CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

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CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

OFFICE OF CLINICAL PHARMACOLOGY REVIEW

NDA	**208-271**
Relevant IND(s)	**67,452**
Submission Date(s)	**06/19/2015, 8/14/2015, 12/15/2015, 12/24/2015, 2/19/2016**
PDUFA Date:	**4/19/2016**
Brand Name	**RELISTOR® Tablets**
Generic Name	**Methylnaltrexone Bromide**
Submission Type	**Original, 505(b)(1)**
Dosage Form, Strength	**Tablet, 150 mg**
Proposed indication	**Treatment of opioid-induced constipation (OIC) in adult patients with chronic non-cancer pain**
Sponsor Recommended Dosing Regimen	**450 mg Once daily**
Sponsor	**Valeant Pharmaceuticals International, Inc., / Salix 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

In this submission, methylnaltrexone bromide (MNTX), mu-opioid receptor antagonist, is being developed as oral tablet for the treatment of opioid-induced constipation in adult patients with chronic non-cancer pain. Currently, methylnaltrexone bromide, under trade name of Relistor, is available as subcutaneous (SC) injection for treatment of opioid-induced constipation in adult patients with chronic non-cancer pain at 12 mg SC dose and for opioid-induced constipation in adult patients with advanced illness. Methylnaltrexone bromide is being developed as oral tablet at 150 mg dosage strength only. The proposed dose is 450 mg (3 X 150 mg oral tablet) once daily. In support of this NDA, the sponsor had submitted 8 *in-vitro* studies (to evaluate drug absorption-, distribution-, and metabolism-related characteristics, and drug-drug interaction potential of methylnaltrexone and its 3 major metabolites), 7 phase I studies (to evaluate the relative bioavailability/bioequivalence, effect of food, effect of hepatic impairment on pharmacokinetic of methylnaltrexone, and pharmacokinetic in patient population), one PK/PD modeling (to evaluate the exposure-response relationship) and one phase III efficacy and safety trial with 150 mg, 300 mg, and 450 mg QD doses.

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 Relationship and Dose Selection

Dose-Response for Efficacy***:*** In the phase III safety and efficacy study MNTX3201, three dose levels, 150 mg, 300 mg, and 450 mg, of oral MNTX tablet were evaluated against placebo in subjects with chronic nonmalignant pain who have OIC. The proportion of subjects responding to study drug during Weeks 1-4, where a responder is defined as ≥ 3 RFBMs/week with an increase of ≥ 1 RFBM/week over baseline for ≥ 3 out of the first 4 weeks of the treatment period (key secondary efficacy endpoint which the agency considers to be more clinically relevant for its approval decision) for placebo, 150 mg, 300 mg, 450 mg were 40.8%, 45.3%, 51.7% and 54.5%, respectively. The observed effect size is small and higher doses were not evaluated. An increase in treatment effect was observed over 150 mg to 450 mg doses, and both 300 mg and 450 mg doses have shown statistically significant improvement over placebo.

Dose-Response for Safety: In dose-ranging phase III study MNTX3201, there appears to be no dose-response for the majority of the adverse event categories (Table). However, dose-response is evident for abdominal pain and diarrhea which is consistent with the mechanism of action and experience with other products in this class of drugs. In addition, based on the cross study comparison, the rate of the abdominal pain AEs does not exceed that of SC 12 mg Relistor. Therefore, the safety profile appears reasonable for the 450 mg oral dose.

Dose Selection: The proposed dose of 450 mg is reasonable as higher doses are not expected to give greater exposures via oral administration nor have they been studied in sufficient numbers to

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form a safety database for 600 mg (n=17). Additionally the difference in response rate between the 150 mg and 300 mg doses (6.4% for the key secondary endpoint and 3.7% for the applicant's primary endpoint) is greater than between the 300 mg and 450 mg doses (2.8% for the key secondary endpoint and 2.6% for the applicant's primary endpoint) suggesting that increasing the dose above 450 mg will yield less additional benefit. Secondly lower doses (i.e. 150, 300 mg) appear to be less ideal in that their efficacy is reduced compared to the 450 mg dose which is already reduced when compared to the approved SC formulation dose of 12 mg (13% difference from placebo for oral 450 mg compared to a 20% difference from placebo for 12 mg SC dose). While there are exposure-response relationships for the adverse events abdominal pain and diarrhea, the AE rates for the high oral dose are lower than the approved SC dose.

Pharmacokinetics:

Methylnaltrexone has an approximately dose proportional increase in C_{max} and AUC between oral doses of 150 mg and 450 mg MNTX. PK variability of methylnaltrexone was relatively high (%CV up to 80% for Cmax and 56% for AUC). There was no significant accumulation of methylnaltrexone following once daily oral dosing of 450 mg MNTX tablet.

Absorption: After a single oral dose administration of 450 mg methylnaltrexone tablets in healthy subjects, the peak plasma concentration of methylnaltrexone was reached in about 1.5-2.5 hours with C_{max} of approximately 43-55 ng/mL. The absolute bioavailability of oral methylnaltrexone was not evaluated.

Food Effect: Administration of oral 450 mg (3 X 150 mg) MNTX tablet with high-fat breakfast reduced the AUC by 43% and C_{max} by 60% and delayed the t_{max} by 2 hours compared to the fasted state (study MNEF1001). In the phase III study MNTX3201, patients were instructed to take 450 mg MNTX tablets (3X150 mg) with water, first thing in the morning on an empty stomach 30 minutes before ingesting any food. The sponsor's proposed label recommends taking the tablet on an empty stomach at least 30 minutes before the first meal of the day. Based on the result of this food effect study and the design of phase III study MNTX 3201, the sponsor's proposed labeling recommendation in regards to food is acceptable.

Protein binding for parent drug and the major metabolites: Plasma protein binding of the parent drug methylnaltrexone ranges 11.0% to 15.3%, as determined previously in NDA 21964 for SC formulation. The in-vitro human plasma protein binding at concentrations of 1, 10, and 50 ng/mL ranged 17.3% to 28.9% for methylnaltrexone sulfate, 20.8% to 29.4% for methyl-6α-naltrexol, and 30.3% to 41.3% for methyl-6β-naltrexol.

Metabolism: Methylnaltrexone is mainly metabolized by sulfotransferase SULT1E1 and SULT2A1 isoforms to form methylnaltrexone sulfate and by aldoketo reductase 1C enzymes form methyl-6-naltrexol isomers. The role of CYP enzymes in metabolizing MNTX is not substantial in vivo based on the mass balance study and in vitro metabolism studies.

