_{3A}	Human 5-HT_4/5-HT_3 Selectivity
	Ki (nM)	Ki (nM)	
TD-8954	0.4	100,000	>250,000
Cisapride	80	2,000	25
Tegaserod	4	4,000	1,000

Source: Sponsor's data

Data from a published study confirmed that TD-8954 is >2,000-fold selective for 5-$HT_{4(c)}$ receptor over other 5-HT receptors, and other non-5-HT receptors (e.g., biogenic amine, peptide, and opiate receptors), transporters, ion channels, and enzymes tested (Beattie, DT. *et al*, 1-13)[31].

According to the sponsor, two metabolites of TD-8954 (THRX-513466, a major metabolite and THRX-913682, a minor metabolite) also have high affinities for the 5-$HT_{4(c)}$ receptor, and a

[31] Beattie, David T., et al. "The Pharmacology of TD-8954, a Potent and Selective 5-HT4 Receptor Agonist with Gastrointestinal Prokinetic Properties." *Front Pharmacol* 2.25 (2011): 1-13.

high degree of selectivity over the human 5-HT_{3A} (>5,000-fold), and all other 5-HT and non-5-HT (>1,000-fold) receptors.

In vitro Cardiac Electrophysiology Studies

Effects on the hERG potassium channel currents in HEK293 cells:
TD-8954, at concentrations of 0, 0.3, 1, 3, 10, and 30 μM, resulted in concentration dependent inhibition of hERG tail currents expressed in HEK293 cells (IC_{50} = 6,200 nM).

Effects on the action potential of canine Purkinje fibers:
Exposure of TD-8954, at concentrations of 0, 0.3, 1, 3, and 10 μM, to canine Purkinje fibers resulted in a prolongation of action potential duration (APD_{60} and APD_{90}) and triangulation[32] of the action potential in concentration- and inverse rate-dependent manners. According to the Sponsor, these results indicated that TD-8954 (≥ 3 μM) is an inhibitor of the delayed rectifier potassium channel in the dog heart.

Effects on the contractility in the coronary artery of pigs, dogs, and humans:
According to the Sponsor, TD-8954 had no contractile activity in isolated coronary artery preparations from pigs and humans (at concentrations up to 30 μM) or dogs (at concentrations up to 10 μM).

In vivo Cardiovascular Safety Pharmacology Studies

In male dogs, at low doses (0.3, 1, and 3 mg/kg) there were no treatment-related hemodynamic or ECG effects observed after oral gavage administration of TD-8954. At higher doses (see table below), the primary treatment-related finding was a dose-dependent elevation in mean heart rate (HR). Diastolic blood pressure (DBP) and mean arterial pressure (MAP) were increased in a non-dose dependent manner. In addition, treatment-related ECG effects in dogs included dose-dependent (with the exception of 90 min post-dose time point) decreases in mean RR interval, and non-dose dependent decreases in mean PR interval and uncorrected QT interval. There were no abnormalities in ECG waveforms or evidence of arrhythmogenesis observed after treatment with doses ≤ 100 mg/kg. QTcF intervals tended to be slightly longer (8-15%) up to 360 min postdose, with significant increases noted in the 30 and 100 mg/kg groups at 180 and 240 min postdose. This trend was not apparent after HR-correction with the QTcVW and QTcQ methods.

[32] "Triangulation" refers to the shape change of the action potential caused by a test agent (i.e., agents that produce action potential triangulation are known to be proarrhythmic).

Table 20: Cardiovascular Effects of TD-8954 in Conscious Beagle Dogs

	10 mg/kg	30 mg/kg	100 mg/kg
HR (postdose)	↑ 92%*† (30 min) ↑ 36%* (360 min)	↑ 142%*† (30 min) ↑ 53%* (360 min)	↑ 163%*† (30 min) ↑ 60%* (360 min)
DBP (postdose)	↑ 51%*† (30 min) ↑ 21% (240 min)	↑ 38%*† (30 min) ↑ 21% (240 min)	↑ 41%*† (30 min) ↑ 26% (240 min)
MAP (postdose)	↑ 43%*† (30 min) ↑ 18% (360 min)	↑ 27%*† (30 min) ↑ 18% (360 min)	↑ 32%* (30 min) ↑ 17% (360 min)
RR interval (postdose)	↓ 48%*† (30 min) ↓ 26%* (360 min)	↓ 59%*† (30 min) ↓ 34%*† (360 min)	↓ 62%*† (30 min) ↓ 37%* (360 min)
PR interval (postdose)	No significant decrease	↓ 25%*† (30 min) ↓ 17%† (360 min)	↓ 31%*† (30 min) ↓ 16% (360 min)
Uncorrected QT interval (postdose)	↓ 19%* (30 min)	↓ 28%*† (30 min) ↓ 13%* (120 min)	↓ 28%*† (30 min) ↓ 22%*† (120 min)

* denotes statistical significance ($p < 0.05$) relative to the time-matched vehicle control
† denotes statistical significance ($p < 0.05$) relative to the corresponding predose baseline (-30 min)

The doses used in this study (10, 30, and 100 mg/kg) are 15, 45, and 150 times higher than the maximum proposed human dose (0.67 mg/kg) in phase 1 studies.

