

**BRIEFING DOCUMENT**  
**GASTROINTESTINAL DRUGS ADVISORY COMMITTEE**  
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## LIST OF ABBREVIATIONS

<b>Abbreviation</b>	<b>Description</b>
5-HT	5-hydroxytryptamine, serotonin
cAMP	cyclic adenosine monophosphate
CIC	chronic idiopathic constipation
IBS-C	constipation-predominant irritable bowel syndrome
$pK_i$	negative decadic logarithm $K_i$
$pEC_{50}$	negative decadic logarithm $EC_{50}$
POI	postoperative ileus
SERT	serotonin (5-HT) transporter

## 1 INTRODUCTION

Theravance, Inc. is developing two agonists highly selective for the human serotonin 5-HT<sub>4</sub> subtype receptor. Potential indications for these agents, velusetrag and TD-8954, include both upper and lower gastrointestinal disorders, such as gastroparesis (including diabetic gastroparesis), gastroesophageal reflux disease, postoperative ileus (POI), chronic idiopathic constipation (CIC), and constipation-predominant irritable bowel syndrome (IBS-C). This briefing document summarizes data from the pharmacology programs for the two compounds describing the selectivity for the 5-HT<sub>4</sub> receptor and lack of significant affinity at a variety of G-protein coupled receptors, enzymes, and ion channels. Data from the preclinical evaluation of cardiovascular safety pharmacology are also reviewed.

The pharmacology programs for velusetrag and TD-8954 included in vitro and in vivo studies to determine the 5-HT<sub>4</sub> agonist potency and prokinetic activity of the compounds. Activation of 5-HT<sub>4</sub> receptors is associated with contraction of guinea pig isolated colonic longitudinal muscle and inhibition of electrically evoked or spontaneous contractions of human isolated colonic circular muscle. 5-HT<sub>4</sub> receptor agonism in vivo results in increased colonic transit in guinea pigs, esophageal relaxation in rats, and enhancement of motility in the upper and lower gastrointestinal tract of dogs.

## 2 VELUSETRAG

In vitro studies, using cell lines stably expressing guinea pig or human recombinant 5-HT<sub>4</sub> receptors to study agonist affinity, potency, and intrinsic activity, confirm that velusetrag is a high-affinity, potent 5-HT<sub>4</sub> receptor agonist with high intrinsic activity. Several different splice variants of the human 5-HT<sub>4</sub> receptor have been identified {1, 2, 3}, and the relative abundance of mRNA transcripts encoding these splice variants is tissue specific {4}. Velusetrag exhibits similar affinity and potency at three alternate splice variants of the human 5-HT<sub>4</sub> receptor (i.e., 5-HT<sub>4(c)</sub>, 5-HT<sub>4(d)</sub>, and 5-HT<sub>4(g)</sub>), which are expressed to varying degrees in the gastrointestinal tract.

In radioligand binding studies, velusetrag exhibits high binding affinity for the human 5-HT<sub>4(c)</sub> (pK<sub>i</sub> = 7.7), 5-HT<sub>4(d)</sub> (pK<sub>i</sub> = 8.3), and 5-HT<sub>4(g)</sub> (pK<sub>i</sub> = 7.8) receptor splice variants and guinea pig 5-HT<sub>4</sub> receptor (pK<sub>i</sub> = 8.0). In cyclic adenosine monophosphate (cAMP) accumulation assays using human embryonic kidney HEK293 cells heterologously expressing the cloned human 5-HT<sub>4(c)</sub>, 5-HT<sub>4(d)</sub>, or 5-HT<sub>4(g)</sub> receptor splice variants, velusetrag is a potent (pEC<sub>50</sub> = 8.3, 8.6, or 8.2, respectively), full (intrinsic activity = 95%, 117%, or 97%,