Elimination: Following a single oral dose administration of 450 mg methylnaltrexone tablets in healthy subjects, the plasma concentration of methylnaltrexone appears to decline in multi-phasic manner with mean terminal half-life of 14-16 hr; the percent of drug recovered in urine as unchanged drug is less than 1.78%; the renal clearance is estimated to be approximately 306 mL/min.

As no mass balance study with oral route of administration was conducted and the relative bioavailability of oral tablet compared to IV formulation was not evaluated to link oral

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formulation to the results of the mass balance study with the IV formulation, the contribution of renal clearance vs. hepatobiliary clearance cannot be estimated for the for the oral formulation of methylnaltrexone .

After intravenous administration of methylnaltrexone, approximately half of the dose was excreted in the urine (53.6%) and 17.3% of administered dose was excreted in the feces up to 168 hours post-dose. Methylnaltrexone is excreted primarily as unchanged drug in the urine and feces. However, radiolabeled recovery in this study was only 70.9 % after 7 days.

Metabolites: Following oral administration of 450 mg MNTX tablets, the AUC ratio of metabolites to the parent drug at steady-state on Day 7 were 79%, 38.5% and 21.4% for methylnaltrexone-sulfate (M2), methyl-6α nalthrexol (M4), and methyl-6β nalthrexol (M5), respectively. Based on the cross-study comparison with SC administration, it appears that the systemic exposure to methylnaltrexone metabolites is greater after oral administration of 450 mg dose than SC administration of 12 mg dose, where the AUC_{0-24} ratios of metabolites to methylnaltrexone at steady-state after 12 mg SC daily dosing was 30%, 19%, and 9% for methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol, respectively. Based on the comparison of C_{max} of MNTX and its major metabolites at the steady state to their respective ki for human mu-opioid receptor, although the overall clinical efficacy of oral MNTX 450 mg dose is probably primary driven by the parent drug MNTX, the active metabolite methyl-6α-naltrexol (M4) may still have some contribution to the efficacy to a certain extent.

Patient vs. Healthy: Following oral administration of 450 mg MNTX tablets, the exposure of methylnaltrexone appears to be lower in OIC patients compared to healthy subjects based on cross-study comparison. Cmax and AUC of methylnatresone in patient population is approximately 25% and 35%-45% lower in OIC patient population compared to healthy subjects, respectively. The most abundant metabolite methylnaltrexone sulfate (M2), which has the least pharmacological activity, has a similar exposure between healthy subjects and OIC patients. Exposure (both AUC and Cmax) of methyl-6α-naltrexol (M4) and methyl-6β-naltrexol (M5), are approximately 2 to 3-fold lower in OIC patients compared to healthy subjects.

Relative BA/BE:

BE between To-Be-Marketed (TBM) Formulation and Phase 3 Formulation: TBM formulation (coated tablet with (b) (4) process) is bioequivalent to the formulation used in pivotal clinical study MNTX 3201 (uncoated tablet with (b) (4) process), as was demonstrated in study MNPK1001 in healthy subjects in crossover study design. In addition, comparable exposures between these two formulations were also shown in patient population in parallel group study design in study MNOC1111.

OSIS Inspection: An inspection was requested for pivotal bioequivalence (BE) study MNPK1001 for both clinical site and bioanalytical site on 08/19/2015. The Division of New Drug Bioequivalence Evaluation (DNDBE) within the Office of Study Integrity and Surveillance (OSIS) conducted the inspection of the bioanalytical site and recommended the analytical data from study MNPK1001 be accepted for Agency review on 01/30/2016. The final classification for this analytical site inspection was voluntary action indicated (VAI). For the clinical site inspection, DNDBE within OSIS recommended accepting data without on-site inspection on 09/02/2016 since OSIS recently inspected the requested clinical site and the inspectional outcome from the inspection was classified as No Action Indicated (NAI).

Relative BA of Oral tablet vs. SC: 450 mg oral dose have approximately 5-fold lower Cmax (32.7 ng/mL vs. 163.81 ng/mL) but 23% higher AUC (327.35 ng.h/mL vs. 265.71 ng.h/mL) compared to 12 mg SC formulation (study MNPK1117). After correcting for the dose different, the dose adjusted relative bioavailability of oral route of administration of MNTX is approximately 3-4% of SC route of administration. Tmax of the oral MNTX is longer than the tmax of SC administration (1.5-2 hours for oral vs. 0.25 hr for SC).

Specific Populations:

Pediatric: No studies were conducted in pediatric patients. A full waiver request for all ages of pediatric population has been submitted in this application due to the low number of pediatric patients with chronic opioid use. DGIEP agrees with this full waiver request.

Hepatic Impairment: Following a single oral dose administration of 3 x 150 mg MNTX tablet, the exposure (both AUC and Cmax) of MNTX in subjects with hepatic impairment are significantly higher than the exposure in subjects with normal hepatic function. The mean MNTX Cmax in subjects with Child-Pugh A, B and C hepatic impairment was 1.7-, 4.8- and 3.7-fold higher than that of in subjects with normal hepatic function, respectively. AUCs of MNTX were similar between the healthy subjects and Child-Pugh A subjects where the AUC of subjects with Child-Pugh B and C hepatic impairment were approximately 2.5- and 2.2- fold higher than that of healthy subjects, respectively. For MNTX metabolites methyl-6α-naltrexol and methyl-6β-naltrexol, the exposures were approximately 3-fold lower in subjects with hepatic impairment than in healthy subject. The sponsor is proposing no dose adjustment in patients with Child-Pugh Class A and proposing 150 mg tablet (reducing the dose by 3-fold) in patients with Child-Pugh Class B and C. The sponsor's proposal is acceptable for the following reasons:

- Safety profile of 1.7-fold higher Cmax in patients with Child-Pugh Class A is adequately covered by the safety profile of currently marketed SC formulation which has approximately 5-fold higher Cmax compared to oral 450 mg MNTX product.
- MNTX has linear PK between 150 mg to 450 mg oral dose.
- Efficacy is primarily driven by the parent drug with some possible contribution of metabolite methyl-6α-naltrexol.