In the 28-day repeat-dose toxicity study in dogs, TD-8954 was administered orally at doses of 5, 25, or 70/40 mg/kg/day (70 mg/kg/day reduced to 40 mg/kg/day due to lack of tolerability at the higher dose). Mean QT values were increased in females by 17% and 14% in the 25 and 75/40 mg/kg/day respectively, when compared to controls. Covariate-adjusted mean QTc values for males were significantly increased (11%) in 75/40 mg/kg/day compared to controls. The no-observed-adverse-effect level (NOAEL) of this study was 5 mg/kg/day, which is 7.5 times higher than the maximum proposed clinical dose (0.67 mg/kg) in phase 1 studies.

In the 13-week repeat-dose toxicity study in dogs, TD-8954 was administered orally at doses of 3, 10 or 30 mg/kg/day. On day 1, mean HR was significantly increased (24% to 41%) in the 10 and 30 mg/kg/day males; however the mean HR was decreased (16% to 25%) in the same groups on day 28 and on week 13. The QTc value was increased by 12%, 11%, and 11% in 30 mg/kg/day males on day 1, day 28, and week 13 respectively, compared to control males. In most cases, the magnitude of QTc changes for males and females treated with TD-8954 were mild (<10%). The NOAEL of this study was 10 mg/kg/day, which is 15 times higher than the maximum proposed clinical dose (0.67 mg/kg) in phase 1 studies.

6.2 Clinical Pharmacology

6.2.1 Metabolism of TD-8954

In vitro CYP inhibition study suggested that CYP3A4 is involved in the metabolism of TD-8954. Information on human ADME (absorption/distribution/metabolism/excretion) study is not available.

6.2.2 Drug-drug Interaction Studies

In vitro, the metabolism of TD-8954 was inhibited in the presence of ketoconazole, a CYP3A4 inhibitor. TD-8954 (10 µM) inhibits CYP isoforms: 1A2, 2C19, 2C9, 2D6, and 3A4 by 10-22% in human liver microsomes.

In vitro study suggests that TD-8954 is a substrate for human P-gp.

6.2.3 Thorough QT Study

No information is available to FDA.

6.2.4 Platelet aggregation study

No information is available to FDA about potential effects of TD-8954 on platelet aggregation.

6.3 Clinical

6.3.1 Completed Studies

A single clinical trial was identified in the literature and consisted of a phase 1 ascending dose study performed in healthy human subjects[33]. GI prokinetic effects of TD-8954 at doses of 0.1–20 mg were observed. The data demonstrated that TD-8954 was associated with an increase in bowel movement frequency and a reduction in the time to first stool compared to placebo. No adverse event data are available in the published reference for this study.

6.3.2 Ongoing Studies

There are currently no known ongoing studies.

6.3.3 Proposed studies

To date, future development plans have not been shared with the FDA.

[33] David T. Beattie, Scott R. Armstrong, Ross G. Vickery, et al. The Pharmacology of TD-8954, a Potent and Selective 5-HT4 Receptor Agonist with Gastrointestinal Prokinetic Properties. *Front Pharmacol.* 2011;2:25.

7 Statistical Considerations for CV Safety Studies

For the assessment of cardiovascular safety, dedicated, randomized cardiovascular (CV) outcome trials are considered to be the gold standard in determining the hazard ratio (HR) of an investigational treatment relative to a well understood control. Such trials are typically designed as event-driven trials with the objective of ruling out an excess risk measured by an upper bound of a 95% confidence interval for the hazard ratio.

Some important issues to consider in the design of a CV trial, from a statistical perspective, are the following:

- The definition of the primary cardiovascular safety outcome of interest, which is driven by clinical considerations. Cardiovascular trials frequently utilize a composite endpoint of non-fatal myocardial infarction, non-fatal stroke and cardiovascular death (major adverse cardiovascular events, or “MACE”)
- The degree of excess risk to rule out for the hazard ratio margin. Excess risk is typically expressed in the form of relative risk or a hazard ratio. The upper bound of a 95% confidence interval for the parameter of interest, such as the hazard ratio, is used to rule out the excess risk.
- The population of interest in the trial. As such trials are event-driven, it is common in CV outcome trials to study a high risk or “enriched” population in order to reduce the number of patients needed to observe the planned number of events for which the trial is designed. This population determines the background risk for the control group.
- The assumed value of the statistical parameter to be assessed in the trial (i.e., the true value of the hazard ratio). In trials where the risk is assumed to be equal between the treatment groups, the hazard ratio is assumed to be 1.

