CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA	**208-745**
Relevant IND(s)	**74883**
Submission Date (s)	**01/29/2016, 9/22/2016**
PDUFA Date:	**01/29/2017**
Brand Name	**TRULANCE™**
Generic Name	**Plecanatide**
Submission Type	**Original, 505(b)(1), NME**
Dosage Form, Strength	**Tablet, 3 mg**
Proposed indication	**Treatment of chronic idiopathic constipation (CIC) in adults**
Sponsor Recommended Dosing Regimen	**3 mg Once daily**
Sponsor	**Synergy Pharmaceuticals Inc**
OND Division	**Division of Gastroenterology and Inborn Errors Products**
OCP Division	**Division of Clinical Pharmacology 3**
OCP Reviewer	**Dilara Jappar, Ph.D.**
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1. Executive Summary

Plecanatide (SP-304) is a synthetic, 16-amino acid peptide with 2 disulfide bonds that is a second-in-class guanylate cyclase-C (GC-C) receptor agonist. The proposed indication for plecanatide is treatment of chronic idiopathic constipation in adults with 3 mg oral dose once daily. In support of this NDA, the sponsor had submitted 7 *in-vitro* studies to evaluate the potential drug-drug interaction, 2 phase I studies to evaluate the food effect and single ascending dose PK in healthy subjects, 3 phase II studies, 2 phase III efficacy and safety studies with 3 mg and 6 mg dose and one long-term phase III safety study to support the safety and efficacy of plecanatide. In addition, the submission contained 4 bioanalytical validation reports. PK samples were also obtained from the patient population in the phase 2 and 3 studies.

1.1 Recommendations

The Office of Clinical Pharmacology has found the submission acceptable from a clinical pharmacology standpoint provided a mutual agreement on labeling languages is reached between the FDA and the sponsor.

1.2 Recommended Post-Marketing Studies

None

1.3 Clinical Pharmacology Highlights

Dose-Response for Efficacy*:*

Exposure-response relationship other than dose response for efficacy for plecanatide was not evaluated since plecanatide did not have measurable systemic exposure.

Phase 2b study (SP304-20210) had evaluated 0.3, 1.0, and 3.0 mg QD oral doses of plecanatide versus placebo in a total of 951 CIC patients over a 12-week treatment period. The 3.0 mg dose provided the highest overall responder rate (19.0% vs. 10.7% for placebo) and a statistically significant treatment difference compared to placebo ($p = 0.009$) while 1 mg dose did not result in statistically significant difference from the placebo ($p = 0.057$). Interestingly, 0.3 mg dose level also resulted in a statistically significant difference from the placebo ($p = 0.016$) although the sponsor claims that 0.3 mg dose was examined in an exploratory nature.

Two dose levels, 3 mg and 6 mg, were evaluated in 2 phase III studies in CIC patients with 12 weeks treatment period. No dose-response was observed between 3 mg and 6 mg doses for primary efficacy endpoint while both dose levels have shown statistically significant improvement over placebo.

Dose-Response for Safety:

Exposure-response relationship other than dose-response for safety for plecanatide was not evaluated since plecanatide did not have measurable systemic exposure.

In the phase 2a study (SP304201-09) with 0.3, 1.0, 3.0, and 9.0 mg QD oral dose levels of plecanatide over 14 days of treatment (versus placebo) in a total of 78 CIC patients, there is no clear trend in AE over plecanatide dose ranges but the 9.0 mg dose appears to be associated with a higher rate of drug-related AEs (33.3%) compared to placebo (10%) or other dose levels (6.4-14.3%). As such, the 9 mg dose was not included in the Phase 2b study.

In Phase 2b dose-ranging study (SP304-20210) with 0.3, 1.0, and 3.0 mg QD oral doses of plecanatide over 12 weeks of treatment in a total of 951 CIC patients (including those on placebo), there appears to be dose-related increase in diarrhea, abdominal pain, number patients

with at least one Treatment-Emergent Adverse Events (TEAE), number of TEAEs and overall drug-related AE.
In the 2 phase III studies, there does not appear to be a clear dose-response for the majority of adverse events between 3 mg and 6 mg doses. However, there were some increase in diarrhea and nasopharyngitis with the higher dose of 6 mg compared to 3 mg.

Dose Selection:
The phase 2b study evaluated 0.3, 1.0, and 3.0 mg doses of plecanatide and had shown that the 3.0 mg dose provided a statistically significant treatment difference compared to placebo ($p = 0.009$) while 1 mg dose did not result in a statistically significant efficacy compared to placebo ($p = 0.057$). Based on the result of this phase 2b study, the 3 mg dose was selected for phase 3 studies. In addition, a 6 mg dose level was added in phase 3 studies to evaluate whether the efficacy could be improved further while maintaining the safety profile within an acceptable range.
In the phase 3 studies, both 3 mg and 6 mg doses resulted in statistically significant improvements over placebo. However, 6 mg dose was not numerically superior to the 3 mg dose on most endpoints although the studies were not sized for a direct comparison of 3 and 6 mg doses levels of plecanatide.
(b) (4) the sponsor had proposed (b) (4) 3 mg (b) (4) dose levels for CIC indication without specifying the distinct subpopulation (b) (4) (b) (4) (b) (4) The (b) (4) proposed dose of 3 mg is consistent with known dose-response relationship for both efficacy and safety.

Pharmacokinetics:
The sponsor attempted to measure the plasma concentration of plecanatide and its active metabolite SP-338 in healthy subjects and in CIC patient population. However, systemic exposures of the parent drug plecanatide or its active metabolite SP-338 were not detectable in human plasma up to 9 mg plecanatide dose (3 time the clinical dose of 3 mg) with LC-MS/MS bioanalytical method (with LLOQ of 1.0 ng/mL for plecanatide and 0.775 ng/mL for SP-338) suggesting insignificant absorption of plecanatide peptide and its metabolite SP-338 following oral route of administration.

Food Effect:
When 9 mg plecanatide tablet was administered with or without food in 24 healthy subjects, only 1 subject had detectable level of plecanatide at 0.5 and 1 hour post-dose under fasted state. Plecanatide concentrations were below the limit of quantitation for all other time points and for all other subjects. The active metabolite was not detected in any subjects. Food had minimal effect on bowel movement frequency, time to first bowel movement, fecal urgency, and fecal incontinence. Administration of 9 mg plecanatide with food had noticeable PD effect in Bristol Stool Form Scale (BSFS) scores and the incidence of abdominal cramping where food (both HF-HC and LF-LC) appear to increase BSFS score resulting in looser stool and increase the incidence of moderate and severe abdominal cramping and degree of abdominal cramping compared to fasted state. In both of the phase III studies, subjects were instructed to take the study drug with or without food at their own choice. In the proposed label, the sponsor is proposing to take plecanatide with or without food. However, based on the result of this food effect study, the agency recommends taking plecanatide with or without food in general but for

patients who experience abdominal cramping, plecanatide tablet should be taken under fasting condition. This is under further discussion with the medical review team.

Protein binding:
Plecanatide do not have detectable level of drug in the plasma at the proposed dose. Therefore, protein binding of plecanatide is not a concern. Nonetheless, plecanatide did not have significant binding to major human plasma proteins human serum albumin (HSA) and human α1-acid glycoprotein (AGP) proteins.

Metabolism:
In the simulated intestinal fluid (SIF), plecanatide is rapidly hydrolyzed at its C-terminus by cleavage of the Leu16 resulting in generation of a biologically active metabolite SP-338. SP-338 is further processed by an internal cleavage at Leu6-Cys7 resulting in a biologically inactive Leu6-clipped SP-338. However, potential metabolites of SP-338 were not quantitated. The potential metabolism by CYPs enzymes in human intestinal microsomes was not evaluated.

CYP inhibition /induction:
As the parent drug SP-304 and its metabolite SP-338 have limited systemic exposure, only CYP enzymes that are expressed in gastrointestinal tract, CYP2C9 and CYP3A, were evaluated for potential inhibition and induction by the parent drug SP-304 and its metabolite SP-338. Based on the in-vitro studies, plecanatide and its major metabolite SP-338 are not likely to inhibit CYP2C9 and CYP3A4 or induce CYP3A4 at the proposed dose.

Transporters:
As the parent drug SP-304 and its metabolite SP-338 have limited systemic exposure, only transporters that are expressed in the gastrointestinal tract, P-gp and BCRP, were evaluated for potential interaction with the parent drug SP-304 and its metabolite SP-338. Based on the in-vitro study on Caco-2 cells, both parent drug plecanatide SP-304 and its active metabolite SP-338 are not substrates or inhibitors of gut transporters P-gp and BCRP.

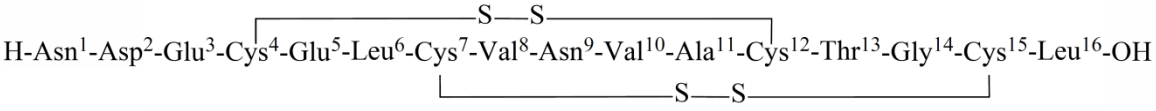
2 Question-Based Review

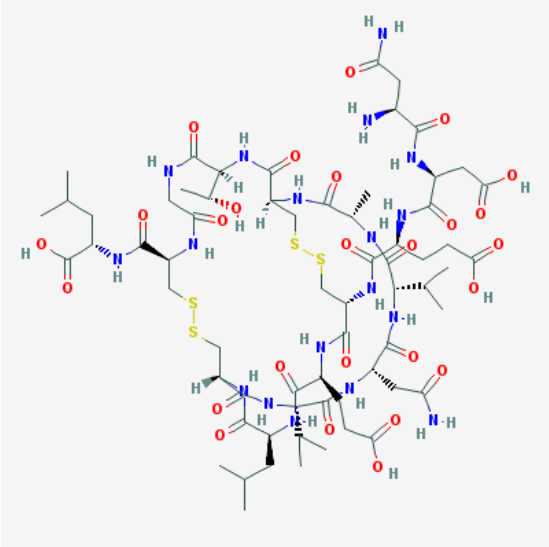
2.1 General Attributes of the drug

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance, and the formulation of the drug product as they relate to clinical pharmacology review?

Plecanatide (SP-304) is a synthetic, 16-amino acid peptide with 2 disulfide bonds. The molecular formula is $C_{65}H_{104}N_{18}O_{26}S_4$ and the molecular weight is 1682 g/mol.

Figure 1: Structure of Plecanatide (SP-304)





Plecanatide is developed as immediate-release oral tablet at 3 mg (b) (4) strength in this application.

2.1.2 What are the proposed mechanism(s) of action and therapeutic indication(s)?

The proposed indication for plecanatide is treatment of chronic idiopathic constipation in adults.

Plecanatide (SP-304) is a synthetic, hexadecapeptide, guanylate cyclase-C (GC-C) receptor agonist. Plecanatide is an analog of the endogenous human uroguanylin peptide (identical with the exception of a single amino acid substitution) which is also a member of the GC-C agonist class. It is proposed that both plecanatide and its active metabolite SP-338 bind to GC-C and act locally on the luminal surface of the intestinal epithelium. Activation of GC-C results in an increase in both intracellular and extracellular concentrations of cyclic guanosine monophosphate (cGMP). Elevation of intracellular cGMP stimulates secretion of chloride and bicarbonate into the intestinal lumen, mainly through activation of the cystic fibrosis transmembrane conductance regulator ion channel (CFTR), resulting in increased intestinal fluid and accelerated transit. In animal models, plecanatide has been shown to increase fluid secretion into the gastrointestinal (GI) tract, accelerate intestinal transit, and cause changes in stool consistency. Similar to uroguanylin, the binding of plecanatide to the intestinal GC-C receptors in the lumen of the GI tract is regulated by intestinal pH levels; therefore, plecanatide is anticipated to exert the bulk of its effect in the acidic environment of the upper GI tract, where it binds more strongly to GC-C receptors.

2.1.3 What are the proposed dosage(s) and route(s) of administration?

The proposed dose of plecanatide tablet is 3 mg once daily by oral route of administration with or without food.

2.1.4 What is the regulatory background?

The first-in-class guanylate cyclase C (GC-C) receptor agonist, Linzess (linaclotide), was approved in 2012. Linaclotide was a chemically synthesized 14-amino acid peptide with 3 disulfide bonds that was also an analog of the endogenous human uroguanylin peptide.

2.2 General Clinical Pharmacology

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

In support of this NDA, the sponsor had submitted 2 phase I studies, 3 phase II studies and 2 phase III efficacy and safety studies, one long-term phase III safety study and 7 *in-vitro* studies. In addition, it contained 4 bioanalytical validation reports. Please see table 1 and table 2 for more information.

Phase I clinical pharmacology program included one single ascending dose study and a food effect study in healthy subjects. *In-vitro* studies characterized the protein binding, metabolic stability in simulated intestinal fluid (SIF) and in human cadaver intestinal fluid, inhibition and induction potential of CYP enzymes expressed in the gastrointestinal tract and potential of plecanatide and its metabolite being substrate or inhibitor of transporters expressed in the gastrointestinal tract. Additionally, four bioanalytical method validation reports along with 5 additional amendments to analyze the concentrations of plecanatide and its metabolite SP-338 in human plasma were submitted.