respectively, relative to 5-HT) agonist. In cAMP accumulation studies using the guinea pig cloned 5-HT<sub>4</sub> receptor, velusetrag is a potent (pEC<sub>50</sub> = 7.9), full (intrinsic activity = 105%) agonist. The potency of velusetrag for the human 5-HT<sub>4(c)</sub> receptor is dependent on receptor expression levels. Thus, in cAMP accumulation assays using a cell line expressing the human 5-HT<sub>4(c)</sub> receptor at 100-fold physiological levels, velusetrag is a full (110%, relative to 5-HT) agonist with high potency (pEC<sub>50</sub> = 9.2). Schild regression analysis generated an affinity estimate (pK<sub>b</sub> value) for the selective 5-HT<sub>4</sub> receptor antagonist GR113808 of 9.7 against velusetrag in HEK293 cells stably expressing human 5-HT<sub>4(c)</sub> receptors, confirming that velusetrag mediates cAMP accumulation via a 5-HT<sub>4</sub> receptor-mediated mechanism. In summary, these in vitro data indicate that velusetrag has high affinity for the human recombinant 5-HT<sub>4</sub> receptor and is a potent, full agonist with respect to receptor-mediated activation of adenylyl cyclase.

The activity of velusetrag has been investigated in animal isolated tissue preparations. Velusetrag produced a concentration-dependent contraction of the guinea pig isolated colon longitudinal muscle–myenteric plexus preparation (pEC<sub>50</sub> = 7.9, intrinsic activity = 81% of the 5-HT maximum). Considering the wealth of literature demonstrating that 5-HT<sub>4</sub> receptor activation results in contraction of this smooth muscle preparation {5, 6} and the presence of antagonists of 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub> receptors in this study, the observed activity of velusetrag is concluded to represent 5-HT<sub>4</sub> receptor activation. Velusetrag produced a concentration-dependent relaxation of the carbachol-precontracted rat isolated esophageal tunica muscularis mucosa (pEC<sub>50</sub> = 7.9, intrinsic activity equivalent to that of 5-HT), a preparation commonly used for evaluating the profile of action of 5-HT<sub>4</sub> receptor agonists {5, 7}. Velusetrag is therefore a potent 5-HT<sub>4</sub> receptor agonist with high intrinsic activity in the guinea pig colon longitudinal muscle–myenteric plexus and rat esophageal tunica muscularis mucosa preparations.

Velusetrag (0.003 to 3 mg/kg), administered subcutaneously, increases colonic transit of carmine red dye in conscious guinea pigs, amounting to up to a 40% reduction in transit time compared with that in vehicle-treated animals. The prokinetic activity of velusetrag is inhibited by the selective 5-HT<sub>4</sub> receptor antagonist piboserod (1 mg/kg subcutaneously).

To characterize further the in vivo activity of velusetrag, digital sonomicrometry was used to monitor 5-HT<sub>4</sub> receptor-mediated relaxation of the rat esophagus. Velusetrag produces a dose-dependent, 5-HT<sub>4</sub> receptor-mediated relaxation of the esophagus in anesthetized rats

after intravenous and intraduodenal administration ( $ED_{50}$  values = 0.03 and 0.54 mg/kg, respectively). Pretreatment with piboserod (1 mg/kg subcutaneously) abolishes the relaxation response to velusetrag.

The prokinetic activity of velusetrag was evaluated in conscious beagle dogs implanted chronically with strain gauges in the fundus, antrum, duodenum, jejunum, and proximal colon using methodology previously described in studies with 5-HT<sub>4</sub> receptor agonists {8, 9, 10, 11}. Velusetrag (3, 10, and 30 µg/kg) produces a dose-dependent increase in contractility in the fundus, antrum, duodenum, and jejunum of fasted beagles after intravenous bolus administration. Oral administration of velusetrag (0.1 and 0.3 mg/kg) is also associated with increased contractility throughout the gastrointestinal tract. The increased contractility observed in this study is entirely consistent with activation of 5-HT<sub>4</sub> receptors by velusetrag in the gastrointestinal tract of the dog.

The in vivo preclinical data indicate that velusetrag is a potent 5-HT<sub>4</sub> receptor agonist in the guinea pig colon, rat esophagus, and dog gastrointestinal tract.