All of the above elements play a role in the number of events needed to reach a defined level of statistical power and the number of patients needed to observe the events. In the sample size calculations that follow, various choices for the amount of risk to rule out and the background rate are presented. All calculations assume equal risk between treatment groups (i.e., the HR=1).

Figure 2 presents sample size calculations in the form of patient years based on HR margins ranging from 1.5 to 4.0, and event rates ranging from 1.0 to 2.5 per 100 patient years of exposure, with 90% power. Figure 2 shows that as the number of events needed to rule out a HR increases, the HR margin decreases. The expected number of patient years is proportional to the number of events needed, and is a function of the background rate of events; a lower background rate implies that more patient years are needed to observe events.

As an example, consider a trial powered at 90% to rule out a HR margin of 2.0 for cardiovascular events. Such a trial would require 88 total events. Assuming a rate of events of 2.5 per 100 patient years, approximately 3,520 total patient years would be required to observe 88 events. The CV event rates ranging from 1 to 2.5 events per 100 patient years used in Figure 2 and Table 21 are based on a hypothetical enriched population with a high CV risk.

Figure 2. Patient years needed to rule out a Hazard Ratio with 90% Power

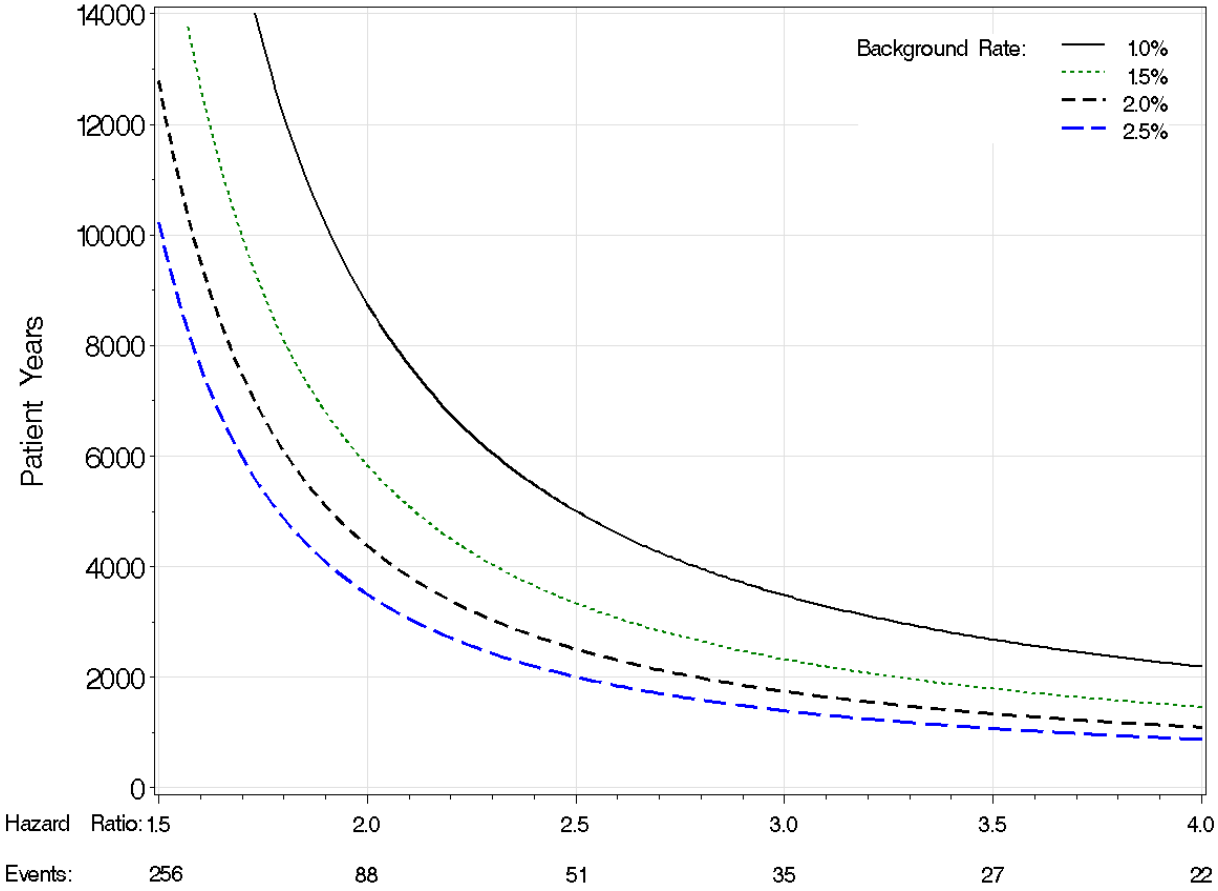

Table 21 shows additional information regarding sample size calculations from those shown in Figure 2. It shows how a combination of HR and background rate translates into a Risk Difference. Table 21 also shows the maximum point estimate for the HR that would need to be observed so that the upper bound of the 95% confidence interval for the HR is less than or equal to the HR margin. To coincide with the previous example, the shaded cells in Table 1 show that an event rate of 2.5 per 100 patient years and a HR margin of 2.0 translate into a risk difference of 2.5%. The shaded cells also show that the point estimate of the HR of CV events between treatment and control needs to be 1.32 or lower in order for the upper bound of the 95% confidence interval of the HR to be smaller than the HR margin of 2.0.

Table 21: Sample Size Calculations Using 90% Power in an Enriched Population

CV Event Rate in Control (per 100 subject years)		Hazard Ratio margin (Treatment / Control)					
		1.5	2.0	2.5	3.0	3.5	4.0
1	Risk Difference	0.5%	1%	1.5%	2%	2.5%	3%
	Total CV events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	25600	8800	5100	3500	2700	2200
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73
1.5	Risk Difference	0.75%	1.5%	2.25%	3%	3.75%	4.5%
	Total CV events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	17067	5867	3400	2333	1800	1467
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73
2	Risk Difference	1%	2%	3%	4%	5%	6%