Table 1: In-Vivo Clinical Pharmacology and Clinical Studies:

Study #	**Objective(s)**	**Study Design**	**Test product; Dosage Regimen; Route of administration**	**Subjects**	**Duration of Treatment**
SP304101-08	Safety and tolerability; identification of the MTD; PK, and effect of a single dose on bowel function	Phase 1,randomized (6:2 withincohort), DB, PBO-ctrl, single-dose, ascending-dose study	Plecanatide oral solution or PBO solution (PBS), with 9 Plecanatide dose cohorts (8 subjects each): 0.1, 0.3, 0.9, 2.7, 5.4, 8.1, 16.2, 24.3, & 48.6 mg Single oral dose No rescue med	71 healthy subjects: 53 plecanatide 18 PBO	1 day
SP304101-09	Effect of food on PD, PK, safety, & tolerability	Phase 1, single-blind, PBO-ctrl, crossover, Randomized (8:2 & to Treatment sequence and then to active drug or PBO), single dose study	Plecanatide tab or PBO tab Single oral 9 mg dose under 3 meal conditions: fasted fed HFHC meal fed LFLC meal No rescue med	30 healthy adult subjects: 24 plecanatide 6 PBO	3 days
SP304201-09	Safety, PK & PD; no efficacy assessments	Phase 2a randomized (15:5 within cohort), DB, PBO-ctrl, Ascending dose cohort, dose-ranging study	Plecanatide cap or PBO cap, with 4 plecanatide dose cohorts (0.3, 1.0, 3.0, and 9.0 mg) Once daily orally (fasted) Rescue med: bisacodyl	78 patients: 58 plecanatide (14 at 0.3 & 1.0 mg, 15 at 3 & 9 mg) 20 PBO	14 days
SP304-20210	Safety & efficacy of plecanatide in adults with CIC	Phase 2b, randomized, DB, PBO-ctrl, dose ranging study	Plecanatide 0.3, 1.0, or 3.0 mg cap or PBO cap Once daily orally Rescue med: bisacodyl	948 patients: 712 plecanatide (567 for 12 wk) 236 PBO	12 weeks
[redacted]					(b) (4)
SP304203-00	Safety and Efficacy	Phase 3, randomized, DB, PBO-ctrl, oral, dose ranging study	Plecanatide 3 or 6 mg tab or PBO tab Once daily orally Rescue med: Bisacodyl	Total 1389 patients: • 474 at 3 mg • 457 at 6 mg • 458 PBO	12 weeks

SP304203-03	Safety and Efficacy	Phase 3, randomized, DB, PBO-ctrl study	Plecanatide 3 or 6 mg tab or PBO tab Once daily orally Rescue med: bisacodyl	Total 1402 patients: • 467 at 3 mg • 469 at 6 mg; • 466 PBO	12 weeks
SP304203-01	Long-term safety and tolerability	Phase 3, open-label	Plecanatide 3 or 6 mg tab Once daily orally Rescue med: Bisacodyl	1782 plecanatide 230 at 3 mg 1552 at 6 mg;	Up to 2 Years

Table 2: In-Vitro Studies

Study #	**Type of Study**	**Title/Objective(s) of the Study**
RSN00008	Protein Binding	Binding of Test Compound SP-304 to HAS and Human AGP
13SYNRP1A	Transporter	Determining the Substrate and Inhibition Potential of Plecanatide (SP-304) for P-gp and BCRP
13SYNRP6A	Transporter	Determining the Substrate and Inhibition Potential of SP-338 for P-gp and BCRP
13SYNRP1B	CYP inhibition /induction by plecanatide	In Vitro Metabolism Studies of Plecanatide (SP-304)
13SYNRP6B	CYP inhibition /induction by metabolite	In Vitro Metabolism Studies of SP-338
SP-PH-018	Stability	Stability of Plecanatide in Human Intestinal Fluid
SP-PH-015	Stability in SIF	Assessment of Plecanatide Stability and Metabolite Identification After Treatment with Simulated Intestinal Fluid in the Presence and Absence of Reducing Agents

2.2.2 What is the basis for selecting the response endpoints and how are they measured in clinical pharmacology and clinical studies?

The proposed indication is treatment of chronic idiopathic constipation in adults. Accordingly, evaluation of clinical efficacy of plecanatide focused on resolving constipation.

Phase I studies:
SAD study SP-SP304101-08
PD: Time to first stool, stool frequency (48-hour period), and stool consistency (48-hour period) using the Bristol Stool Form Scale (BSFS)

Food Effect study SP304101-09
PD: bowel movement frequency, stool consistency, time to first bowel movement, fecal urgency, fecal incontinence and soiling, and abdominal cramping.

Phase II studies:
Phase 2a study SP-SP304201-09:
PD: Stool frequency was assessed as a combination of Complete Spontaneous Bowel Movement (CSBM) and Spontaneous Bowel Movement (SBM); Stool Consistency was assessed with 7-point Bristol Stool Form Scale; Ease of Passage (Straining) measured using the 7-point Ease-of-Passage Scale; Completeness of Evacuation, The time to first bowel movement

Phase 2b study SP-SP304202-10:
The primary efficacy endpoint was the proportion of patients who were overall CSBM responders.
The secondary efficacy endpoints included change from baseline in 12-week CSBM frequency rate, time to first CSBM, change from baseline in the 12-week SBM frequency rate, time to first SBM, stool consistency as measured by the Bristol Stool Form Scale (BSFS), days of rescue medication use, and straining.

Phase III studies (*SP304203-00 and SP304203-03*):
The primary efficacy endpoint was the proportion of patients who were durable overall complete spontaneous bowel movement (CSBM) responders over the 12-week treatment period. A CSBM weekly responder was defined as patient how had ≥3 CSBM in a given week and an increase from baseline of ≥ 1 CSBM for that same week. An overall CSBM responder was defined as a patient who was a weekly responder for at least 9 of the 12 treatment weeks, and a durable overall CSBM responder was also a weekly responder in at least 3 of the last 4 weeks.

Secondary efficacy endpoints included:

- Change from baseline in frequency rate of CSBMs and Spontaneous bowel movements
- Change from baseline in stool consistency based on the Bristol Stool Form Scale
- Change from baseline in Straining Score
- Treatment satisfaction
- Patient reported symptoms associated with constipation in the Daily Symptom Diary

2.2.3 Are the active moieties in the plasma and urine appropriately identified and measured to assess pharmacokinetic parameters and exposure-response relationships?

Yes, concentrations of plecanatide and its active metabolite SP-338 in plasma were analyzed by appropriately validate LC-MS/MS bioanalytical methods with LLOQ of 1.0 ng/mL for plecanatide and LLOQ of 0.775 ng/mL for SP-338. Please see the analytical section 2.9 for more details. Plasma levels for both plecanatide and its metabolite SP-338 were undetectable in plasma up to 9 mg of oral dose.

2.3 Exposure-Response Evaluation

2.3.1 What are the characteristics of the exposure-response relationships for efficacy?

Exposure-response relationship for efficacy for plecanatide was not evaluated since plecanatide did not have measurable systemic exposure.

Phase 2a study (SP304201-09) was a randomized, double-blind, placebo-controlled, dose-ranging study that evaluated 0.3, 1.0, 3.0, and 9.0 mg QD oral doses of plecanatide over 14-day days of treatment in a total of 78 CIC patients. As the primary objective study was to evaluate safety, the study was not powered to demonstrate statistical differences between SP-304 and placebo regarding the efficacy. Nonetheless, multiple parameters assessed during the study indicated that SP 304 had a greater effect on symptoms of CIC and greater patient satisfaction compared to placebo. SP-304 patients reported a greater degree of improvement compared to baseline in complete spontaneous bowel movement, spontaneous bowel movement, stool consistency, and straining versus placebo patients; however, no clear dose-response relationship was noticeable for most efficacy endpoint except that a trend toward shorter mean time to first bowel movement was observed with increasing dose of SP-304 (23.7, 19.5, 17.4, and 11.0 hours for the 0.3, 1.0, 3.0, and 9.0 mg SP-304 dose levels, respectively).

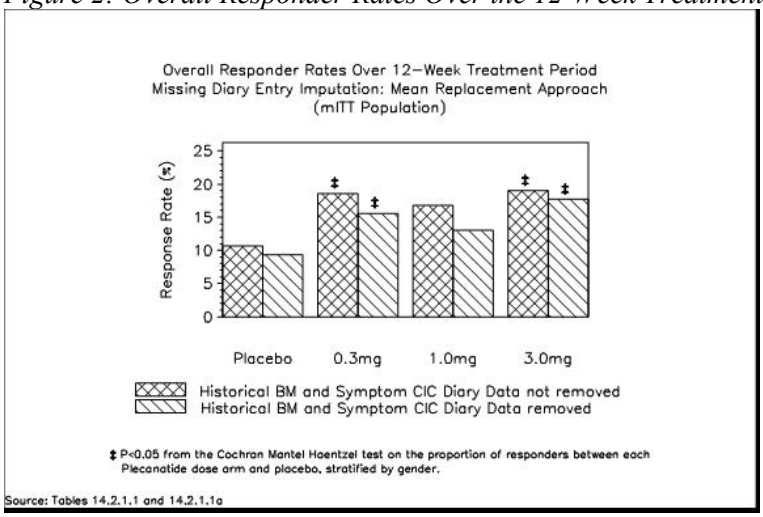
Phase 2b Study (SP304-20210) was a randomized, double-blind, placebo-controlled, dose-ranging study that evaluated 0.3, 1.0, and 3.0 mg QD oral doses of plecanatide versus placebo over 12 weeks of treatment in a total of 951 CIC patients. The primary efficacy endpoint was the proportion of patients who were durable overall CSBM responders over the 12-week treatment period, which was the same primary endpoint included in the two phase 3 studies. On the

primary endpoint, the 3.0 mg dose provided the highest overall responder rate versus placebo (19.0% vs. 10.7%, respectively) and a statistically significant treatment difference compared placebo (p = 0.009) while 1 mg dose did not result in statistically significant difference from the placebo (p = 0.057). Interestingly, 0.3 mg dose level also resulted in a statistically significant difference from the placebo (p = 0.016) although the sponsor claims that 0.3 mg dose was examined in an exploratory nature. The 3.0-mg dose of plecanatide also yielded the best responses in the secondary efficacy endpoints.

Table 3: Durable Overall CSBM Responder Rates Over 12-Week Treatment Period (Study SP304-20210):

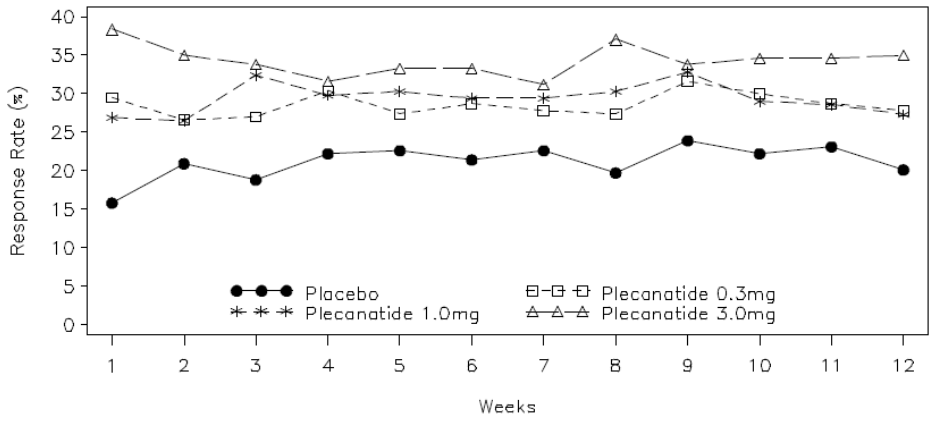
		Plecanatide		
	Placebo (n = 234) n (%)	**0.3 mg (n = 237) n (%)**	**1.0 mg (n = 238) n (%)**	**3.0 mg (n = 237) n (%)**
Overall responder[a]				
n	234	237	238	237
Responder	25 (10.7)	44 (18.6)	40 (16.8)	45 (19.0)
Nonresponder	209 (89.3)	193 (81.4)	198 (83.2)	192 (81.0)
95% CI on response rate[b]	(6.73, 14.64)	(13.62, 23.52)	(12.06, 21.56)	(13.99, 23.98)
Comparison between plecanatide and placebo				
Treatment difference[c]		7.9	6.1	8.3
95% CI for the difference[b]		(1.54, 14.22)	(−0.06, 12.31)	(1.93, 14.68)
P value[d,g]		0.016	0.057	0.009

Figure 2: Overall Responder Rates Over the 12-Week Treatment Period



The 3.0 mg dose also provided the highest weekly responder rate of any treatment at each treatment period week, maintaining a weekly CSBM responder rate between 31.2% and 38.4% where the weekly CSBM response rates for placebo patients ranged from 15.8% to 23.9%.

Figure 3: Study SP304-20210 CSBM Weekly Responder Rates



Phase 3 studies: The sponsor had evaluated the safety and efficacy of two dose levels, 3 mg and 6 mg, of oral plecanatide tablets in a 2 phase III, multicenter, randomized, double-blind, placebo-controlled, parallel-group study in subjects with CIC. A no dose-proportional increase in primary efficacy endpoint was observed between 3 mg and 6 mg while both dose levels have shown statistically significant improvements over placebo.

Table 4: Durable Overall CSBM Responder Rates in the Two Placebo Controlled Studies: at least 9 of 12 weeks and at least 3 of the last 4 weeks (ITT Population)

	Placebo	Plecanatide 3 Mg [P-Value]	Treatment Difference [95% Ci]	Plecanatide 6 Mg [P-Value]	Treatment Difference [95% CI]
	Study 1 (SP304203-00)				
Durable Overall CSBM Responder	10.2%	21.0% [<0.001]	10.8% [6.1%, 15.4%]	19.5% [<0.001]	9.3% [4.7%, 14.0%]
	Study 2 (SP304203-03)				
Durable Overall CSBM Responder	12.8%	20.1% [0.004]	7.3% [2.4%, 12.1%]	20.0% [0.004]	7.2% [2.4%, 12.1%]

CI = confidence interval

2.3.2 What are the characteristics of the exposure-response relationships for safety?

Exposure-response relationship for safety for plecanatide was not evaluated since plecanatide did not have measurable systemic exposure.

In the phase 2a study (SP304201-09) with 0.3, 1.0, 3.0, and 9.0 mg QD oral doses of plecanatide over 14 days of treatment in a total of 78 CIC patients, although there is no clear trend in AE over plecanatide dose ranges or in comparison with placebo, 9.0 mg dose appears to be associated with a higher rate of drug-related AEs (33.3%) compared to the placebo (10%) or other dose levels (6.4-14.3%).

In the phase 2b dose-ranging study (SP304-20210) with 0.3, 1.0, and 3.0 mg QD oral doses of plecanatide over 12 weeks of treatment in total of 951 CIC patients, there appears to be dose-related increase in diarrhea, abdominal pain, number patients with at least one Treatment-Emergent Adverse Events (TEAE), number of TEAEs and overall drug-related AE.