In the hepatic impairment study with SC administration in healthy subjects and patients with mild and moderate hepatic impairment (n=8 per group), the Cmax and AUC in patients with mild and moderate hepatic impairment were similar to those of healthy subjects. Therefore, no dose adjustment was recommended in patients with mild and moderate hepatic impairment for SC formulation. The effect of severe hepatic impairment on the PK of SC methylnaltrexone was not evaluated. In this submission, the sponsor is proposing to monitor for methylnaltrexone-related adverse reactions in patients with severe hepatic impairment for SC formulation and if considering dose adjustment, reduce the SC dose by half based on the weight of the patients. The sponsor's proposal appears to be reasonable based on the available data from the hepatic impairment study with oral formulation in which moderate and severe hepatic impairment have similar degree of effect on the PK of methylnaltrexone. Nonetheless, efficacy of SC cannot be assured at lower dose as efficacy of SC for OIC was only evaluated and established at 12 mg.

Renal Impairment: There was no dedicated PK study to evaluate the effect of renal impairment on the PK of methylnaltrexone following oral dose administration of MNTX tablets. A renal impairment study was conducted previously in NDA 21964 following a single SC administration of 0.30 mg/kg MNTX dose and had shown that the Cmax did not change significantly as a function of renal function while the AUC increased by 30%, 75% and 90% in patients with mild, moderate and severe renal impairment, respectively, compared to the subjects with normal renal

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function. The current label for SC formulation recommends reducing the dose by half for SC administration for patients with severe renal impairment only. (b) (4) [redacted] Therefore, the agency recommends 150 mg dose in patients with severe renal impairment as the expected exposure from this dose is comparable to the exposure from 300 mg dose in subjects with normal renal function. Please note that 300 mg dose had demonstrated statistically significant efficacy over placebo in phase 3 trials. In addition, since the extent of increase in AUC in patients with moderate renal impairment (75%) is similar to that of severe renal impairment (90%), we recommend the same dose adjustment, 150 mg, for the patients with moderate renal impairment.

In-vitro Drug-Drug Interaction Evaluation:

CYP Inhibition /Induction by the Parent Drug Methylnaltrexone:
Inhibition and induction of CYP enzymes by the parent drug methylnaltrexone was already addressed previously in NDA 21964 for SC formulation. Methylnaltrexone did not significantly inhibit (up to 100 uM) or induce (25 uM) the activity of cytochrome P450 (CYP) isozymes CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, or CYP3A4, while it was a weak inhibitor of CYP2D6. In addition, methylnaltrexone did not induce CYP2E1. As the Cmax of 450 mg oral dose is (32-54 ng/mL = 73-124 nM) is about 200 fold lower than the highest previously tested concentration for inhibition (100 uM) and induction (25 uM), the lack of interaction of methylnaltrexone with CYP enzyme from previous studies is still applicable to the 450 mg oral dose to rule out the potential of methylnaltrexone to inhibit or induce CYP enzymes in the liver.
The available data from previous studies is not adequate to rule out the potential of methylnaltrexone to inhibit CYP3A4 in the gut when methylnaltrexone is administered as 450 mg oral tablet. Potential of methylnaltrexone to inhibit CYP3A4 was evaluated only up to 100 uM and no inhibition was observed at that concentration (IC50 >100 uM). As Igut is approximated to be 4128 uM, the potential of methylnaltrexone to inhibit CYP3A4 in the gut cannot be ruled out with the limited available data when methylnaltrexone is given as 450 mg oral dose. However, there is no strong scientific evidence either to suggest the potential of MNTX to inhibit CYP3A4 in gut since there is no trend of inhibition of CYP3A4 with increasing concentration of MNTX when MNTX was evaluated as an inhibitor of CYP3A4 across concentration of 0.015 to 100 uM. Therefore, IC50 is much greater than 100 uM.

CYP Inhibition/Induction by the Metabolites:
Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol do not induce CY1A2, CYP2B6 and CY3A4/5 enzyme at both mRNA level and enzyme activity level in freshly isolated human hepatocytes at up to 60 μM concentration, which is approximately 1000-fold higher than expected Cmax of these metabolites at the clinical dose of 450 mg MNTX oral tablets.

Methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol did not cause direct, time-dependent or metabolism-dependent inhibition of CYP1A2, CYP2B6 ,CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 in human liver microsomes at concentrations up to 4500 ng/mL for methylnaltrexone sulfate, (200-fold higher than Cmax) , 1000 ng/mL for methyl-6α-naltrexol (80-fold higher than Cmax) and 500 ng/mL for methyl-6β-naltrexol (100-fold higher than Cmax), which is approximately 80-200-fold higher than expected Cmax of these metabolites at the clinical dose of 450 mg MNTX oral tablets.

Substrate for Transporters:

Methylnaltrexone and metabolites were investigated as substrates of the transporters P-gp, BCRP, OCT1, OCT2, OAT1, OAT3, OATP1B1, OATP1B3, MATE1, and MATE2-K.
In previous studies in NDA 21964 for SC formulation, it was already demonstrated that methylnaltrexone was a substrate of OCT1 but not a substrate of OAT1or P-gp.

In this submission, it was shown that methylnaltrexone and its metabolites methyl-6α-naltrexol, and methyl-6β-naltrexol are substrates for OCT1 and OCT2. In addition, methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol appears to be substrates for MATE1 and MATE2-K. In a previously conducted in vivo clinical drug interaction study with cimetidine, an inhibitor of OCT1, OCT2 , MATE1 and MATE2-K, Cmax and AUC of methylnaltrexone increased by 10% when 24 mg methylnaltrexone as an IV infusion over 20 minutes was administered before and after multiple doses of cimetidine 400 mg every 6 hours. The lack of significant interaction with cimetidine from the previous studies alleviates the need for a further in-vivo study to address the potential interaction of methylnaltrexone and its metabolites with OCT2, MATE1 and MATE2-K.
Metabolites methylnaltrexone sulfate and methyl-6α-naltrexol could potentially be substrate for BRCP. However, efflux ratio of methylnaltrexone sulfate and methyl-6α-naltrexol across MDCKII-BCRP cells and their respective uptake inhibition by BCRP model inhibitor were not consistent across different concentration (0.3 uM, 1 uM and 10 uM).