	Total CV events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	12800	4400	2550	1750	1350	1100
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73
2.5	Risk Difference	1.25%	2.5%	3.75%	5%	6.25%	7.5%
	Total CV events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	10240	3520	2040	1400	1080	880
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73

The rate of CV events observed in Zelnorm (tegaserod) trials and observational studies is estimated at between 1 and 5 events per 1000 patient years[34]. Note that different studies have used different CV event definitions.

Table 22 presents sample size calculations for a trial with event rates between 1 per 1000 and 5 per 1000 patient years. Note that the number of events needed to rule out a HR are unaffected by the event rate and are unchanged from Table 21. However, the number of patient years needed to observe these events is larger because of the lower event rate. A trial in a non-enriched population with a lower event rate requires more patient years than a trial in an enriched population in order to observe the same number of events and achieve the same statistical power to rule out a pre-specified HR.

[34] The following event rates have been observed in trials and observational studies for tegaserod (Zelnorm):

- The Zelnorm meta-analysis observed 13 adverse CV events among 11,614 subjects treated with Zelnorm and 1 adverse event among 7,031 subjects on placebo (RR 7.9).
- J. Anderson, *et al.* (2009) observed a rate of composite CV endpoint of 3.5 per 1000 untreated subjects, and 4.6 per 1000 subjects treated with tegaserod in a case-control study. OR 1.06 (0.56 to 2.02).
- J. Loughlin, *et al.* (2010) observed an incidence of CV events (including acute coronary syndrome, MI and coronary revascularization) of 4.83 per 1000 patient years among subjects on tegaserod, and 5.18 per 1000 patient years on comparators. HR 0.95 (0.73 to 1.23)

Table 22: Sample Size Calculations Using 90% Power in a Non-Enriched Population

Event Rate in Control		Hazard Ratio margin (Treatment / Control)					
		1.5	**2.0**	**2.5**	**3.0**	**3.5**	**4.0**
1 per 1000 Patient years	Risk Difference	0.05%	0.1%	0.15%	0.2%	0.25%	0.3
	Total events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	256000	88000	51000	35000	27000	22000
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73
3 per 1000 Patient years	Risk Difference	0.15%	0.3%	0.45%	0.6%	0.75%	0.9
	Total events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	85333	29333	17000	11667	9000	7333
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73
5 per 1000 Patient years	Risk Difference	0.25%	0.5%	0.75%	1%	1.25%	1.5
	Total events needed for 90% Power	256	88	51	35	27	22
	Total patient years for 90% Power	51200	17600	10200	7000	5400	4400
	Maximum point estimate to rule out HR	1.17	1.32	1.44	1.55	1.65	1.73

As shown in Table 22, designing a CV safety trial in a non-enriched population at average CV risk would require very large sample sizes to rule out modest increases in risk.