Table 5: Most Frequent (Overall Incidence ≥ 2%) Treatment-Emergent Adverse Events Incidences, by Preferred Term in Phase 2b study

	Placebo	0.3 mg	1.0 mg	3.0 mg

Diarrhea	3 (1.3%)	13 (5.5%)	20 (8.4%)	23 (9.7%)
Abdominal pain	11 (4.7%)	6 (2.5%)	9 (3.8%)	12 (5.1%)
Patients with at least one TEAE	96 (40.7%)	99 (41.8%)	103 (43.3%)	106 (44.7%)
Number of TEAEs	186	183	198	234
Drug-related AEs	6 (2.5%)	12 (5.1%)	16 (6.7%)	21 (8.9%)

In 2 phase III studies, there does not appear to be a clear dose-response for the majority of adverse event categories between 3 mg and 6 mg dose levels. However, there were some increase in diarrhea and nasopharyngitis with 6 mg dose compared to 3 mg dose.

Table 6: Adverse Events (Preferred Terms)Occurring in ≥0.5% of Patients in the Primary Pool by System Organ Class (ITT-S Population)

	Placebo	3 mg	6 mg
Diarrhea	12 (1.3%)	43 (4.6%)	47 (5.1%)
Nasopharyngitis	14 (1.5%)	11 (1.2%)	20 (2.2%)

2.3.3 Does this drug prolong the QT or QTc interval?

Since plecanatide has negligible systemic exposure in human, the sponsor had requested for a waiver for requirement to conduct a thorough QT study, and the agency had agreed that there is no need for conducting a thorough QT study during IND stage. Please see advice letter dated 6/18/2014 for IND 74883.

2.3.4 Is the dose and dosing regimen selected by the sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

The phase 2b study had evaluated 0.3, 1.0, and 3.0 mg doses of plecanatide and had shown that the 3.0 mg dose provided a statistically significant treatment difference compared to placebo ($p = 0.009$) while 1 mg dose did not result in a statistically significant efficacy compared to placebo ($p = 0.057$). Based on the result of this phase 2b study, the 3 mg dose was selected for phase 3 studies. In addition, a 6 mg dose level was added in phase 3 studies to evaluate whether the efficacy could be improved further while maintaining the safety profile within an acceptable range.

In the phase 3 studies, both 3 mg and 6 mg doses resulted in statistically significant improvements over placebo. However, 6 mg dose was not numerically superior to the 3 mg dose on most endpoints although the studies were not sized for a direct comparison of 3 and 6 mg doses of plecanatide.

(b) (4) the sponsor had proposed (b) (4) 3 mg (b) (4) dose levels for CIC indication without specifying a subpopulation (b) (4) (b) (4) (b) (4) The (b) (4) proposed 3 mg dose is consistent with the known dose-response relationship for both efficacy and safety.

PK characteristics of drug

Plecanatide is not orally bioavailable in humans with essentially no measurable plasma concentration of plecanatide and its major metabolite SP-338 in both healthy subjects and CIC patients.

2.5.1 What are the single dose and multiple dose PK parameters of the parent drug and its relevant metabolites in healthy subjects?

The sponsor attempted to measure plecanatide and its active metabolites SP-338 concentration in plasma in healthy subjects in two phase I studies (SAD study and food effect study). However, systemic exposures of the parent plecanatide or its active metabolite SP-338 were not detectable in human plasma up to 9 mg plecanatide dose (3 time the clinical dose of 3 mg) with LC-MS/MS bioanalytical method (with LLOQ of 1.0 ng/mL for plecanatide and 0.775 ng/mL for SP-338) suggesting insignificant absorption of plecanatide peptide and its metabolite SP-338 following the oral route of administration in healthy subjects.

SAD Study SP-SP304101-08 :

This was a phase 1, first-in-human, single-site, randomized, double-blind, placebo-controlled, single-ascending-dose study to evaluate the safety, tolerability, PK, and PD of SP-304 oral solution in healthy subjects following single dose administration. A total of 9 cohorts were evaluated in this study at dose levels of 0.1, 0.3, 0.9, 2.7, 5.4, 8.1, 16.2, 24.3, and 48.6 mg SP-304. Each cohort consisted of up to 8 subjects randomly assigned in a 3:1 allocation to receive a single dose of either SP-304 (N=6) or placebo (N=2) under fasted conditions. PK samples were collected at pre-dose and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36, and 48 hours post-dose for determination of SP-304 plasma concentration. None of the plasma samples assayed in this study had a detectable plasma level of SP-304 (LLOQ of 10.0 ng/mL).

Food effect Study SP304101-09:

This was a phase 1, single-center, single-blind, randomized, crossover, 3 sequence, single-dose study of the effect of food on the PD, PK, safety, and tolerability of 9 mg plecanatide tablets and placebo administered orally to 30 healthy adult subjects. This study consisted of 3 treatment periods and 3 treatment conditions (fasted, fed with high fat-high calories meal, and low fat-low calories meal). For each treatment period, PK blood samples were collected at pre-dose and at 0.5, 1, 2, 4, 8, 12, 24, and 48 hours post-dose to determine the plasma concentration of plecanatide and SP-338. Twenty-three of the 24 plecanatide-treated subjects had plecanatide (LLOQ of 1.0 ng/mL) plasma concentrations that were BLOQ for all time-points during all 3 treatment periods. Only one subject had measurable concentrations following treatment of plecanatide at 0.5 hour and 1 hour post-dose in the fasted state only (1.99 ng/mL at 0.5 hr and 2.18 ng/mL at 1.0 hr post dose). Plasma concentrations of metabolite SP-338 (LLOQ of 0.775 ng/mL) were BLOQ for all subjects at all time-points. Therefore, no PK parameters were estimated.

How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

The sponsor attempted to measure plecanatide and its active metabolites SP-338 concentration in the plasma in CIC (b) (6) patient population in 3 phase II studies and in one Phase III study (SP304203-03). However, systemic exposures of parent drug plecanatide or its active metabolite SP-338 were not detectable up to 9 mg dose with LC-MS/MS bioanalytical method (with LLOQ of 1.0 ng/mL for plecanatide and 0.775 ng/mL for SP-338) suggesting an insignificant absorption of plecanatide peptide and its metabolite SP-338 following the oral route of administration in CIC patients.

CIC patients:

Phase IIa study SP-SP304201-09:
This was a phase 2a, randomized, double-blind, placebo-controlled, dose ranging study with 0.3 mg, 1.0 mg, 3.0 mg and 9.0 mg SP-304 capsules dose levels with once daily dosing for 14 days in CIC patients. A total of 20 patients per dose cohort were randomized in a 3:1 ratio to SP- 304 (N=15) or placebo (N=5). PK samples were collected on Days 1 and Day14 at pre-dose and at 0.5, 1 and 2 hours post-dose to determine the plasma PK profile of plecanatide following once daily dosing of SP-304 (0.3 mg, 1.0 mg, 3.0 mg and 9.0 mg) capsule for 14 days. SP-304 plasma levels were below the BLQ (LLOQ of 10 ng/ml) at all time-points for all dose levels up to 9 mg SP-304 capsule.

Phase IIb study SP304202-10:
This was a randomized, double-blind, placebo-controlled, repeat-dose, oral, dose-ranging study with once daily dosing of 0.3 mg, 1.0 mg and 3.0 mg plecanatide capsules in 951 CIC patients with 12 weeks of treatment. Plasma samples were assayed for plecanatide concentrations at pre-dose at Weeks 4 and 12. Plecanatide concentrations in all samples assayed were below the BLQ (LLOQ of 1.0 ng/ml), and thus, standard PK parameters could not be estimated.

Phase III study SP304203-03:
This was a randomized, multicenter, double-blind, parallel-group, placebo-controlled study to assess the safety and efficacy of plecanatide 3 mg and 6 mg against placebo with once daily dosing for 12 weeks in 1410 CIC patients. Intensive PK sampling was collected in subset of 95 patients (approximately 32 patients per dose arm). Samples were collected from patients randomized to all treatments to maintain comparable trial conditions and the study blind, but only samples from patients randomized to active treatment were analyzed. Blood samples were collected at before dosing and post-dose on Day 28 (Week 4) at 0.5, 1, 2, 3, 4, 8, 12 hours post-dose, and on Day 29 at 24 hours and on Day 31 at 72 hours post-dose. If the plasma concentration of plecanatide was not quantifiable after 8 hours, the 12-, 24- and 72-hour samples were not assayed. None of the plasma samples assayed in this study had a detectable plasma level of plecanatide (LLOQ of 1.0 ng/mL) or its major metabolite SP-338 (LLOQ of 0.775 ng/mL).

2.4.3 Based on PK parameters, what is the degree of linearity (dose proportionality) or nonlinearity in the dose-concentration relationship?

The sponsor attempted to evaluate the PK profile of plecanatide over a range of doses to assess the dose linearity. However, since plecanatide plasma concentrations were all below BLOQ at all dose levels, PK linearity of plecanatide could not be assessed.

2.4.4 How do the PK parameters change with time following chronic dosing? (This may include time to steady-state; prediction of multiple dose PK from single dose PK; accumulation ratio.)

The sponosr attemdpted to evaluate the plecanatide PK following both single dose and multiple dose administration to assess its accumulation potential. However, plecanatide and its metabolites SP-338 concentrations were all below BLOQ level even after multiple dosing for 4 weeks in CIC patients.

2.4.5 What are the ADME characteristics of the drug?

2.4.5.1 What are the characteristics of drug absorption?

Bi-directional permeability of 20 μM plecanatide and its metabolite SP-338 across Caco-2 cell monolayers were evaluated in triplicate at 37 °C. The permeability of plecanatide and SP-338 across Caco-2 cell monolayer were low where Papp was 0.13 $x10^{-6}$ cm/s for plecanatide and 0.0456 x 10^{-6} cm/s for SP-338. In addition, the efflux ratio of plecanatide and its metabolite SP-338 across Caco-2 were less than 2 suggesting that plecanatide and SP-338 are not a substrates for P-gp and BCRP transporters.

2.4.5.2 What are the characteristics of drug distribution?

Plecanatide do not have detectable amount of drug in the plasma. Therefore, protein binding of plecanatide is not a concern. Nonetheless, plecanatide did not have a significant binding to major human plasma proteins human serum albumin (HSA) and human α1-acid glycoprotein (AGP) proteins.

Study RSN00008 : Binding of plecanatide to human serum albumin (HSA) and human α1-acid glycoprotein (AGP) proteins was evaluated using a fluorescence probe displacement method. A total of 5 assays for binding to HSA (human serum albumin) or AGP (α1-acid glycoprotein) were conducted for the test and control compounds. Interactions of the compounds for other binding proteins, such as lipoproteins or low abundance specific serum binding proteins, are not assessed by these assays.

Binding site	Probe/measurement	Positive control
HSA site IIA	Dansyl amide (DA) probe	Iophenoxate
HSA site IIIA	Dansyl sarcosine (DS) probe	Iophenoxate
HSA in general	Quenching of intrinsic HSA trp fluorescence (QIF)	Iophenoxate
AGP site I	Quinaldine red (QR) probe	Chlorpromazine
AGP in general	Quenching of intrinsic AGP trp fluorescence (QIF)	Chlorpromazine

The test compound and control compounds were tested at concentrations ranging from 0-125μM. DMSO was used as the negative vehicle control for the assays.

Table 7: Protein binding of plecanatide

	HSA/Site IIA		HSA/Site IIIA		HSA/QIF		AGP/Site I		AGP/QIF	
Compound	Kd (μM)	% bound @10 uM	Kd (μM)	% bound @10 uM	Kd (μM)	% bound @10 uM	Kd (μM)	% bound @10 uM	Kd (μM)	% bound @10 uM
SP-304	NB	NB	195	75.3	NB	VB	NB	NB	NB	NB
Iophenoxate	6.8	98.9	13.2	97.8	2.7	99.5	223	9.9	NB	NB
Chlorpromazine	NB	NB	NB	NB	180	76.7	3.1	84.9	19.6	52.3

The test compound, SP-304, did not demonstrate significant binding to HSA, and no binding to AGP was detected using these fluorescent assay methods. The control compounds, chlorpromazine and iophenoxate, demonstrated expected protein binding behavior.

Reviewer's Comment:

- *This study did not use the commonly utilized methodology of equilibrium dialysis approach to evaluate the potential binding of SP-304 to human plasma protein. Since plecanatide has almost no detectable level in human plasma with LOQ of 1 ng/mL, this does not raise a major review issue.*
- *Only two major plasma proteins HAS and AGP were evaluated for potential binding in this study instead of total plasma protein. As these two protein account for more than 60% of the plasma protein in human plasma and plecanatide has almost no detectable level in human plasma, this does not raise a review issue.*
- *The evaluated concentration of 10 μM for % protein binding was reasonable as plecanatide had almost no detectable level in plasma.*

2.4.5.3 What are the characteristics of *in-vitro* drug metabolism?

In the simulated intestinal fluid (SIF), plecanatide is rapidly hydrolyzed at its C-terminus by cleavage of the Leu16 resulting in generation of a biologically active metabolite SP-338. SP-338 is further processed by an internal cleavage at Leu6-Cys7 resulting in a biologically inactive Leu6-clipped SP-338. However, potential metabolites of SP-338 were not quantitated. The potential metabolism by CYP enzymes in human intestinal microsomes was not evaluated.

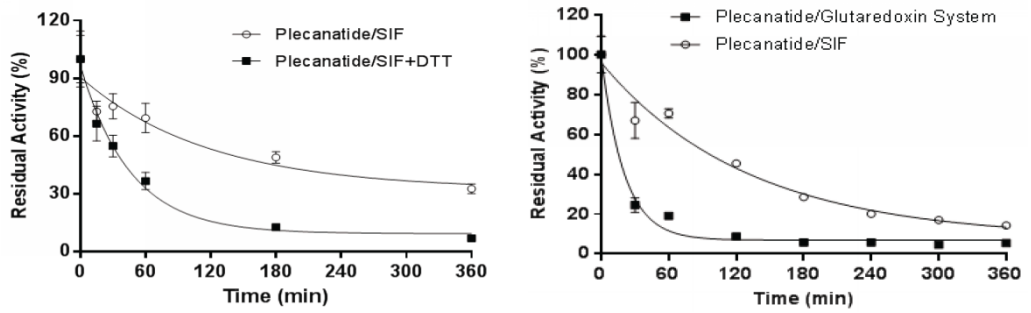
Study SP-PH-015:

The stability of plecanatide was evaluated in simulated intestinal fluid (SIF) in the presence and absence of a reducing agent, dithiothreitol (DTT) and a reducing power regenerating system, glutaredoxin. Plecanatide was incubated in SIF, SIF plus 1mM DTT at 2 mM concentration and with glutaredoxin system at 1mM concentration for 6 hours at room temperature. After the incubation time, iodoacetamide (IAA) was added to the samples to alkylate reduced cysteine residues. Plecanatide stability was evaluated at the end of 6-hours of incubation time by assessing the residual biological activity of plecanatide which was assessed by testing for their ability to stimulate cyclic guanosine monophosphate (cGMP) synthesis in T84 human colon carcinoma cells and was expressed a percentage of residual activity ± S.D as compared to vehicle control (considered as 100%). Half-life of the peptide(s) for each treatment was calculated from non-linear fit plots of time versus residual activity.