Inhibition of Transporters:
Methylnaltrexone and metabolites were investigated as inhibitors of the transporters P-gp, BCRP, OCT1, OCT2, OAT1, OAT3, OATP1B1, OATP1B3, MATE1, and MATE2-K.
Methylnaltrexone did not inhibit P-gp, BCRP (up to 500 uM), MRP2 (up to 100 uM), OATP1B1 and OTAP1B3 (up to 1800 uM). OCT2 was inhibited and IC50 was estimated to be greater than 100 uM. Estimated IC50 was greater than 10 uM for OCT1, OCT2, OAT1, OAT3, MATE1 and MATE2-K. For all metabolites, the IC50 values are estimated to be greater than the highest tested concentration of 10 uM. Based on Cmax values and estimated IC50 values for all transporter, it is unlikely that there will be any in-vivo inhibition of transporters by methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol.

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?

Methylnaltrexone bromide is white to almost white crystalline powder with molecular formula of $C_{21}H_{26}NO_4Br$ and a molecular weight of 436.36 g/mol. Methylnaltrexone bromide is being developed as oral tablet at 150 mg strength in this application.

Figure 1: Structure of Methylnaltrexone bromide

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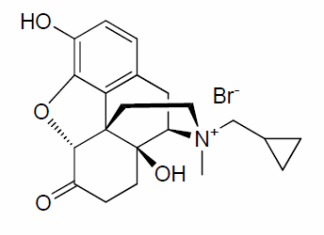

Dissociation Constant: pKa = 8.34

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

The proposed mechanism of action is that methylnaltrexone is a peripherally –acting selective mu-opioid receptor antagonist with restricted ability to cross the blood-brain barrier which allow it to act on tissues such as the gastrointestinal tract, thereby decreasing the constipating effects of opioids without impacting opioid-mediated analgesic effects on the central nervous system.

The proposed indication for Relistor oral tablet is treatment of opioid-induced constipation in adult patients with chronic non-cancer pain.

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

The proposed dose of methylnaltrexone bromide tablet is 450 mg (3 tablets of 150 mg tablet) once daily by oral route of administration at least 30 minutes before meal.
The sponsor's proposed oral dose is (b) (4) and 150 mg once daily in patients with moderate to severe hepatic impairment.

2.1.4 What is the regulatory background?

Original NME for methylnaltrexone was approved on 04/28/2008 (NDA 21964) as subcutaneous (SC) injection with a brand name of Relistor for treatment of opioid-induced constipation in patients with advanced illness who are receiving palliative care, when response to laxative therapy has not been sufficient. Subsequently, an efficacy supplement was approved for an additional indication for treatment of opioid-induced constipation (OIC) in adult patients with chronic non- cancer pain for methylnaltrexone 12 mg SC injection on 09/29/2014.
In this submission, the sponsor had developed an oral formulation of methylnaltrexone as a tablet and proposing for an indication of treatment of opioid induced constipation (OIC) in adult patients with chronic non-cancer pain only.

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 7 phase I studies, one phase III efficacy and safety study and 8 *in-vitro* studies. Please see table 1 and table 2 for more detailed information.

Phase I clinical pharmacology program included relative BA/BE studies, food effect studies, hepatic impairment study. In addition, it had one phase 1b study in patient population to evaluate the PK, efficacy and safety of TBM formulation vs. phase 3 formulation after a single dose administration. A PK/PD modeling was also evaluated from the result of this study. *In-vitro* studies characterized the protein binding, metabolism pathway, and substrate, inhibitor or inducer potential of methylnaltrexone and its metabolites for various enzymes and transporters. Additionally, three bioanalytical method validation reports along with 10 additional addendum

and amendments to analyze the concentration of methylnaltrexone and its 3 metabolites in plasma and urine were submitted.

One randomized, double-blinded, parallel-group, placebo-controlled, multi-center, phase III study (MNTX3201) evaluated efficacy and safety of 150 mg, 300 mg and 450 mg QD dosing in patients with opioid-induced constipation with chronic non-cancer pain. Duration of treatment in this study was 28 days of once daily dosing (QD) followed by 56 days of dosing as need (PRN). Pharmacokinetic was not evaluated in this phase 3 study.

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

Study #	**Objective(s)**	**Study Design**	**Test product; Dosage Regimen; Route of administration**	**Subjects**
MNPK1001	Formulation Exploration BE study (includes pivotal BE study)	Open-label, multiple-cohort, single-dose, two-way crossover	Single doses of 450 mg (3 x 150 mg) MNTX oral tablets from 6 test formulations. The reference tablet was the (b) (4) formulation used in Study MNTX 3201	180 healthy subject
MNFE1001	Food effect	Open label, single dose, crossover	Single dose of oral 450 mg (3x150 mg) MNTX (b) (4) tablets (TBM)	32 Healthy subjects
MNPK1114	Food effect	Open label, single dose, crossover	Single dose of 450 mg (3 x 150 mg) MNTX oral tablets ((b) (4) formulation)	32 Healthy subjects
MNPK1004	Hepatic impairment	Open label, single dose, parallel group	Single dose of 450 mg (3 x 150 mg) MNTX oral (b) (4) tablets (TBM)	24 Hepatic impaired subjects (mild, moderate, and severe) and matched healthy subjects
MNOC1111	PK, efficacy and Safety in Patient population, Phase 1b.	Open Label Phase: Open-label, single SC dose, to assess for clinical response Double Blind Phase (OL responders only): Randomized, placebo-controlled, single-dose	Open Label Phase: • Single-dose of 12 mg SC MNTX Double Blinded Phase: • 450 mg (3 x 150 mg) MNTX (b) (4) tablets (TBM) • 450 mg (3 x 150 mg) MNTX (b) (4) tablets (phase 3 formulation) • matching placebo tablets	138 Patients with chronic non-cancer pain who have OIC
MNPK1117	Relative BA to SC formulation	Open label, cross-over	Single doses of oral MNTX tablets • 150 mg (1 × 150 mg tablet) • 300 mg (2× 150 mg tablets) • 450 mg (3 × 150 mg tablets) Single doses of MNTX SC 12 mg	48 Healthy subjects

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MNPK1118	Multiple dose PK study (stage 1) Relative BA study (stage 2)	Open-label, 2-stage, single and multiple-dose	Stage 1: Once daily doses of 450 mg (3 x 150 mg) MNTX oral film-coated tablets for 7 days; Stage 2: Single dose of 450 mg (3 x 150 mg) MNTX oral film-coated tablets (test formulation) single dose of 450 mg (3 x 150 mg) MNTX uncoated oral tablets with (b) (4) (phase 3 formulation)	16 healthy subjects in Stage 1 16 healthy subjects in Stage 2
MNTX 3201	Efficacy, safety & tolerability	Randomized, double-blind, parallel-group, placebo-controlled	Oral MNTX 150 mg tablets ((b) (4) formulation) or placebo; Randomized 1:1:1:1 to 150 mg, 300 mg, 450 mg, or placebo; Dosing QD during the first 4 weeks and PRN for the remaining 8 weeks	804 Patients with chronic non- cancer pain who have OIC