Velusetrag is highly selective for 5-HT<sub>4</sub> receptor over other serotonergic and non-serotonergic receptors, enzymes, and channels. It exhibits very low binding affinity for the human recombinant 5-HT<sub>3A</sub> receptor ( $pK_i$  = 4.2) and other 5-HT receptor subtypes ( $\leq 35\%$  inhibition of specific binding at 10 µM). The binding selectivity of velusetrag for the human 5-HT<sub>4(c)</sub> receptor over the human 5-HT<sub>3A</sub> receptor is approximately 3000-fold. The binding affinities of velusetrag for a variety of receptors ( $n$  = 58), transporters ( $n$  = 3), and ion channels ( $n$  = 11) and its effects on the activity of enzymes ( $n$  = 4) have been evaluated. Velusetrag has low binding affinity for sigma receptor binding sites ( $pK_i$  = 6.6); guinea pig peripheral histamine H<sub>1</sub> receptors ( $pK_i$  = 6.3); the human 5-HT transporter (SERT;  $pK_i$  = 5.9); rat  $\alpha_1$  adrenoceptors ( $pK_i$  = 5.5); guinea pig histamine H<sub>2</sub> receptors ( $pK_i$  = 4.8); and human recombinant muscarinic M<sub>1</sub> ( $pK_i$  = 5.9), M<sub>2</sub> ( $pK_i$  = 6.1), M<sub>3</sub> ( $pK_i$  = 5.8), M<sub>4</sub> ( $pK_i$  = 5.9), and M<sub>5</sub> ( $pK_i$  = 5.4) receptors. The binding selectivity of velusetrag for the human 5-HT<sub>4(c)</sub> receptor over sigma, H<sub>1</sub>, SERT,  $\alpha_1$ , and H<sub>2</sub> receptors is 10-, 30-, 70-, 200-, and 800-fold, respectively. The selectivity over muscarinic receptors is 40- to 200-fold. The affinity of velusetrag for the sigma receptor is consistent with that described in the literature for several other 5-HT<sub>4</sub> ligands {12, 13}. With respect to other binding sites, velusetrag exhibits  $\geq 400$ -fold selectivity for binding to 5-HT<sub>4</sub> receptors. Velusetrag (3 µM) has no effect on rat neuronal (Na<sub>v</sub>1.2a) or human cardiac (Na<sub>v</sub>1.5) voltage-gated sodium currents.

With respect to functional in vitro selectivity, velusetrag (at 30  $\mu\text{M}$ ) has no significant agonist or antagonist activity at histamine  $\text{H}_1$  receptors in the guinea pig trachea. Velusetrag (up to 10  $\mu\text{M}$ ) also has no agonist or antagonist activity on human muscarinic  $\text{M}_2$  or  $\text{M}_4$  receptor-mediated exchange of GDP for [ $^{35}\text{S}$ ]GTP $\gamma\text{S}$  in membranes prepared from Chinese hamster ovary (CHO-K1) cells heterologously expressing the respective cloned receptors. Velusetrag (up to 10  $\mu\text{M}$ ) has no effect on human muscarinic  $\text{M}_1$  or  $\text{M}_3$  or chimpanzee  $\text{M}_5$  receptor-mediated increases in intracellular calcium in CHO-K1 cells heterologously expressing the respective cloned receptors. At concentrations up to 10 or 30  $\mu\text{M}$ , velusetrag fails to constrict human, canine, and porcine coronary arteries, consistent with its high degree of 5-HT $_4$  receptor selectivity {14}.

In summary, velusetrag is a high-affinity, potent agonist at the human recombinant 5-HT $_4$  receptor, with a high degree of selectivity over all non-5-HT $_4$  receptors, ion channels, transporters, and enzymes against which it has been tested. Velusetrag has demonstrated prokinetic activity in several in vitro and in vivo assays.