Table 8: Plecanatide biological activity half-life in SIF in the Presence and Absence of Dithiothreitol and in Glutaredoxin System

	SIF	SIF + DTT	Glutaredoxin System
Half-life of biological activity	81.6-90.6 min	31.8 min	14 min

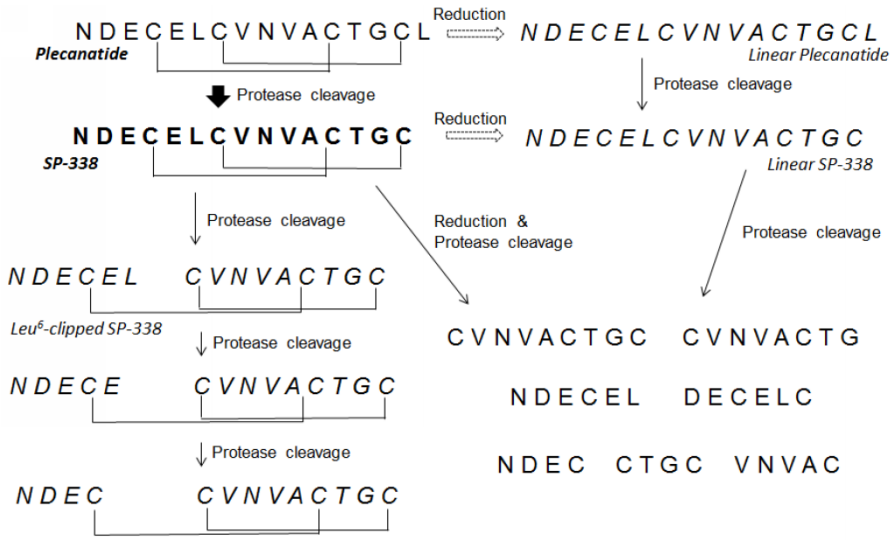
Figure 4: Time Dependent Loss of Biological Activity of Plecanatide Following Treatment with SIF and SIF Containing 1 mM DTT and Glutaredoxin System



In addition to evaluating for biological activity, the samples of plecanatide collected over the course of treatment with SIF in the presence and absence of 1 mM DTT were also analyzed by LC-MS to detect and identify potential metabolites of plecanatide degradation. An untreated sample (vehicle control) comprised of 1 mM plecanatide incubated in SIF without pancreatin and DTT in the presence of 100 mM IAA was included as controls.

LC-MS analysis suggests that plecanatide is rapidly hydrolyzed in SIF at its C-terminus by cleavage of the Leu16 resulting in generation of a biologically active metabolite SP-338. SP-338 is further processed by an internal cleavage at Leu6-Cys7 resulting in Leu6-clipped SP-338. The two peptide chains of this putative metabolite are held together by disulfide linkages. Study SP-pH-011 had shown that while the metabolite SP-338 had biological cGMP stimulation activity with slightly lower potency compared to plecanatide, Leu6-clipped SP-338 is biologically inactive as it failed to produce measurable levels of cGMP in T84 cell bioassay. Leu6-clipped SP-338 is presumably further processed by carboxypeptidase, which trims the alpha chain removing the Leu6 and Glu5-Leu6. These metabolites are likely to be inactive as they are derivatives of Leu6-clipped SP-338.
In presence of a reducing agent (1 mM DTT) in SIF, several peptide fragments of 4-9 amino acids were identified suggesting complete reduction and proteolytic degradation of the parent plecanatide or its active metabolite SP-338. A series of low molecular weight peaks, expected to be fragments consisting of 2-4 amino acids produced as a result of proteolytic digestion, were also observed.

Figure 5: Summary of Putative Degradation Pattern of Plecanatide in SIF, SIF+DTT



Study SP-PH-018:
Plecanatide was incubated up to 2 hours with human intestinal fluid from duodenal, jejunal and ileal, which was collected from a female cadaver within 8 hours of post-mortem. Plecanatide stability was assessed by monitoring its ability to stimulate cGMP activity in T84-cell bioassay. There was no appreciable reduction in biological activity of plecanatide following incubation with human cadaver intestinal fluids, suggesting lack of proteolytic (peptidases) and reduction capability in the obtained cadaver intestinal fluids. However, the earlier study SP-PH-015 has demonstrated a steady loss of activity in simulated intestinal fluid in the presence or absence of reducing agents.

2.6 Intrinsic Factors

2.6.1 What intrinsic factors influence exposure (PK of parent and/or relevant metabolites) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Pediatric:
No studies were conducted in pediatric patients. The sponsor requested a waiver for birth to (b) (4) (b) (4) and deferral for 6 month to (b) (4) years of age.

Hepatic impairment:
No dedicated hepatic impairment study was conducted in this submission. As plecanatide has no appreciable amount of absorption in plasma, the lack of hepatic impairment study is acceptable.

Renal impairment
No dedicated renal impairment study was conducted in this submission. As plecanatide has no appreciable amount of absorption in plasma, the lack of renal impairment study is acceptable.

2.7 Extrinsic Factors

2.7.1 Drug-Drug Interactions

2.7.1.1 Is the drug inhibitor and/or an inducer of CYP enzymes? Were relevant metabolites evaluated for inhibitor or induction potential, *in-vitro*?

As the parent drug SP-304 and its metabolite SP-338 have limited systemic exposure, only CYP enzymes that are expressed in gastrointestinal tract, CYP2C9 and CYP3A, were evaluated for potential inhibition and induction by the parent drug SP-304 and its metabolite SP-338. Based on the in-vitro studies, plecanatide and its major metabolite SP-338 do not significantly inhibit CYP2C9 and CYP3A4 at 5 µM concentration or induce CYP3A4 at up to 3 µM concentration.

Parent Drug Plecanatide SP-304 (Study 13SYNRP1B)

Inhibition:

Human liver microsomal (0.25 mg protein/mL) from a pool of 11 individual was incubated with corresponding selective CYP model substrates (at approximately Km) in presence and absence (negative control) of the 5 µM test compound SP-304 in duplicate at 37°C. The reaction mixture was equilibrated in a shaking water bath at 37ºC for 5 minutes. The reaction was initiated by the addition of NADPH, followed by incubation at 37ºC for 10 (CYP3A) or 20 (CYP2C9) minutes. The reaction was terminated by the addition of two volumes of ice-cold acetonitrile (ACN) containing an internal standard (IS, deuterium-labeled CYP probe metabolite). Positive controls were performed in parallel using known positive inhibitors. The inhibitory effects of test compound on the metabolism of CYP-specific probe substrates were determined by comparing the rate of metabolite formation in the absence and presence of different concentrations of test compound.

Table 9: Incubation Concentrations of Probe Substrates and Positive Controls

CYP	Model Substrate	Metabolites	Positive control
2C9	Diclofenac (6 uM)	4'-hydroxydiclofenac	Sulphaphenazole (5.0 uM)
3A4	Testosterone (75 uM)	6β-hydroxytestosterone	Ketoconazole (1.0 uM)
3A4	Midazolam (1.4 uM)	1'-hydroxymidazolam	Ketoconazole (1.0 uM)

% Inhibition = 100 × (Enzyme activity in the absence of the inhibitor – Enzyme activity in the presence of the inhibitor)/Enzyme activity in the absence of the inhibitor.

Table 10: Inhibition of CYP Activities in HLM by 5 µM Plecanatide (SP-304)

CYP	Probe Substrate	Metabolite	% Inhibition (n=2)[a]
CYP2C9	Diclofenac	4'-OH diclofenac	0 (-4.84)
CYP3A	Testosterone	6β-OH testosterone	0 (-5.44)
	Midazolam	1'-OH midazolam	6.43

[a] Negative values of % inhibition were treated as zero.

Table 11: Inhibition of CYP Activities in HLM by Positive Inhibitors

CYP	**Positive Inhibitor**	**Inhibitor Concentration**	**% Inhibition (n=2)[a]**
CYP2C9	Sulfaphenazole	5 µM	86.8
CYP3A Testosterone)	Ketoconazole	1 µM	96.5
CYP3A (Midazolam)	Ketoconazole	1 µM	89.7

[a] QC acceptance criterion: ≥50% inhibition by positive inhibitor.

Reviewer's Comment:

- *As SP-304 does not have measurable concentration in plasma with LOQ of 1.0 ng/mL, only evaluating the inhibition of CYPs that are expressed in gut (CYP3A and CYP2C9) was appropriate.*
- *The choices of CYP-specific model substrates and their respective concentrations to evaluate the inhibitory potential of test compound on each CYP isoforms were acceptable.*
- *Choices of model inhibitors as positive controls appear to be reasonable. The positive controls had expected level of inhibition to demonstrate the appropriateness of the test system.*
- *Inhibition potential of CYP3A by plecanatide was only evaluated at one concentration and thus Ki was not estimated in this study. The tested concentration plecanatide at 5 µM is lower than the highest potential concentration of plecanatide in gut in CIC patients taking 3 mg tablet.*
    - *Igut= dose/250 mg=3 mg/1685 mg/mol/250 mL = 7.13 µM*
- *Plecanatide (SP-304) at 5 µM did not inhibit CYP3A and CYP2C9. Therefore, Ki >5 µM for both CYP3A and CYP2C9. Since R= 1+I_{gut}/Ki = 1+7.13µM/5µM = 2.4 < 11, likelihood of plecanatide to inhibit CYP3A or CYP2C9 in gut are less likely.*

Induction:

Fresh human hepatocytes (from 3 donors) were treated once daily for three consecutive days with test compound SP-304 at final concentrations of 0.03, 0.3, and 3 µM (~0.1%, 1%, and 10% of [I]2 from 9 mg dose/250 mL) at 37°C. Positive controls with induction medium spiked with rifampicin (RIF) at 50 µM for CYP3A and vehicle controls were treated in parallel. All experiments were conducted in triplicate (n=3).

After the treatment, the activity of target enzyme CYP3A4 was assessed by incubating the hepatocytes with model probe substrate midazolam 20 µM (probe substrate for CYP3A4/5) and measuring the appearance rate of their respective metabolites 1'-Hydroxymidazolam by LC/MS/MS. The level of mRNA was also quantified from the treated cells to evaluate the effect of SP-304 on CYP3A mRNA levels. Specifically, total RNA was isolated from the treated cells using the RNeasy mini kit and treated with RNase-free DNase. The concentration of RNA was determined using a Quibit Fluorometer with a Quan-It RNA assay kit. cDNA was synthesized from up to 1 µg of the total RNA harvested from the cells using a Quantitect RT kit (Qiagen). Analysis of gene expression by qPCR was performed using the LightCycler 480 System

Table 12: Induction of CYP3A Enzyme Activity by Plecanatide (SP-304)

Donor	Compound	Treatment	1'-OH Midazolam Formation (pmol/well/min, n=3)[a]		Fold-Induction[b]	% of RIF-Treated Cells[c]
			Mean	SD		
1	Control	Vehicle	1.19	0.44	1.0	0
		RIF	14.1	2.2	12	100
	Plecanatide (SP-304)	0.03 µM	1.31	0.19	1.1	0.94
		0.3 µM	1.35	0.14	1.1	1.3
		3 µM	1.76	0.38	1.5	4.5
2	Control	Vehicle	1.75	0.15	1.0	0
		RIF	5.04	0.89	2.9	100
	Plecanatide (SP-304)	0.03 µM	1.24	0.43	0.71	-15
		0.3 µM	1.16	0.63	0.66	-18
		3 µM	1.36	0.44	0.78	-12
3	Control	Vehicle	0.582	0.03	1.0	0
		RIF	3.60	0.40	6.2	100
	Plecanatide (SP-304)	0.03 µM	0.607	0.03	1.0	0.83
		0.3 µM	0.599	0.03	1.0	0.54
		3 µM	0.582	0.05	1.0	-0.02

[a] Data were expressed as the mean ± SD from three individual incubations and measurements (n=3).
[b] Fold-induction was calculated from the ratio of the normalized enzyme activity (formation rate of the probe substrate metabolite) of the test compound or the positive control to that of the vehicle control.
[c] Percentage of enzyme activity relative to the positive control. Negative values were treated as zero.

Table 13: Induction of CYP3A4 mRNA by Plecanatide (SP-304)

Donor	Compound	Treatment	Fold-Induction[a] (n=3)	
			Mean	SD
1	Control	Vehicle	1.0	0.02
		RIF	74	4.1
	Plecanatide (SP-304)	0.03 µM	1.2	0.04
		0.3 µM	1.1	0.05
		3 µM	1.4	0.21
2	Control	Vehicle	1.0	0.07
		RIF	13	0.36
	Plecanatide (SP-304)	0.03 µM	0.94	0.10
		0.3 µM	0.94	0.03
		3 µM	0.85	0.21
3	Control	Vehicle	1.0	0.05
		RIF	85	2.25
	Plecanatide (SP-304)	0.03 µM	0.81	0.02
		0.3 µM	0.74	0.03
		3 µM	0.97	0.07

[a] Fold-induction was calculated from the normalized mRNA level ($2^{-\Delta\Delta Ct}$) of the test compound or the positive control relative to that of the vehicle control from triplicate measurements.