Table 2: In-Vitro Studies

Study Identifier	**Type of Study**	**Title/Objective(s) of the Study**
XS-0641	Protein Binding	Assessment of methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol protein binding in human plasma
XT128007	Transporter	Evaluation of Methylnaltrexone as an Inhibitor of Human P-gp, BCRP, OCT2 and MRP2 and Substrate of Human BCRP, MRP2, OATP1B1, OATP1B3, OAT1 and OAT3 Transporters
XS-0621	Transporter	Evaluation of Methylnaltrexone as an Inhibitor of OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K and Substrate of MATE1 and MATE2-K; Evaluation of Methylnaltrexone Sulfate, Methyl-6α-Naltrexol and Methyl-6β-Naltrexol as Inhibitors and Substrates of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K
XT123038	CYP induction by metabolites	Evaluation of Methylnaltrexone Sulfate, Methyl-6α-Naltrexol and Methyl-6β-Naltrexol as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes
XT125033	CYP inhibition by metabolites	In Vitro Evaluation of Methylnaltrexone Sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol as Inhibitors of Cytochrome P450 (CYP) Enzymes in Human Liver Microsomes
RPT-63601	Direct Glucuronidation	Evaluation of the Potential For Direct Glucuronidation of N-Methylnaltrexone (MNTX) In Human, Sprague-Dawley Rat, And Beagle Dog Microsomes
RPT-63643	Metabolism by CYP450	Identification of Cytochrome P450 Drug-Metabolizing Enzymes Involved in the Metabolism of N-Methylnaltrexone Utilizing CDNA-Expressed Human Enzymes
RPT-63756	Metabolic Stability	Metabolic Stability Study of Methylnaltrexone in Rat, Dog, And Human Hepatic Microsomes

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 opioid-induced constipation in adult patients with chronic non-cancer pain. Accordingly, evaluation of clinical efficacy of methylnaltrexone focused resolving constipation.

The primary and secondary endpoints in the phase III trial MNTX3201 were as following:

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- Primary: Average percentage of dosing days that resulted in rescue-free bowel movements (RFBMs) within 4 hours of dosing during Weeks 1 – 4. RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement.
- Key secondary efficacy endpoints in hierarchical order:
    - Responder endpoint: Proportion of subjects who responded to study drug during Weeks 1 to 4, where a responder is defined as ≥ 3 RFBM/week, with an increase of ≥ 1 RFBM/week over baseline, for ≥ 3 out of the first 4 weeks of the treatment period.
    - Change in weekly number of RFBMs from baseline over the entire first 4 weeks (28 days) of dosing.

Note that the primary efficacy endpoint has not been agreed upon by the agency and recommendations were made during development to change the primary endpoint (See the medical review by Dr. Dina Zand for further details). As such the agency is considering the key secondary endpoint to be more clinically relevant for approval as it is consistent with prior approvals for Relistor and other products in this class for opioid induced constipation.

The primary and secondary endpoints in phase 1b study with only a single dose administration were slightly different than those in phase III study.

- Primary: Proportion of subjects with a RFBM within 4 hours after a single oral dose of study drug (double-blind treatment phase). A RFBM was defined as a bowel movement with no laxative use within 24 hours prior to the bowel movement.
- Secondary:
    - Clinical equivalence of the phase 3 formulation and the to-be-marketed formulation with regard to the primary efficacy endpoint
    - Proportion of subjects with a RFBM within 24 hours after the single oral dose of study drug.
    - Time to the first RFBM within 24 hours after the single oral dose of study drug.
    - Additional efficacy endpoints evaluate stool consistency (Bristol Stool Form Scale), straining, and sensation of complete evacuation and are presented in the body of the study report

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 methylnaltrexone and its 3 metabolites in plasma and urine were measured by appropriately validated LC-MS/MS bioanalytical methods. Please see the analytical section 2.9 for more details.

2.3 Exposure-Response Evaluation

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

The sponsor had evaluated the safety and efficacy of three dose levels, 150 mg, 300 mg and 450 mg, of oral MNTX tablet formulation in a phase 3, multicenter, randomized, double-blind, placebo-controlled, parallel-group study in subjects with chronic nonmalignant pain who have OIC.

The applicant's primary and key secondary endpoint findings for the phase 3 trial are shown in Table 3, Figure 2 and Table 4. The primary efficacy endpoint has not been agreed upon by the agency and recommendations were made during development to change the primary endpoint

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(See the medical review by Dr. Dina Zand for further details). As such, the agency is considering the key secondary endpoint to be more clinically relevant as it is consistent with prior approvals for Relistor and is recommended as the primary endpoint for other products being developed in this class for opioid induced constipation. This is in part due to the more durable nature of the endpoint, considering response over the double blind duration of the trial.

The applicant's primary efficacy endpoint is defined as:

> "The primary efficacy endpoint was the average percentage of dosing days that resulted in RFBMs within 4 hours of dosing during Weeks 1 – 4. A RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement. The weekly number of RFBMs was calculated as 7 × total RFBMs in a week/all non-missing assessment days."

The applicant's key secondary efficacy endpoint is defined as:

> "Responder endpoint: Proportion of subjects who responded to study drug during Weeks 1 to 4, where a responder is defined as ≥ 3 RFBMs/week, with an increase of ≥ 1 RFBM/week over baseline, for ≥ 3 out of the first 4 weeks of the treatment period."

A dose-proportional increase in key secondary efficacy endpoint was observed over 150 mg to 450 mg doses, and both 300 mg and 450 mg dose have shown statistically significant improvement over placebo.