### **3 TD-8954**

In vitro studies, using a cell line stably expressing human recombinant 5-HT $_{4(c)}$  receptors, have confirmed that TD-8954 is a high-affinity 5-HT $_4$  receptor ligand ( $\text{pK}_i = 9.4$ ) {15}. TD 8954 is highly selective for the 5-HT $_4$  receptor over all other serotonergic and non-serotonergic receptors, enzymes, and channels. Data from in vitro studies using cell lines stably expressing human recombinant 5-HT $_{4(c)}$  receptors have confirmed that TD-8954 is a potent agonist at the 5-HT $_4$  receptor ( $\text{pEC}_{50} = 9.3$ ) with high intrinsic activity (83% of the 5-HT maximum response) {16}. TD-8954 produced a potent, concentration-dependent, 5-HT $_4$  receptor-mediated, contraction of the guinea pig isolated colonic longitudinal muscle/myenteric plexus preparation ( $\text{pEC}_{50} = 8.6$ ) {17} and produced a concentration-dependent attenuation of spontaneous and electrical field stimulation-evoked contractile activity of human isolated colonic circular muscle {18}. In vivo studies showed that TD-8954 was associated with a dose-dependent increase in colonic transit of carmine red dye in conscious guinea pigs {19}, verifying the prokinetic action of the compound. In digital sonomicrometry studies, TD-8954 produced a dose-dependent, 5-HT $_4$  receptor-mediated relaxation of the esophagus in anesthetized rats following intravenous and intraduodenal administration {20}. In conscious, fasted beagle dogs with strain gauge transducers surgically implanted on the antrum, duodenum, jejunum, and proximal colon, TD-8954 (0.01

and 0.03 mg/kg) produced a dose-dependent increase in contractility of the antrum, duodenum, and jejunum following oral administration {21}.

TD-8954 (1  $\mu$ M) had only minimal binding (<14% inhibition of specific radioligand binding) at the 5-HT<sub>1A</sub>, 5-HT<sub>1B</sub>, 5-HT<sub>1D</sub>, 5-HT<sub>2A</sub>, 5-HT<sub>2B</sub>, 5-HT<sub>2C</sub>, 5-HT<sub>5A</sub>, 5-HT<sub>6</sub>, and 5-HT<sub>7</sub> receptors, and was >270,000-fold more selective for the human 5-HT<sub>4</sub> receptor over the 5-HT<sub>3A</sub> receptor (pK<sub>i</sub> values of 9.4 and <4.0, respectively) {22, 23}. The binding selectivity of TD-8954 for the human 5-HT<sub>4</sub> receptor over human recombinant M<sub>1</sub>, M<sub>2</sub>, M<sub>3</sub>, M<sub>4</sub>, and M<sub>5</sub> muscarinic receptors was  $\geq$ 2000 {24}. TD-8954 had greater than 2500-fold selectivity for the human 5-HT<sub>4</sub> receptor over all other non-5-HT receptors, transporters, ion channels, and enzymes at which it has been tested {25}. TD-8954, at a concentration (3  $\mu$ M) approximately 6000- or 1200-fold higher than its 5-HT<sub>4</sub> receptor agonist EC<sub>50</sub> value for 5-HT<sub>4</sub> receptor-mediated cAMP accumulation in a human recombinant cell line or contraction of the guinea pig isolated colon, respectively, had no effect on rat neuronal (Na<sub>v</sub>1.2a) or human cardiac (Na<sub>v</sub>1.5) voltage-gated sodium channel function {26}. In summary, TD-8954 is a high-affinity, potent agonist at the human recombinant 5-HT<sub>4</sub> receptor, with a high degree of selectivity over all non-5-HT<sub>4</sub> receptors, ion channels, transporters, and enzymes against which it has been tested. TD-8954 has demonstrated prokinetic activity in several in vitro and in vivo assays. In human isolated colonic circular muscle, TD-8954 is associated with inhibition of spontaneous and electrically evoked contractions, actions consistent with its 5-HT<sub>4</sub> agonist properties and prokinetic action.

#### **4 SUMMARY**

Two highly selective 5-HT<sub>4</sub> agonists, velusetrag and TD-8954, are being developed as potential treatments for upper and lower gastrointestinal disorders, such as gastroparesis (including diabetic gastroparesis), gastroesophageal reflux disease, postoperative ileus (POI), chronic idiopathic constipation (CIC), and constipation-predominant irritable bowel syndrome (IBS-C). Both agents demonstrate a high degree of selectivity for the 5-HT<sub>4</sub> receptor and lack of significant affinity at a variety of G-protein coupled receptors, enzymes, and ion channels. In animal and human isolated tissue preparations, including human colonic circular smooth muscle preparations, both agents were potent 5-HT<sub>4</sub> receptor agonists with moderate or high intrinsic activity, and both agents demonstrated prokinetic activity in in vivo studies.

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