Reviewer's Comment:

- *The choice for CYP for evaluation for induction, positive controls (model inducers) and CYP-specific model substrate to evaluate the CYP enzyme activities were appropriate.*
- *The test conditions were appropriate for measuring the target enzyme CYP3A4 activities as the treatment of human hepatocytes with positive controls CYP3A inducer rifampin caused anticipated and appropriate in CYP activity and mRNA levels.*
- *Parent drug SP-304 up to 3 µM concentration did not induce CYP3A activities as the percent of change in target enzyme activity is not greater than 40% of the positive control with a known inducer. It also did not induce 3A4 mRNA.*
- *The tested concentration of 0.03, 0.3, and 3 µM for SP-304 are lower than the highest potential concentration of plecanatide in gut in CIC patients taking 3 mg tablet (I_{gut}=*

dose/250 mL=3 mg / 1685 mg/mol /250 mL=7.13 µM). Nonetheless, as there is no concentration dependent increase in induction potential of CYP3A by SP-304 at 0.03, 0.3 and 3 µM, the likelihood of SP-304 to inhibit CYP3A at 7.13µM is remote.

Metabolite SP-338 (Study 13SYNRP6B)

Inhibition:
Pooled human liver microsomal (HLM) from a pool of 200 individual (0.25 mg protein/mL) was incubated with corresponding selective CYP model substrates (at approximately Km) in presence and absence (negative control) of the 5 µM test compound SP-338 in duplicate at 37ºC. The reaction mixture was equilibrated in a shaking water bath at 37ºC for 5 minutes. The reaction was initiated by the addition of NADPH, followed by incubation at 37ºC for 10 (CYP3A) or 20 (CYP2C9) minutes. The reaction was terminated by the addition of two volumes of ice-cold acetonitrile (ACN) containing an internal standard (IS, deuterium-labeled CYP probe metabolite). Positive controls were performed in parallel using known positive inhibitors. The inhibitory effects of test compound on the metabolism of CYP-specific probe substrates were determined by comparing the rate of metabolite formation in the absence and presence of different concentrations of test compound.

Table 14: Incubation Concentrations of Probe Substrates and Positive Controls

CYP	Model Substrate	Metabolites	Positive control
2C9	Diclofenac (10 uM)	4'-hydroxydiclofenac	Sulphaphenazole (5.0 µM)
3A4	Testosterone (55 uM)	6β-hydroxytestosterone	Ketoconazole (1.0 µM)
3A4	Midazolam (2.5 uM)	1'-hydroxymidazolam	Ketoconazole (1.0 µM)

% Inhibition = 100 × (Enzyme activity in the absence of the inhibitor – Enzyme activity in the presence of the inhibitor)/Enzyme activity in the absence of the inhibitor.

Table 15: Inhibition of CYP Activities in HLM by SP-338

CYP	Probe Substrate	Metabolite	% Inhibition (n=2)
CYP2C9	Diclofenac	4'-OH diclofenac	16
CYP3A	Testosterone	6β-OH testosterone	1.1
	Midazolam	1'-OH midazolam	6.3

Table 16: Inhibition of CYP Activities in HLM by Positive Inhibitors

CYP	Positive Inhibitor	Inhibitor Concentration	% Inhibition (n=2)[a]
CYP2C9	Sulfaphenazole	5 µM	84
CYP3A (Testosterone)	Ketoconazole	1 µM	97
CYP3A (Midazolam)	Ketoconazole	1 µM	87

[a] QC acceptance criterion: ≥50% inhibition by positive inhibitor.

Reviewer's Comment:

- *The choices of CYP-specific model substrates and their respective concentrations to evaluate the inhibitory potential test compound on each CYP isoforms were acceptable.*
- *Choices of model inhibitors as positive controls appear to be reasonable. The positive controls had expected level of inhibition to demonstrate the appropriateness of the test system.*
- *Metabolite SP-3338 at 5 µM did not inhibit CYP3A and CYP2C9.*

- *The tested concentration metabolite SP-338 at 5 µM is reasonable as the actual concentration of SP-338 in gut is unknown and the tested concentration of 5 µM is close to the highest potential concentration of parent drug plecanatide in gut in CIC patients taking 3 mg tablet (Igut= 7.13 µM).*

Induction:

Fresh human hepatocytes (from 3 donors) were treated once daily for three consecutive days with test compound SP-304 at final concentrations of 0.03, 0.3, and 3 µM at 37oC. Positive controls with induction medium spiked with rifampicin (RIF) at 50 µM for CYP3A and vehicle controls were treated in parallel. All experiments were conducted in triplicate (n=3).

After treatment, the activity of target enzyme CYP3A4 was assessed by incubating the hepatocytes with model probe substrate midazolam 20 µM(probe substrate for CYP3A4/5) and measuring the appearance rate of their respective metabolites 1'-Hydroxymidazolam by LC/MS/MS. Level of mRNA was also quantified from the treated cells to evaluate the effect of SP-3338 on CYP3A mRNA levels. Specifically, total RNA was isolated from the treated cells using the RNeasy mini kit and treated with RNase-free DNase. The concentration of RNA was determined using a Quibit Fluorometer with a Quan-It RNA assay kit. cDNA was synthesized from up to 1 µg of the total RNA harvested from the cells using a Quantitect RT kit (Qiagen). Analysis of gene expression by qPCR was performed using the LightCycler 480 System.

Table 17: Induction of CYP3A Enzyme Activity by SP-338

Donor	Compound	Treatment	1'-OH Midazolam Formation (pmol/well/min, n=3)		Fold-Induction[a]	% of RIF-Treated Cells[b]
			Mean	SD		
1	Control	Vehicle	1.10	0.10	1.0	0
		RIF	11.7	0.61	11	100
	SP-338	0.03 µM	0.992	0.03	0.90	0
		0.3 µM	0.993	0.02	0.90	0
		3 µM	1.00	0.09	0.91	0
2	Control	Vehicle	0.934	0.35	1.0	0
		RIF	4.07	1.8	4.4	100
	SP-338	0.03 µM	0.639	0.16	0.68	0
		0.3 µM	0.622	0.14	0.67	0
		3 µM	0.683	0.18	0.73	0
3	Control	Vehicle	3.71	0.23	1.0	0
		RIF	13.3	0.62	3.6	100
	SP-338	0.03 µM	3.33	0.10	0.90	0
		0.3 µM	2.72	0.10	0.73	0
		3 µM	2.69	0.16	0.72	0

[a] Fold-induction was calculated from the ratio of the normalized enzyme activity (formation rate of the probe substrate metabolite) of the test article or the positive control to that of the vehicle control.
[b] Percentage of enzyme activity relative to the positive control. Negative values are treated as zero.

Table 18: Induction of CYP3A4 mRNA by SP-338

Donor	Compound	Treatment	Fold-Induction[a] (n=3)		% of RIF-Treated Cells[b]
			Mean	SD	
1	Control	Vehicle	1.0	0.05	0
		RIF	36	2.8	100
	SP-338	0.03 µM	1.0	0.24	0
		0.3 µM	1.1	0.07	0.3
		3 µM	0.75	0.10	0
2	Control	Vehicle	1.0	0.15	0
		RIF	8.9	0.77	100
	SP-338	0.03 µM	0.36	0.06	0
		0.3 µM	0.41	0.04	0
		3 µM	0.46	0.03	0
3	Control	Vehicle	1.0	0.03	0
		RIF	6.6	0.08	100
	SP-338	0.03 µM	0.67	0.04	0
		0.3 µM	0.75	0.02	0
		3 µM	0.71	0.03	0

[a] Fold-induction was calculated from the normalized mRNA level ($2^{-\Delta\Delta Ct}$) of the test article or the positive control relative to that of the vehicle control from triplicate measurements.
[b] Percentage of mRNA fold-induction relative to the positive control. Negative values are treated as zero.

Reviewer's Comment:

- *The choice for CYP for evaluation for induction, positive controls (model inducers) and CYP-specific model substrate to evaluate the CYP enzyme activities were appropriate.*
- *The test conditions were appropriate for measuring the target enzyme CYP3A4 activities as the treatment of human hepatocytes with positive controls CYP3A inducer Rifampin caused anticipated and appropriate in CYP activity and mRNA levels.*
- *Metabolite SP-338 up to 3 µM concentration did not induce CYP3A activities as the percent of change in target enzyme activity is not greater than 40% of the positive control with known inducer. It also did not induce 3A4 mRNA.*
- *The tested concentration of 0.03, 0.3, and 3 µM for SP-338 are reasonable as the actual concentration of SP-338 in gut is unknown and the tested concentration of metabolite up to 3 µMis close to the highest potential concentration of parent drug plecanatide in gut in CIC patients taking 3 mg tablet (Igut= 7.13 µM).*

2.7.1.2 Are the drug and relevant metabolites substrates and/or an inhibitors of transport processes?

As both parent drug and tis metabolite SP-338 have limited systemic exposure, only transporter that are expressed in gastrointestinal tracts, P-gp and BCRP, were evaluated for potential interaction with plecanatide and its metabolite Sp-338. Based on the in-vitro study in Caco-2 cell, both parent drug plecanatide SP-304 and its active metabolite SP-338 are not substrates or inhibitors of gut transporters P-gp and BCRP.

Parent Drug plecanatide SP-344 (Study 13SYNRP1A)

Potential of plecanatide for being a substrate and/or inhibitor for P-go or BCRP was evaluated in Caco-2 cell monolayer. Prior to each assay, Caco-2 cells were certified by evaluating permeability of control compound and measuring transepithelial electrical resistance (TEER) to ensure the integrity of the cell monolayer. .

Substrate:

Potential of plecanatide (SP-304) being a substrate of P-gp and /or BCRP was assessed by evaluating the bi-directional permeability of 20 µMplecanatide across Caco-2 cells (apical-to-basolateral (AP-to- BL) and basolateral-to-apical (BL-to-AP)) in triplicate at 37 ± 1 °C. For the dosing solution, plecanatide was co-dosed with 200 µM lucifer yellow (LY) to determine the monolayer integrity.

Table 19: Experimental conditions to evaluate the substrate potential of plecanatide

	P-gp	BCRP
Test-System	Caco-2	Caco-2
[plecanatide] (µM)	20	20
Permeability control	Lucifer yellow (200 µM)	Lucifer yellow (200 µM)
Test article sampling time (min)	D: 5, 120 R: 60, 120	D: 5, 120 R: 60, 120
Analysis method	LC-MS/MS	LC-MS/MS

A - Apical B-Basal D-Donor R-Receiver

The permeability of lucifer yellow was measured (apical to basolateral) after the incubation to determine integrity of the monolayer.

Table 20: Permeability and Recovery of Plecanatide in Caco-2 Cells (P-gp and BCRP Substrate Assessment)

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio
		P_{app} ($x10^{-6}$ cm/s)[a]	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)[a]	Recovery (%)	
20 µM Plecanatide	Replicate 1	0.160	78.3	0.247[b]	74.6[b]	1.0
	Replicate 2	0.0972	84.2	0.147	86.1	
	Replicate 3	0.133	102	0.112	85.1	
	Average	0.130	88.2	0.130	85.6	
	S.D.	0.0313	12.5	N.D.[a]	N.D.[a]	

[a]: Data were calculated using concentrations of plecanatide only as SP-338 was not detectable.
[b]: Data were not included for calculation of the average of the P_{app} value and the recovery as the corresponding monolayer failed the integrity test (see table 5.12.). Only those concentrations obtained from cell monolayers which passed the LY criterion were included for the average P_{app} calculation.
[c] ND: Not Determined.

Table 21: Monolayer Integrity of Caco-2 Cells (P-gp and BCRP Substrate Assessment)

Treatment	Replicates	AP-to-BL		BL-to-AP	
		P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
20 µM Plecanatide	Replicate 1	0.79	Pass	0.84	Fail
	Replicate 2	0.28	Pass	0.71	Pass
	Replicate 3	0.58	Pass	0.72	Pass

[a] Passing criteria: P_{app} of LY ≤ 0.8×10^{-6} cm/s.

Reviewer's Comment:

- *The selected concentration of 20 µM was acceptable as it is higher than the highest potential plecanatide concentration in gut (Igut) with clinical dose of 3 mg (Igut= dose/250 mg=3 mg/1682 mg/mol/250 mL = 7.13 µM).*
- *The test to evaluate the potential of plecanatide being substrate for P-gp and BCRP did not include positive and negative controls to validate the test systems for presence of P-gp and BCRP transporters. However, since the Caco-2 cell test system that evaluated the potential*

of plecanatide being inhibitor of efflux transporters had positive controls to validate the system.

- *Based on the efflux ratio of 1, plecanatide does not appear to be substrate for P-gp nor BCRP. Permeability of plecanatide across Caco-2 cells in both directions were less than 1X10^{-6} cm/s suggesting very low permeability.*

Inhibition:

Inhibition potential of P-gp and BCRP by plecanatide was evaluated by measuring the bi-directional permeability of model substrates (digoxin for P-gp and cladribine for BCRP) across Caco-2 cell monolayers in presence and absence of 2 µM plecanatide in triplicated at 37 ± 1 °C

Table 22: Experimental conditions to evaluate the inhibition potential of plecanatide

	P-gp	BCRP
Test-System	Caco-2	Caco-2
Plecanatide concentration	2 µM	2 µM
Positive control substrate	Digoxin (10 µM)	Cladribine (10 µM)
Positive control inhibitor 1	Valspodar (1 µM)	Ko143 (10 µM)
Permeability control	Lucifer yellow (200 µM)	Lucifer yellow (200 µM)
Sampling time (min)	D: 5, 120; R: 120	D: 5, 120; R: 120
Analysis method	LC-MS/MS	LC-MS/MS

A- Apical B- Basal D-Donor R-Receiver

The permeability of lucifer yellow was measured (apical to basolateral) after the incubation to determine integrity of the monolayer.

Table 23: Permeability and Recovery of Digoxin in Caco-2 Cells (P-gp Inhibitor Assessment)

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio	% of Inhibition
		P_{app} (x10^{-6} cm/s)	Recovery (%)	P_{app} (x10^{-6} cm/s)	Recovery (%)		
10 µM Digoxin	**Replicate 1**	0.978	91.2	10.8	85.7	13	N.A.[a]
	Replicate 2	0.806	83.5	12.5	84.2		
	Replicate 3	0.890	92.9	12.3	96.3		
	Average	0.891	89.2	11.9	88.7		
	S.D.	0.0862	4.97	0.888	6.57		
10 µM Digoxin + 1 µM Valspodar	**Replicate 1**	4.950	85.8	4.47	77.2	1.0	100
	Replicate 2	3.781	96.1	4.23	90.1		
	Replicate 3	4.373	88.5	4.63[b]	101[b]		
	Average	4.368	90.1	4.35	83.6		
	S.D.	0.5847	5.31	N.D.[c]	N.D.[c]		
10 µM Digoxin + 2 µM Plecanatide	**Replicate 1**	1.228	78.1	19.4	86.3	18	-39.6
	Replicate 2	0.735	88.5	11.5	81.2		
	Replicate 3	0.839	85.5	20.1	89.0		
	Average	0.934	84.0	17.0	85.5		
	S.D.	0.2598	5.37	4.77	3.96		

[a] N.A.: not applicable.