Table 3: Applicants Primary Efficacy Endpoint for the phase 3 trial MNTX-3201: Average Percentage of Dosing Days that Resulted in RFBMs within 4 Hours of Dosing during Weeks 1 – 4 (ITT Population; Observed Cases)

	Placebo	MNTX 150 mg	MNTX 300 mg	MNTX 450 mg
Percentage (%) of dosing days that resulted in RFBMs within 4 hours of dosing during Weeks 1 – 4				
N	201	201	201	200
Mean (SD)	18.18 (16.995)	21.05 (20.116)	24.64 (21.311)	27.40 (23.453)
Median (min, max)	14.29 (0.0, 92.9)	14.81 (0.0, 96.4)	21.43 (0.0, 92.9)	23.54 (0.0, 100.0)
LS mean[a]	18.23	21.06	24.75	27.37
LS mean difference (vs placebo)[a]	—	2.83	6.51	9.13
95% CI for difference (vs placebo)[a]	—	-1.21, 6.86	2.47, 10.55	5.09, 13.18
Raw p-value (vs placebo)[a]	—	0.1692	0.0016	< 0.0001
Nonparametric analysis p-value[b]	—	0.1185	0.0010	< 0.0001
Linear dose response[a]				< 0.0001
Quadratic dose response[a]				0.9426

Source: Table 14.2.1.1a and 14.2.1.1c. Abbreviations: ANCOVA = analysis of covariance; CI = confidence interval; ITT = intent-to-treat; LS = least squares; Max = maximum; Min = minimum; MNTX or MOA-728methylnaltrexone; RFBM = rescue-free bowel movement.

Notes: RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement. If a subject did not receive concomitant daily opioid treatments during the entire treatment period, the subject was considered to have no RFBMs for the treatment period.

a From ANCOVA model with treatment as effect and analysis region as covariate.

b Based on nonparametric ANCOVA analysis with treatment as effect and analysis region as strata, using macro (NParCov) developed by Gary Koch and Richard Zink.

Figure 2: Applicants Primary Efficacy Endpoint for the phase 3 trial MNTX-3201: Primary Efficacy Endpoint: Average Percentage of Dosing Days that Resulted in RFBMs within 4 Hours of Dosing During Weeks 1 – 4 (ITT Population)

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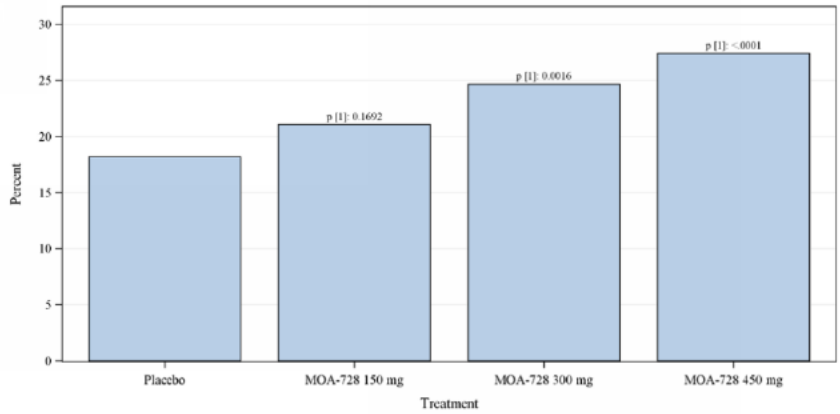


(Source: Applicant's Clinical Study Report, Trial MNTX-3201, Figure 5)

Table 4: Applicant's First Key Secondary Efficacy Endpoint (Endpoint of FDA Focus): Proportion of Subjects Responding to Study Drug during Weeks 1-4 (ITT Population, Study MTX-3201)

Responders[a]	Placebo	MNTX 150 mg	MNTX 300 mg	MNTX 450 mg
N	201	201	201	200
LOCF Approach[b]				
Yes, n (%)	82 (40.8)	91 (45.3)	104 (51.7)	109 (54.5)
Percentage Difference (vs Placebo)	—	4.5	10.9	13.7
95% CI for Percentage Difference (vs Placebo)	—	-5.2, 14.1	1.3, 20.6	4.0, 23.4
Odds Ratio (Active/Placebo)[c]	—	1.23	1.54	1.79
95% CI for Odds Ratio[c]	—	0.82, 1.84	1.03, 2.29	1.20, 2.66
Raw p-value (vs Placebo)[c]	—	0.3076	0.0339	0.0043
Worst Case Approach[d]				
Yes, n (%)	77 (38.3)	86 (42.8)	99 (49.3)	103 (51.5)
Percentage Difference (vs Placebo)	—	4.5	10.9	13.2
95% CI for Percentage Difference (vs Placebo)	—	-5.1, 14.1	1.3, 20.6	3.5 22.8
Odds Ratio (Active/Placebo)[c]	—	1.23	1.55	1.77
95% CI for Odds Ratio[c]	—	0.82, 1.84	1.04, 2.32	1.19, 2.65
Raw p-value (vs Placebo)[c]	—	0.3185	0.0321	0.0052

Source: Table 14.2.2.1a. Abbreviations: CI = confidence interval, ITT = intent-to-treat, LOCF = last observation carried forward; MNTX or MOA-728 = methylnaltrexone, RFBM = rescue-free bowel movement.

a A responder was defined as having ≥ 3 RFBMs/week, with ≥ 1 RFBM/week increase from baseline for ≥ 3 weeks of Weeks 1–4. The weekly number of RFBMs was calculated as 7 × total RFBMs in a week/all nonmissing assessment days in the week. The weekly number of RFBMs was set to missing for any week when the subjects completed < 4 diary days. RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement. If a subject did not receive concomitant daily opioid treatments during a week, the subject was considered no change from baseline for the week.

b LOCF approach: the last nonmissing 4 days with diary data was imputed for the week with < 4 diary days.

c Based on logistic regression model with treatment as effect and analysis region as a covariate.

d Worst case approach: missing weeks were classified as nonresponse for that week.

(Source: Applicant's Clinical Study Report, Trial MNTX-3201, Figure 5)

Table 5: Cross-Study Comparison of Efficacy with SC formulation: Key Secondary Efficacy Endpoint (Endpoint of FDA Focus): Proportion of Subjects Responding to Study Drug during Weeks 1-4

	N	Yes, N(%)	% difference from placebo	P-value
Oral Relistor Program (*Study MTX-3201*)				
Placebo	201	82 (40.8%)	--	
150 mg MNTX	201	91 (45.3%)	4.5%	0.3076

300 mg MNTX	201	104 (51.7%)	10.9%	0.0339
450 mg MNTX	200	109 (54.5%)	13.7%	0.0043
SC Relistor Program				
Placebo	162	62 (38%)		
12 mg SC MNTX	150	88 (59%)	20%	<0.001

Methylnaltrexone Pharmacokinetic Exposures by Dose:

Table 6 indicates the AUC and Cmax values as the mean value for the respective dose for various studies. A range indicates the range of mean values for each study with that dose. Presenting the data in this way is intended to illustrate the PK variability where relevant.