[b]: Data were not included for calculation of the average of the P_{app} value and the recovery as the corresponding monolayer failed the integrity test (see table 5.16.). Only those concentrations obtained from cell monolayers which passed the LY criterion were included for the average P_{app} calculation.

[c] N.D.: not determined.

Table 24: Monolayer Integrity of Caco-2 Cells (P-gp Inhibitor Assessment)

Treatment	AP-to-BL		BL-to-AP	
	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
10 µM Digoxin	0.6	Pass	0.7	Pass
	0.5	Pass	0.5	Pass
	0.6	Pass	0.5	Pass
10 µM Digoxin + 1 µM Valspodar	0.5	Pass	0.5	Pass
	0.4	Pass	0.5	Pass
	0.3	Pass	1.0	Fail
10 µM Digoxin + 2 µM Plecanatide	0.6	Pass	0.6	Pass
	0.4	Pass	0.7	Pass
	0.4	Pass	0.6	Pass

[a] Passing criteria: P_{app} of LY $\leq 0.8 \times 10^{-6}$ cm/s.

Table 25: Permeability and Recovery of Cladribine in Caco-2 Cells (BCRP Inhibitor Assessment)

Treatment	Replicates	AP-to-BL		BL-to-AP		Efflux Ratio	% of Inhibition
		P_{app} ($x10^{-6}$ cm/s)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Recovery (%)		
10 µM Cladribine	**Replicate 1**	1.47	100	19.9	97.8	20	N.A.[a]
	Replicate 2	0.850	98.9	21.7	103		
	Replicate 3	0.783	106	19.6	104		
	Average	1.03	102	20.4	102		
	S.D.	0.379	4.09	1.14	3.33		
10 µM Cladribine + 10 µM Ko143	**Replicate 1**	1.38	101	1.88	98.7	1.1	99.5
	Replicate 2	2.57	102	1.98	106		
	Replicate 3	1.73	102	2.39	101		
	Average	1.89	102	2.08	102		
	S.D.	0.609	0.579	0.274	3.80		
10 µM Cladribine + 2 µM Plecanatide	**Replicate 1**	1.20	96.4	25.2	101	21	-9.48
	Replicate 2	0.850	101	22.6	106		
	Replicate 3	1.23	106	22.8	103		
	Average	1.09	101	23.5	103		
	S.D.	0.211	4.81	1.46	2.75		

[a] N.A.: not applicable.

Table 26: Monolayer Integrity of Caco-2 Cells (BCRP Inhibitor Assessment)

Treatment	Replicates	AP-to-BL		BL-to-AP	
		P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
10 µM Cladribine	**Replicate 1**	0.2	Pass	0.4	Pass
	Replicate 2	0.2	Pass	0.4	Pass
	Replicate 3	0.2	Pass	0.4	Pass
10 µM Cladribine + 10 µM Ko143	**Replicate 1**	0.2	Pass	0.2	Pass
	Replicate 2	0.3	Pass	0.2	Pass
	Replicate 3	0.3	Pass	0.3	Pass
10 µM Cladribine + 2 µM Plecanatide	**Replicate 1**	0.3	Pass	0.5	Pass
	Replicate 2	0.2	Pass	0.4	Pass
	Replicate 3	0.2	Pass	0.3	Pass

[a] Passing criteria: P_{app} of LY $\leq 0.8 \times 10^{-6}$ cm/s.

Reviewer's Comment:

- *All The test systems to evaluate the inhibition potential was appropriately validated with positive controls with model substrates and known model inhibitors. Model substrates has significant efflux ratio > 2 and were significantly inhibited in presence of model inhibitors (positive controls).*
- *Inhibition of P-gp and BCRP transporters was assessed at 2 µM. The test concentration for the inhibitor assessment was selected based on 0.1x[I]2. Plecanatide (SP-304) at 2 µM did not inhibit P-gp and BCRP. Therefore, Ki >2 µM for both P-gp and BCRP. Since R= $1+I_{gut}/Ki$ = 1+7.13uM/2µM = 4.5 < 11, likelihood of plecanatide to inhibit P-gp and BCRP in gut are less likely.*

Metabolite SP-338 (Study 13SYNRP6A)

Potential of plecanatide for being a substrate and/or inhibitor for P-go or BCRP was evaluated in Caco-2 cell monolayer. Prior to each assay, Caco-2 cells were certified by evaluating permeability of control compound and measuring transepithelial electrical resistance (TEER) to ensure the integrity of the cell monolayer. .

Substrate:

Potential of SP-338 being a substrate of P-gp and /or BCRP was assessed by evaluating the bi-directional permeability of 0.5, 2 and 10 µM SP-338 across Caco-2 cells (apical-to-basolateral [AP-to- BL] and basolateral-to-apical [BL-to-AP]) in triplicate at 37 ± 1 °C. For the dosing solution, SP-338 was co-dosed with 200 µM lucifer yellow (LY) to determine the monolayer integrity.

Table 27: Experimental conditions to evaluate the substrate potential of metabolite SP-338

	P-gp	BCRP
Test-System	Caco-2	Caco-2
[SP-338] (µM)	0.5, 2 and 10 µM	0.5, 2 and 10 µM
Permeability control	Lucifer yellow (200 µM)	Lucifer yellow (200 µM)
Test article sampling time (min)	D: 5, 120 R: 60, 120	D: 5, 120 R: 60, 120
Analysis method	LC-MS/MS	LC-MS/MS

A - Apical B-Basal D-Donor R-Receiver

The permeability of lucifer yellow was measured (apical to basolateral) after the incubation to determine integrity of the monolayer.

Table 28: Permeability and Recovery of SP-338 in Caco-2 Cells (P-gp and BCRP Substrate Assessment)

Treatment	Replicates	A-to-B		B-to-A		Efflux Ratio
		P_{app} ($x10^{-6}$ cm/s)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Recovery (%)	
0.5 µM SP-338	1	N.D.[a]	94.0[d]	N.D.[a]	92.5[d]	N.A.[b]
	2	N.D.[a]	97.8[d]	N.D[a]	93.5[d]	
	3	N.D.[a]	86.8[d]	N.D.[a]	99.1 [c,d]	
	Average	N.A.[b]	92.9	N.A.[b]	93.0	
	S.D.	N.A.[b]	5.59	N.A.[b]	N.A.[b]	
2 µM SP-338	1	N.D.[a]	82.0[d]	N.D.[a]	86.5[d]	N.A.[b]
	2	N.D.[a]	77.5[d]	N.D.[a]	81.0[d]	
	3	N.D.[a]	92.5[d]	N.D.[a]	81.5[d]	
	Average	N.A.[b]	84.0	N.A.[b]	83.0	
	S.D.	N.A.[b]	7.70	N.A.[b]	3.04	
10 µM SP-338	1	0.0225[e]	87.1[e]	0.0625	89.8	1.05
	2	0.0319	91.5	0.0336	92.2	
	3	0.0824	85.0	0.788c	101c	
	Average	0.0456	87.9	0.0480	91.0	
	S.D.	0.0322	3.29	N.A.[b]	N.A[b]	

[a] N.D.: Not determined as the analyte was below the LLOQ of the bioanalytical method.
[b] N.A.: Not applicable.
[c] Data were not included for calculation of the average of the recovery and/or the P_{app} value as the corresponding monolayer failed the integrity test (see Table 15). Only those concentrations obtained from cell monolayers which passed the LY criterion were included for the average recovery and P_{app} value calculation.
[d] The recovery values were calculated using the LLOQ, 1 nM, as the measured concentration of SP 338 in the receiver was less than the LLOQ.
[e] The measured concentration of SP-338 at 60 minutes was less than the values of the LLOQ; the measured concentration of SP-338 at 120 minutes was used to estimate the P_{app} value and the recovery of SP-338. The actual P_{app} value and the recovery value were less than the reported ones

Table 29: Monolayer Integrity of Caco-2 Cells (P-gp and BCRP Substrate Assessment)

Treatment	Replicates	A-to-B		B-to-A	
		P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
0.5 µM SP-338	1	0.3[b]	Pass	0.3	Pass
	2	0.3[b]	Pass	0.3	Pass
	3	0.3[b]	Pass	1.3[b]	Fail
2 µM SP-338	1	0.3[b]	Pass	0.3	Pass
	2	0.3[b]	Pass	0.3	Pass
	3	0.3[b]	Pass	0.3	Pass
10 µM SP-338	1	0.3[b]	Pass	0.3	Pass
	2	0.3[b]	Pass	0.3	Pass
	3	0.8	Pass	2.0	Fail

[a] Passing criteria: P_{app} of LY $\leq 0.8 \times 10^{-6}$ cm/s.
[b] The P_{app} values were calculated using the nominal concentration of LLOQ, 0.3125 µM at 120 minutes as LY concentrations obtained both at 60 minutes and 120 minutes were below LLOQ

Reviewer's Comment:

- *The selected concentration of metabolite Sp-338 up to 10 µM was acceptable as it is higher than the highest potential parent drug plecanatide concentration in gut (Igut) with clinical dose of 3 mg (Igut= dose/250 mg=3 mg/1682 mg/mol/250 mL = 7.13 µM).*
- *The test to evaluate the potential of SP-338 being substrate for P-gp and BCRP did not include positive and negative controls to validate the test systems for presence of P-gp and BCRP transporters. However, the Caco-2 cell test system that evaluated the potential of plecanatide being inhibitor of efflux transporters had positive controls to validate the system.*
- *Based on the efflux ratio of 1.05, SP-338 does not appear to be substrate for P-gp nor BCRP.*
- *Apparent permeability could not be assessed for SP-338 at 0.5 and 2 µM in Caco-2 cell test system as the analyte was below the LLOQ of 1 nM , indicating its low permeability.*
- *Permeability of SP-338 across Caco-2 cells in both directions were less than $1X10^{-6}$ cm/s suggesting very low permeability.*

Inhibition:
Inhibition potential of P-gp and BCRP by metabolite SP-338 was evaluated by measuring the bi-directional permeability of model substrates (digoxin for P-gp and cladribine for BCRP) across Caco-2 cell monolayers in presence and absence of 2 µM plecanatide in triplicated at 37 ± 1 °C

Table 30: Experimental conditions to evaluate the inhibition potential of SP-338

	P-gp	BCRP
Test-System	Caco-2	Caco-2
SP-338 concentration	2 µM	2 µM
Positive control substrate	Digoxin (10 µM)	Cladribine (10 µM)
Positive control inhibitor	Valspodar (1 µM)	Ko143 (10 µM)
Permeability control	Lucifer yellow (200 µM)	Lucifer yellow (200 µM)
Sampling time (min)	D: 5, 120; R: 120	D: 5, 120; R: 120
Analysis method	LC-MS/MS	LC-MS/MS

A-Apical B- Basal D-Donor R-Receiver
The permeability of lucifer yellow was measured (apical to basolateral) after the incubation to determine integrity of the monolayer.

Table 31: Permeability and Recovery of Digoxin in Caco-2 Cells (P-gp Inhibitor Assessment)

Treatment	Replicates	A-to-B		B-to-A		Efflux Ratio	% of Inhibition
		P_{app} ($x10^{-6}$ cm/s)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Recovery (%)		
10 µM Digoxin	1	1.03	78.0	16.5	80.1	17.7	N.A.[a]
	2	0.879	82.8	15.4	90.1		
	3	0.790	87.8	15.8	88.9		
	Average	0.900	82.9	15.9	86.4		
	S.D.	0.122	4.93	0.549	5.48		
10 µM Digoxin + 1 µM Valspodar	1	5.25	77.6	5.29	86.0	0.920	100
	2	4.85	82.9	4.22	86.2		
	3	6.02	87.5	5.32	94.8		
	Average	5.37	82.7	4.94	89.0		
	S.D.	0.592	4.95	0.623	5.01		
10 µM Digoxin + 2 µM SP-338	1	0.787	82.8	21.7	87.4	20.3	15.9
	2	0.823	85.4	16.6	87.8		
	3	1.25	92.1	19.9	89.2		
	Average	0.954	86.8	19.4	88.1		
	S.D.	0.259	4.82	2.59	0.908		

[a] N.A.: not applicable.

Table 32: Monolayer Integrity of Caco-2 Cells (P-gp Inhibitor Assessment)

Treatment	A-to-B		B-to-A	
	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
10 µM Digoxin	0.3[b]	Pass	0.3	Pass
	0.4	Pass	0.4	Pass
	0.3[b]	Pass	0.4	Pass
10 µM Digoxin + 1 µM Valspodar	0.3[b]	Pass	0.4	Pass
	0.4	Pass	0.3	Pass
	0.3[b]	Pass	0.4	Pass
10 µM Digoxin + 2 µM SP-338	0.3[b]	Pass	0.3	Pass
	0.3	Pass	0.4	Pass
	0.4	Pass	0.4	Pass

[a] Passing criteria: P_{app} of LY ≤ 0.8 × 10^{-6} cm/s.
[b] The P_{app} values were calculated using the nominal concentration of LLOQ, 0.3125 µM at 120 minutes as LY concentrations obtained both at 60 minutes and 120 minutes were below LLOQ.

Table 33: Permeability and Recovery of Cladribine in Caco-2 Cells (BCRP Inhibitor Assessment)

Treatment	Replicates	A-to-B		B-to-A		Efflux Ratio	% of Inhibition
		P_{app} ($x10^{-6}$ cm/s)	Recovery (%)	P_{app} ($x10^{-6}$ cm/s)	Recovery (%)		
10 µM Cladribine	1	1.53	92.0	20.3	98.3	13.6	N.A.[a]
	2	1.32	99.1	16.9	100		
	3	1.20	98.4	17.7	103		
	Average	1.35	96.5	18.3	101		
	S.D.	0.166	3.92	1.80	2.29		
10 µM Cladribine + 10 µM Ko143	1	1.51	91.5	1.95	97.5	1.26	97.9
	2	1.58	94.6	2.00	100		
	3	1.66	94.5	2.04	102		
	Average	1.58	93.5	2.00	100		
	S.D.	0.0733	1.75	0.0449	2.32		
10 µM Cladribine + 2 µM SP-338	1	1.07	93.5	18.5	98.1	12.7	6.62
	2	1.14	95.1	17.0	96.6		
	3	2.08	96.1	19.2	97.4		
	Average	1.43	94.9	18.2	97.4		
	S.D.	0.562	1.32	1.13	0.784		

[a] N.A.: not applicable.

Table 34: Monolayer Integrity of Caco-2 Cells (BCRP Inhibitor Assessment)

Treatment	Replicates	A-to-B		B-to-A	
		P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]	P_{app} ($x10^{-6}$ cm/s)	Pass/Fail[a]
10 µM Cladribine	1	0.3[b]	Pass	0.8	Pass
	2	0.3[b]	Pass	0.3	Pass
	3	0.3[b]	Pass	0.4	Pass
10 µM Cladribine + 10 µM Ko143	1	0.3[b]	Pass	0.3	Pass
	2	0.3	Pass	0.4	Pass
	3	0.3[b]	Pass	0.4	Pass
10 µM Cladribine + 2 µM SP-338	1	0.6	Pass	0.4	Pass
	2	0.4	Pass	0.4	Pass
	3	0.6	Pass	0.4	Pass

[a] Passing criteria: P_{app} of LY $\leq 0.8 \times 10^{-6}$ cm/s.
[b] The P_{app} values were calculated using the nominal concentration of LLOQ, 0.3125 µM at 120 minutes as LY concentrations obtained both at 60 minutes and 120 minutes were below LLOQ.

Reviewer's Comment:

- *All the test systems to evaluate the inhibition potential was appropriately validated with positive controls with model substrates and known model inhibitors. Model substrates has significant efflux ratio > 2 and were significantly inhibited in presence of model inhibitors (positive controls).*
- *Inhibition potential of P-gp and BCRP transporters by SP-338 was assessed at 2 µM. SP-338 at 2 µM did not inhibit P-gp and BCRP. Therefore, Ki >2 µM for both P-gp and BCRP. Since highest potential concentration of parent drug plecanatide in gut (Igut) with 7.13 µM with clinical dose of 3 mg, the highest potential concentration of metabolite in gut is < 7.13 uM. With the worst case scenario, $R= 1+I_{gut}/Ki = 1+7.13uM/2\mu M = 4.5 < 11$, likelihood of plecanatide to inhibit P-gp and BCRP in gut are less likely.*
- *SP-338 at 2 µM did not inhibit P-gp and BCRP.*

2.8 General Biopharmaceutics

2.8.1 What are the solubility and the permeability?

The solubility profile of plecanatide over a pH range of (b) (4) is summarized in Table 32. Please refer to section 2.4.5.1for information on permeability across Caco-2 cell monolayers.

Table 35: Solubility Profile of plecanatide

(b) (4)

2.8.2 What is the composition of the final to-be-marketed formulation (drug substance and drug product)? If there are multiple dose strengths, are the active and inactive ingredients proportionally similar in composition among different dose strengths?

Plecanatide tablets are immediate-release tablet developed at 3 mg (b) (4) dose strengths. (b) (4)

(b) (4)

Table 36: Composition of To-Be Marketed (TMB) and Phase III Formulation of Plecanatide Tablets

Component	Quality Standard	Function	Dosage Strength (mg/tablet)	
			3	(b) (4)
Plecanatide[a]	In-house standard	Drug substance	3.0	
Microcrystalline cellulose[b]	USP-NF	(b) (4)	(b) (4)	
Magnesium stearate[c]	USP-NF			
(b) (4)	USP-NF			
Total (mg)				

qs = quantity sufficient; USP -NF = United States Pharmacopeia-National Formulary; wt/wt% = weight percentage
[a] The drug substance is corrected for assay (wt/wt%).
[b] Microcrystalline cellulose USP-NF meets compendial requirements (b) (4)
(b) (4) (Module 3.2.P.4.1).
(b) (4)

2.8.3 Was the proposed to-be-marketed formulation used in the pivotal clinical and bioavailability studies?

The to-be-marketed (TBM) formulation is the same formulation as the formulation used in phase III studies to establish safety and efficacy. Therefore, no bioequivalence assessment is needed.

2.8.4 Were there any major changes to the drug substance and/or drug product during the development process? Are there *In-vivo* bioequivalence (BE) or comparability studies to compare PK or PD of various formulations?

The initial phase 1 SAD study (SP-SP304101-08) used an oral solution formulation of plecanatide (b) (4) The phase 2a study (SP-SP304201-09) used capsule formulation where 1, 3, and 9 mg dose strengths used a capsule (b) (4) and 0.3 mg strength used a capsule (b) (4) The plecanatide formulation that was used in CIC phase 2b study was a capsule formulation (b) (4) the phase 3 studies formulation which is the same formulation as the commercial formulation (TBM). Since plecanatide and its metabolite SP-338 do not have measurable plasma concentrations despite the formulation and dose strength, relative BA/BE study to compare the plasma exposure of plecanatide for various formulations used during the drug development process is not an option.

Table 37: Plecanatide Formulations Used in Phase 2b and Phase 3 Clinical Programs

		Phase 2b	Phase 2b	Phase 2b	Phase 3	Phase 3	Phase 2b
Clinical Usage		CIC study SP304202-10 (Capsule) (b) (4)			CIC studies SP304203-00, SP304203-01, SP304203-03 (b) (4)	CIC studies SP304203-00, SP304203-01, SP304203-03 (b) (4) (b) (4)	Food Effect study SP304101-09 (b) (4)
Dosage Form		Capsule/Tablet			Tablet		
Dosage Strength		**0.3 mg**	**1 mg**	**3 mg**	**3 mg**	**6 mg**	**9 mg**
Component	**Function**	**Quantity (mg)**					
Plecanatide[a]	Drug substance	0.3	1.0	3.0	3.0	6.0	9.0
Microcrystalline cellulose[b,c] USP-NF		(b) (4)					
Magnesium stearate[d] USP-NF							
Total (mg)		100	100	100	100	100	100

CIC = chronic idiopathic constipation, (b) (4) USP-NF = United States Pharmacopoeia-National Formulary; wt/wt% = weight percentage

[a] The drug substance will be corrected for purity (wt/wt%).

[b] Microcrystalline cellulose USP-NF (b) (4)

[c] Microcrystalline cellulose USP-NF, (b) (4)

2.8.5 What is the effect of food on the bioavailability (BA) of the drug from the dosage form?

When 9 mg plecanatide tablet was administered with or without food in 24 healthy subjects, only 1 subject had detectable level of plecanatide at 0.5 and 1 hour post-dose under fasted state. Plecanatide concentrations were below the limit of quantitation for all other time points and for all other subjects. The active metabolite SP-338 was not detected in any subjects. Food had minimal effects on BM frequency, time to first bowel movement, fecal urgency, and fecal incontinence.

Administration of 9 mg plecanatide with food had noticeable PD effect in BSFS scores and the incidence of abdominal cramping where food (both HF-HC and LF-LC) appear to increase BSFS score and increase the incidence of moderate and severe abdominal cramping and degree of abdominal cramping compared to fasted state. In both of the phase III studies, subjects were instructed to take the study drug with or without food at their own choice. In the proposed label, the sponsor's proposal is to take plecanatide with or without food. However, based on the result of this food effect study, the agency recommends taking plecanatide with or without food in general but for patients who experience abdominal cramping, plecanatide tablet should be taken under fasting condition. This is under further discussion with medical review team.

Study SP304101-09:

This was a phase 1, single-center, single-blind, randomized, crossover, 3 sequence, single-dose study of the effect of food on the PD, PK, safety, and tolerability of 9 mg plecanatide tablets and placebo administered orally to 30 healthy adult subjects. This study consisted of 3 treatment periods and 3 treatment conditions (fasted, fed with high fat-high calories meal, and low fat-low calories meal). Each treatment period included a single dose of 9 mg of plecanatide or placebo. Each period was separated by 7 days of washout period. In each treatment period, subjects were randomized to receive a single dose of study medication or placebo in 4:1 ratio. For each treatment period, PK blood samples were collected at pre-dose and at 0.5, 1, 2, 4, 8, 12, 24, and 48 hours post-dose to determine the plasma concentration of plecanatide and metabolite SP-338. PD assessment included bowel movement frequency, stool consistency, time to first bowel movement, fecal urgency, fecal incontinence and soiling, and abdominal cramping.

- HF-HC Meal Content: 2 eggs fried in butter, 2 strips of pork bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes, and 8 ounces of whole milk
- LF-LC Meal Content: ½ cup eggbeaters prepared with cooking spray, 2 ounces of fresh banana, 1 slice of whole multigrain bread, 1 teaspoon margarine, 1 tablespoon low-sugar jam and 8 ounces of skim milk

	HF-HC	LF-LC
Total Calories	~1,000	~350
Total Fat Content	~60 gr (60%)	~7 gr (17%)
Calories (% of Total Calories) from Protein	150 (15%)	60 (17%)
Calories (% of Total Calories) from Carbohydrates	250 (25%)	230 (66%)
Calories (% of Total Calories) from Fat	600 (60%)	60 (17%)

Results:

PK: Twenty-three of the 24 plecanatide-treated subjects had plecanatide *(LLOQ of 1.0 ng/mL)* plasma concentrations that were BLOQ for all time-points during all 3 treatment periods. *Only o*ne subject had measurable plasma concentrations of plecanatide at 0.5 hour and 1 hour post-dose in the fasted state only (1.99 ng/mL at 0.5 hr and 2.18 ng/mL at 1.0 hr post dose). Plasma concentrations of metabolite SP-338 (LLOQ of 0.775 ng/mL) were BLOQ for all subjects at all time-points. Therefore, no PK parameters were estimated.

PD:

- Bowel movement frequency: Food does not appear to effect BM frequency.

Table 38: Summary of Bowel Frequency and Changes in Frequency

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Pre-dose						
Arithmetic Mean (SD)	0.92 (0.319)	1.02 (0.375)	0.96 (0.440)	1.08 (0.204)	1.25 (0.524)	1.08 (0.492)
% CV	34.75	36.76	45.94	18.84	41.95	45.38
Geometric Mean	0.92	0.95	0.92	1.07	1.14	1.00
Median (Range)	1.00 (0.0 to 1.5)	1.00 (0.5 to 2.0)	1.00 (0.0 to 2.0)	1.00 (1.0 to 1.5)	1.25 (0.5 to 2.0)	1.00 (0.5 to 2.0)
≥ 5 stools, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Overall post-dose						
Arithmetic Mean (SD)	1.17 (0.732)	1.63 (0.824)	1.35 (0.801)	1.00 (0.548)	1.33 (0.753)	1.00 (0.316)
% CV	62.77	50.72	59.12	54.77	56.46	31.62
Geometric Mean	0.98	1.45	1.34	0.89	1.16	0.95
Median (Range)	1.00 (0.5 to 3.0)	1.50 (0.5 to 3.5)	1.00 (0.0 to 3.0)	1.00 (0.5 to 2.0)	1.00 (0.5 to 2.5)	1.00 (0.5 to 1.5)
≥ 5 stools	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Change from pre-dose to overall post-dose						
Arithmetic Mean (SD)	0.25 (0.659)	0.60 (0.859)	0.40 (0.821)	-0.08 (0.665)	0.08 (0.492)	-0.08 (0.376)
Median (Range)	0.25 (-0.5 to 2.0)	0.50 (-0.5 to 2.5)	0.00 (-1.0 to 2.5)	0.00 (-1.0 to 1.0)	0.25 (-0.5 to 0.5)	0.00 (-0.5 to 0.5)
≥ 2 times/day, n (%)	1 (4.2)	3 (12.5)	1 (8.3)	0 (0)	0 (0)	0 (0)

- Stool consistency: Stool consistency was assessed by using the Bristol Stool Form Scale (BSFS) score. Food (both HF-HC and LF-LC) appears to increase BSFS compare to fasted state for subjects who were treated with 9 mg plecanatide.

Table 39: Summary of BSFS Scores and Changes

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Pre-dose BSFS Scores						
n	24	24	23	6	6	6
Arithmetic Mean (SD)	3.06 (1.237)	3.44 (1.174)	2.96 (1.391)	3.22 (0.981)	3.44 (0.867)	3.13 (0.494)
% CV	40.49	34.16	47.05	30.45	25.17	15.80
Geometric Mean	2.80	3.22	2.67	3.11	3.36	3.09
Median (Range)	3.00 (1.0 to 5.5)	3.00 (1.0 to 5.5)	3.00 (1.0 to 7.0)	3.00 (2.0 to 5.0)	3.25 (2.5 to 5.0)	3.00 (2.5 to 4.0)
≥ 6, n (%)	0 (0)	0 (0)	1 (4.3)	0 (0)	0 (0)	0 (0)
No BM, n	0	0	1	0	0	0
Overall post-dose BSFS Scores						
n	24	24	22	6	6	6
Arithmetic Mean (SD)	3.68 (1.607)	5.15 (1.209)	4.38 (1.440)	3.13 (1.022)	3.48 (1.050)	3.36 (0.618)
% CV	43.64	23.48	32.85	32.69	30.13	18.40
Geometric Mean	3.32	5.00	4.12	3.00	3.37	3.32
Median (Range)	3.90 (1.0 to 7.0)	5.38 (3.0 to 6.7)	4.58 (2.0 to 6.3)	3.00 (2.0 to 5.0)	3.20 (2.5 to 5.5)	3.00 (3.0 to 4.5)
≥ 6, n (%)	2 (8.3)	10 (41.7)	6 (27.3)	0 (0)	0 (0)	0 (0)
No BM, n	0	0	2	0	0	0
Change in BSFS Scores from pre-dose to overall post-dose						
n	24	24	21	6	6	6
Arithmetic Mean (SD)	0.63 (1.570)	1.71 (1.131)	1.59 (1.644)	-0.10 (0.638)	0.04 (0.650)	0.24 (0.260)
Median (Range)	0.33 (-2.0 to 4.0)	1.50 (0.0 to 4.3)	1.17 (-1.0 to 5.0)	0.00 (-1.3 to 0.5)	0.25 (-1.2 to 0.5)	0.21 (0.0 to 0.5)
No BM, n	0	0	3	0	0	0

- Time to first bowel movement appears to be shortest for subjects with HF-HC food regardless of treatment with 9 mg plecanatide or with placebo. In general, time to first bowel movement is highly variable.