Two notable points from an exposure perspective regarding the dose are:

1) There is approximately a 4-fold difference in Cmax between the proposed dose of 450 mg PO compared to the approved 12 mg SC dose
2) Above 450 mg a less than proportional increase in exposure suggests increasing dose may not yield higher exposures. Cmax and AUC do not appear to increase for 17 subjects with 600 mg dose

Table 6: Pharmacokinetic Exposures of Methylnaltrexone Across the Range of Studied Oral Doses in Comparison to the 12 mg SC Dose.

	150 mg PO	300 mg PO	450 mg PO DC	600 mg PO	12 mg SC
AUC (ng·hr/mL)	107	231	105[1] - 373[2]	105[3]	186 - 269
Cmax (ng/mL)	13.2	26.2	35[1] – 48[3]	44[3]	133 - 174

(Source: Applicant's Summary of Clinical Pharmacology, Studies [1]MNOC1111, [2]MNPK1117, [3]MNTX-2201)

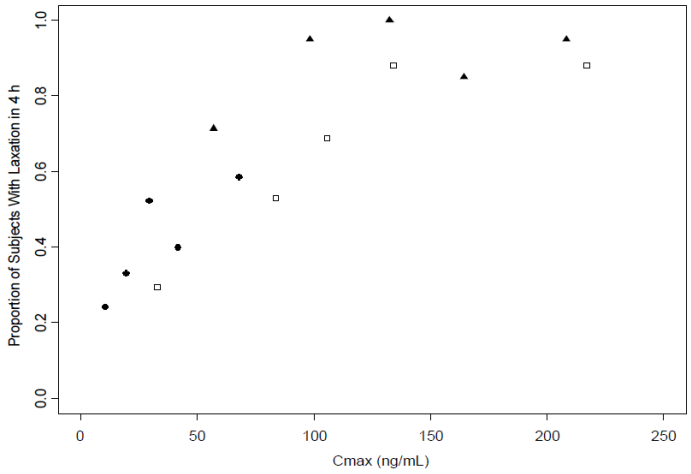
Additionally, the exposure-response for the applicant's primary endpoint indicates the efficacy with the oral dose appears lower than with the IV or SC formulations (Figure 3).

Several aspects with regards to exposure-response and dose response prevent conclusions being drawn from these analyses which make the interpretation of these analysis difficult:

- Exposure-Response was developed on Phase 1 and 2 studies for only the Primary Endpoint and this included non-relevant patient populations (i.e. methadone maintenance and post-surgery pain).
- PK data was not collected in Phase 3 trial MNTX-3201 to use this data for exposure-response analyses.
- Exposure response for the key secondary endpoint (FDA's clinically relevant endpoint): The secondary endpoint data was not collected in the Phase 2 trials where the PK information was available, so the applicant simulated PK for the phase 3 study, where the secondary endpoint was available, to conduct this analysis. This approach would be acceptable when there is a good covariate model to explain inter-individual variability and the unexplained variability is small (i.e. the covariates explain majority of the difference in PK between subjects). However, the applicant did not provide sufficient description of their PK model in their response to justify these simulations.

- The exposure response plot for primary endpoint (Figure 3) over predicts the observed response in each dose group for the phase 3 study when considering the expected Cmax exposure for each dose group in Table 6.

Figure 3: Observed Proportion of Subjects Who Experienced Laxation Within 4 Hours After Administration of MNTX Versus the Midpoint of Each Cmax Quintile By Route of Administration



Circles – oral administration
Triangles – subcutaneous administration
Open squares – intravenous administration

(Source: Applicant's Summary of Clinical Pharmacology, Figure 6)

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

In dose-ranging phase 3 study MNTX3201, there appears to be no dose-response for the majority of adverse event categories (Table 7). However, dose-response is evident for abdominal pain and diarrhea (Figure 4) which is consistent with the mechanism of action and experience with other products in this class of drugs. Based on the cross trial comparison, the rate of the abdominal pain AEs does not exceed that of SC 12 mg relistor. Therefore, the safety profile appears reasonable for the 450 mg dose. Please refer to the clinical review by Dr. Dina Zand for a detailed review of safety findings.

Table 7: Treatment-Emergent Adverse Events in ≥ 2% of Subjects Treated with MNTX or Placebo (Safety Population, Trial 3201).