Table 40: Summary of Time to First Bowel Movement

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Time to First Bowel Movement, hours						
n	24	24	23	6	6	6
Arithmetic Mean (SD)	6.5 (8.67)	3.6 (5.07)	11.1 (30.51)	8.9 (5.10)	7.1 (3.91)	14.1 (9.33)
%CV	134.0	140.8	273.9	57.3	55.3	66.1
Geometric Mean	3.5	2.3	3.7	5.5	5.9	10.5
Median (Range)	3.8 (0 to 35)	1.8 (1 to 25)	2.8 (1 to 149)	9.7 (0 to 15)	7.2 (2 to 13)	12.2 (2 to 26)
No BM, n	0	0	1	0	0	0

- Fecal urgency: The degree of fecal urgency was scored by the subject as: none = 0, mild = 1, moderate = 2, and severe = 3. There is no consistent effect of food on fecal urgency.

Table 41: Summary of Number (Percentages) of Subjects Reporting Fecal Urgency – Worst

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Pre-dose						
None, n (%)	19 (79.2)	20 (83.3)	17 (73.9)	5 (83.3)	5 (83.3)	5 (83.3)
Mild, n (%)	3 (12.5)	1 (4.2)	3 (13.0)	0 (0)	0 (0)	0 (0)
Moderate, n (%)	2 (8.3)	2 (8.3)	2 (8.7)	1 (16.7)	1 (16.7)	1 (16.7)
Severe, n (%)	0 (0)	1 (4.2)	1 (4.3)	0 (0)	0 (0)	0 (0)
No BM, n (%)	0	0	1	0	0	0
Overall post-dose						
None, n (%)	17 (70.8)	10 (41.7)	11 (50.0)	5 (83.3)	5 (83.3)	5 (83.3)
Mild, n (%)	3 (12.5)	5 (20.8)	5 (22.7)	0 (0)	0 (0)	0 (0)
Moderate, n (%)	4 (16.7)	5 (20.8)	4 (18.2)	1 (16.7)	1 (16.7)	1 (16.7)
Severe, n (%)	0 (0)	4 (16.7)	2 (9.1)	0 (0)	0 (0)	0 (0)
No BM, n (%)	0	0	2	0	0	0

Table 42: Summary of Degree of Fecal Urgency – Daily Average Scores

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	**Fed HF-HC (n = 24)**	**Fed LF-LC (n = 24)**	**Fasted State (N = 6)**	**Fed HF-HC (n = 6)**	**Fed LF-LC (n = 6)**
Pre-dose						
n	24	24	23	6	6	6
Arithmetic Mean (SD)	0.24 (0.536)	0.25 (0.608)	0.33 (0.691)	0.33 (0.816)	0.33 (0.816)	0.17 (0.408)
%CV	220.56	243.17	207.25	244.95	244.95	244.95
Geometric Mean	1.06	1.41	1.03	2.00	2.00	1.00
Median (Range)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.3)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.0)	0.00 (0.0 to 1.0)
No BM, n	0	0	1	0	0	0
Overall post-dose						
n	24	24	22	6	6	6
Arithmetic Mean (SD)	0.31 (0.612)	0.84 (0.875)	0.56 (0.762)	0.33 (0.816)	0.33 (0.816)	0.33 (0.816)
%CV	196.00	103.89	136.81	244.95	244.95	244.95
Geometric Mean	0.89	1.30	0.89	2.00	2.00	2.00
Median (Range)	0.00 (0.0 to 2.0)	0.80 (0.0 to 2.4)	0.17 (0.0 to 2.5)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.0)
No BM, n	0	0	2	0	0	0

- fecal incontinence and soiling: Food does not appear to effect fecal incontinence and soiling.

Table 43: Summary of Number (Percentages) of Subjects Reporting Fecal Incontinence – Worst

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	**Fed HF-HC (n = 24)**	**Fed LF-LC (n = 24)**	**Fasted State (N = 6)**	**Fed HF-HC (n = 6)**	**Fed LF-LC (n = 6)**
Pre-dose						
Yes	1 (4.2)	0 (0)	0 (0)	0 (0)	1 (16.7)	0 (0)
No	23 (95.8)	24 (100.0)	23 (100.0)	6 (100.0)	5 (83.3)	6 (100.0)
No BM	0	0	1	0	0	0
Overall post-dose						
Yes	1 (4.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	23 (95.8)	24 (100.0)	22 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
No BM	0	0	2	0	0	0

Table 44: Summary of Number (Percentages) of Subjects Reporting Soiling – Worst

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	**Fed HF-HC (n = 24)**	**Fed LF-LC (n = 24)**	**Fasted State (N = 6)**	**Fed HF-HC (n = 6)**	**Fed LF-LC (n = 6)**
Pre-dose						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	24 (100.0)	24 (100.0)	23 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
No BM	0	0	1	0	0	0
Overall post-dose						
Yes	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No	24 (100.0)	24 (100.0)	22 (100.0)	6 (100.0)	6 (100.0)	6 (100.0)
No BM	0	0	2	0	0	0

- Abdominal cramping: The degree of abdominal cramping was scored by the subject as: none = 0, mild = 1, moderate = 2, and severe = 3. Food (both HF-HC and LF-LC) appear to increase in incidence of moderate and severe abdominal cramping and degree of abdominal cramping compared to fasted state when treated with 9mg plecanatide.

Table 45: Summary of Number (Percentages) of Subjects Reporting Abdominal Cramping – Worst

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Pre-dose						
None	20 (83.3)	20 (83.3)	16 (69.6)	6 (100.0)	6 (100.0)	6 (100.0)
Mild	3 (12.5)	2 (8.3)	6 (26.1)	0 (0)	0 (0)	0 (0)
Moderate	1 (4.2)	2 (8.3)	1 (4.3)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No BM	0	0	1	0	0	0
Overall post-dose						
None	17 (70.8)	10 (41.7)	10 (45.5)	6 (100.0)	6 (100.0)	6 (100.0)
Mild	3 (12.5)	4 (16.7)	5 (22.7)	0 (0)	0 (0)	0 (0)
Moderate	4 (16.7)	7 (29.2)	5 (22.7)	0 (0)	0 (0)	0 (0)
Severe	0 (0)	3 (12.5)	2 (9.1)	0 (0)	0 (0)	0 (0)
No BM	0	0	2	0	0	0

Table 46: Summary of Degree of Abdominal Cramping – Daily Average

	Plecanatide 9 mg			Placebo		
	Fasted State (N = 24)	Fed HF-HC (n = 24)	Fed LF-LC (n = 24)	Fasted State (N = 6)	Fed HF-HC (n = 6)	Fed LF-LC (n = 6)
Pre-dose						
n	24	24	23	6	6	6
Arithmetic Mean (SD)	0.21 (0.509)	0.19 (0.491)	0.28 (0.470)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
%CV	244.31	252.34	170.81	-	-	-
Geometric Mean	1.19	1.07	0.82	-	-	-
Median (Range)	0.00 (0.0 to 2.0)	0.00 (0.0 to 2.0)	0.00 (0.0 to 1.5)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)	0.0 (0.0 to 0.0)
No BM, n	0	0	1	0	0	0
Overall post-dose						
n	24	24	22	6	6	6
Arithmetic Mean (SD)	0.27 (0.492)	0.76 (0.840)	0.58 (0.704)	0.00 (0.000)	0.00 (0.000)	0.00 (0.000)
%CV	182.44	110.34	121.40	-	-	-
Geometric Mean	0.83	1.09	0.90	-	-	-
Median (Range)	0.00 (0.0 to 1.7)	0.55 (0.0 to 2.3)	0.42 (0.0 to 2.2)	0.00 (0.0 to 0.0)	0.00 (0.0 to 0.0)	0.00 (0.0 to 0.0)
No BM, n	0	0	2	0	0	0

2.9 Analytical Section

2.9.1 What bioanalytical methods are used to assess concentrations of the measured moieties?

The concentrations of parent drug plecanatide (SP-304) in human plasma were determined using two validated liquid chromatography mass spectrometry (LC/MS/MS) methods validated at (b) (4) lab with slightly different sensitivity.

Initial validation study report No 1901 with LLOQ of 10 ng/mL (initial report dated 7/10/2008, Amendment 1 dated 9/18/2009, Amendment 2 dated 12/17/2009) was used in:

- phase 1 study SP304101-08
- phase 2a study SP304201-09

Subsequently, an improved LC/MS-MS with validation study report No 2431 with LLOQ of 1.0 ng/mL was used in (initial report dated 9/12/2012, amendment 1 dated 8/02/013, Amendment 2 dated 9/16/2014):

- phase 1 food-effect study SP304101-09
- phase 2b study SP304-20210
- (b) (4)
- phase 3 study SP304203-03

The concentrations of metabolite SP-338 in human plasma were determined using validated liquid chromatography mass spectrometry (LC/MS/MS) methods validated at (b) (4) lab with validation study report No 2432 (Initial report dated 11/30/2012, amendment 1 dated 09/16/2014). Method 2432 was used to evaluate samples from the following studies for SP-338 plasma concentrations

- phase 1 food-effect study SP304101-09
- (b) (4)
- Phase 3 study SP304203-03

No urine samples were analyzed.

2.9.2 What is the range of the standard curve? How does it relate to the requirements for clinical studies? What curve fitting techniques were used? What are the lower and upper limits of quantification (LLOQ/ULOQ)?

Analyte	Matrix	Validation report	Range of Standard Curve	LLOQ	ULOQ
SP-304	Plasma	1901	10 to 250 ng/mL (8 levels)	10 ng/mL	250 ng/mL
SP-304	Plasma	2431	1.00 to 250 ng/mL (8 levels)	1.00 ng/mL	250 ng/mL
SP-338	Plasma	2432	0.775 to194 ng/mL (8 levels)	0.775 ng/mL	194 ng/mL

1901: A best fit calibration curve using a quadratic regression with 1/x weighting was generated for calibration standards using the respective peak area ratios (analyte/internal standard) versus the concentration of the calibration standards.

2431: A best fit calibration curve using a linear regression with 1/x weighting was generated for calibration standards using the respective peak area ratios (analyte/internal standard) versus the concentration of the calibration standards. The mean coefficient of determination for SP-304 calibration standards was 0.9963.

2432: A best fit calibration curve using a linear regression with 1/x weighting was generated for calibration standards using the respective peak area ratios (analyte/internal standard) versus the concentration of the calibration standards. The mean coefficient of determination for SP-338 calibration standards was 0.9989.

1901: For plasma SP-340 samples with dilution factor of 10 were evaluated and were within acceptable range with %theoretical and precision for SP-304 were 102% and 3.8%,

2431: 500 ng/mL diluted ten-fold to 50 ng/mL has acceptable criteria with The % theoretical and precision for SP-304 were 101% and 7.7%,

2432: A 389 ng/mL QC sample for SP-338 was diluted ten-fold (10X) with human plasma were within acceptable range with %theoretical and precision for SP-304 were 90.6% and 3.5%.

2.9.3 What are the accuracy, precision and selectivity at these limits?

Analyte	Matrix	Validation report		Intra-assay	Inter-Assay
SP-304	Plasma	1901	Precision (CV%)	0.79 to 5.9%	1.8% to 3.9%
			Accuracy	-5.8 % to 11%	-2% to 6%
SP-304	Plasma	2431	Precision (CV%)	4.7% to 16%	6.8% to 16%
			Accuracy	-7.7% to 18%	-5.9% to 9%
SP-338	Plasma	2432	Precision (CV%)	2.4% to 11%	3.4% to 13%
			Accuracy	0% to 17.4%	6.7% to11.2%

All methods had adequate selectivity:

- 1901: Of the 6 individual lots of blank human plasma, there were no detectable peaks observed at the retention times of SP-304 or at the retention time of the internal standard.
- 1901: 100 ng/mL acetaminophen, 100 ng/mL acetylsalicylic acid, and 100 ng/mL of caffeine did not interfere with detection of SP-304.
- 2431: Of the 6 individual lots of blank human plasma, there were no detectable peaks observed at the retention times of SP-304 or at the retention time of the internal standard.
- 2431: 200 ng/mL each acetaminophen, ibuprofen, caffeine, chlorpheniramine, naproxen, and pseudoephedrine did not interfere with detection of SP-304
- 2432: There were no detectable peaks observed at the retention time of the SP-338 or at the retention time of the internal standard in either the 0 ng/mL samples or the control blanks prepared in six different individual lots of matrix.
- 2432: 200 ng/mL each acetaminophen, ibuprofen, caffeine, chlorpheniramine, naproxen, and pseudoephedrine did not interfere with detection of SP-338.

2.9.4 What is the sample stability under the conditions used in the study (long-term, freeze-thaw, sample-handling, sample transport, autosampler)?

All samples were analyzed within the time period for which the long-term stability has been established.

Analyte	Study report	Matrix	Freeze-thaw -70°C (Cycles)	At Room temperature (benchtop)	At 4°C (autosampler)	At -20°C	At -70°C
SP-304	1901	Plasma	5	18 hr	12.5 hr		531 Days
SP-304	2431	Plasma	4	20.5 hr	499 hr	134 Days	794 days @ 3.0 ng/mL 1419 days @ 30 and 220 ng/mL
SP-338	2432	Plasma	5	49.1 hr	992 hr	41 Days	917 days

Study 1901: SP-304 is stable in whole blood at refrigerated temperature for at least 120 minutes
Study 2431: SP-304 is stable in whole blood at refrigerated temperature for at least 120 minutes
Study 2432: SP-338 is stable in whole blood at refrigerated temperature for at least 120 minutes

2.9.5 What is the plan for the QC samples and for the reanalysis of the incurred samples?

1901: QC samples for SP-304 in human plasma were 30.0, 80.0, 220, and 500 ng/mL.
2431: QC samples for SP-304 in human plasma were 1.00, 3.00, 80.0, and 220 ng/mL.
2432: QC samples for SP-338 in human plasma were 0.776, 2.32, 62.0, and 171 ng/mL

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/s/

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