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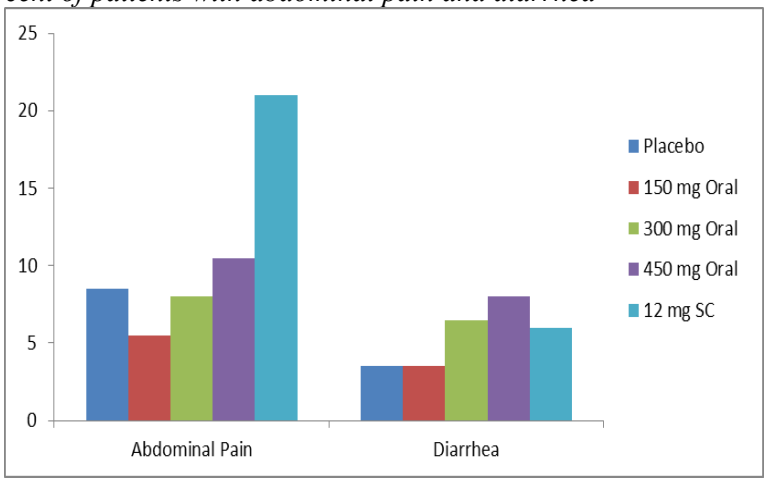
System Organ Class Preferred Term	Placebo n (%)	MNTX 150 mg n (%)	MNTX300 mg n (%)	MNTX 450 mg n (%)	All MNTX n (%)
Subjects Randomized (N)	**201**	**201**	**201**	**200**	**602**
Any system organ class	127 (63.2)	117 (58.2)	120 (59.7)	118 (59.0)	355 (59.0)
Gastrointestinal disorders					
Abdominal discomfort	4 (2.0)	2 (1.0)	0	1 (0.5)	3 (0.5)
Abdominal distension	6 (3.0)	6 (3.0)	3 (1.5)	7 (3.5)	16 (2.7)
Abdominal pain	17 (8.5)	11 (5.5)	16 (8.0)	21 (10.5)	48 (8.0)
Abdominal pain upper	7 (3.5)	4 (2.0)	6 (3.0)	6 (3.0)	16 (2.7)
Diarrhea	7 (3.5)	7 (3.5)	13 (6.5)	16 (8.0)	36 (6.0)
Flatulence	9 (4.5)	11 (5.5)	7 (3.5)	10 (5.0)	28 (4.7)
Nausea	18 (9.0)	13 (6.5)	16 (8.0)	12 (6.0)	41 (6.8)
Vomiting	9 (4.5)	3 (1.5)	6 (3.0)	7 (3.5)	16 (2.7)
Infections and infestations					
Influenza	5 (2.5)	4 (2.0)	6 (3.0)	2 (1.0)	12 (2.0)
Sinusitis	4 (2.0)	5 (2.5)	7 (3.5)	2 (1.0)	14 (2.3)
Upper respiratory tract infection	9 (4.5)	9 (4.5)	7 (3.5)	8 (4.0)	24 (4.0)
Urinary tract infection	7 (3.5)	7 (3.5)	8 (4.0)	7 (3.5)	22 (3.7)
Injury, poisoning, and procedural complications	9 (4.5)	12 (6.0)	9 (4.5)	11 (5.5)	32 (5.3)
Muscle strain	4 (2.0)	0	2 (1.0)	1 (0.5)	3 (0.5)
Musculoskeletal and connective tissue disorders					
Arthralgia	4 (2.0)	7 (3.5)	5 (2.5)	4 (2.0)	16 (2.7)
Back pain	7 (3.5)	12 (6.0)	6 (3.0)	5 (2.5)	23 (3.8)
Nervous system disorders					
Headache	8 (4.0)	2 (1.0)	8 (4.0)	9 (4.5)	19 (3.2)
Tremor	1 (0.5)	7 (3.5)	4 (2.0)	3 (1.5)	14 (2.3)
Psychiatric disorders	9 (4.5)	11 (5.5)	19 (9.5)	10 (5.0)	40 (6.6)
Anxiety	3 (1.5)	6 (3.0)	9 (4.5)	7 (3.5)	22 (3.7)
Respiratory, thoracic, and mediastinal disorders					
Rhinorrhea	3 (1.5)	5 (2.5)	4 (2.0)	4 (2.0)	13 (2.2)
Skin and subcutaneous tissue disorders					
Hyperhidrosis	4 (2.0)	6 (3.0)	8 (4.0)	6 (3.0)	20 (3.3)
Vascular disorders	6 (3.0)	5 (2.5)	5 (2.5)	5 (2.5)	15 (2.5)
Hot flush	4 (2.0)	2 (1.0)	2 (1.0)	2 (1.0)	6 (1.0)

(Source: Applicant's Clinical Study Report, Trial 3201, Table 24)

Figure 4: Cross-Study Comparison of Safety of Oral MNTX (Study MNTX3201) with SC formulation: Percent of patients with abdominal pain and diarrhea



2.3.3 Does this drug prolong the QT or QTc interval?

No QT studies were conducted with oral Relistor tablet. Based on the information from the previous submission to Relistor SC, methylnaltrexone does not prolong the QTc interval to any clinically relevant extent when 0.3 mg/kg and 0.64 mg/kg doses of methylnaltrexone bromide administered by intravenous infusion over 20 minutes. As the Cmax of 450 mg oral tablet is about 22-fold lower than the Cmax of 0.64 mg/kg IV infusion that did not prolong QT, potential of oral Relistor to prolong QT is minimal.

Table 8: Exposure comparison for QT analysis:

	0.30 mg/kg IV infusion[a]	0.64 mg/kg IV infusion[a]	0.15 mg/kg SC	12 mg SC	450 mg oral tablet (TBM)
Cmax (ng/mL)	462 ± 82	1062 ± 258	117 ± 32.7	140 ± 35.6	48.14 ± 41
AUC24 (ng·hr/mL)	335 ± 50	739 ± 139	175 ± 36.6	218 ± 28.3	
AUCinf (ng·hr/mL)	340 ± 52	748 ± 142			382.19 (193)

a: PK values were taken from QT study report CSR-71901 to NDA 21964

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?

Yes, the proposed dose appears to be acceptable as higher doses are not expected to give greater exposures via oral administration (Table 6)) while lower doses would result in lower efficacy compared to 450 mg which is already lower than already approved the efficacy observed with 12 mg SC injection of methylnaltrexone. Additionally the difference in response rate between the 150 mg and 300 mg doses (6.4% for the key secondary endpoint and 3.7% for the applicant's primary endpoint) is greater than between the 300 mg and 450 mg doses (2.8% for the key secondary endpoint and 2.6% for the applicant's primary endpoint) suggesting that increasing the dose above 450 mg will yield less additional benefit. Secondly lower doses (i.e. 150, 300 mg) appear to be less ideal in that their efficacy is reduced compared to the 450 mg dose (*(Source: Applicant's Summary of Clinical Pharmacology, Studies [1]MNOC1111, [2]MNPK1117, [3]MNTX-2201)* , Table 4). While acknowledging the concerns related to cross study comparison of efficacy, the efficacy of oral 450 mg Relistor is already reduced when compared to the approved SC formulation dose of 12 mg (13% difference from placebo for oral 450 mg compared to a 20% difference from placebo for 12 mg SC dose). While there are exposure-response relationships for the adverse events abdominal pain and diarrhea (Table 7 and Figure 4), the AE rates for the high oral dose are lower than the approved SC dose.

2.3.5 Do Patients with abdominal pain have a higher response rate and can potentially benefit from a lower dose of relistor?

No, patients with abdominal pain AEs do not exhibit a higher response rate. Using the four week secondary responder endpoint (proportion of subjects who responded to study drug during weeks 1 to 4 where a responder is defined as ≥3 RFBMs/week, with an increase of ≥1 RFBM/week over baseline, for ≥3 out of the first 4 weeks of the treatment period), the response rate for patients with abdominal pain was 30.1% compared with 28.6% for subjects without abdominal pain. This analysis was performed based on the hypothesis that abdominal pain was associated with either too much antagonism of the gut opioid receptor or that abdominal pain was reflective of GI motility.

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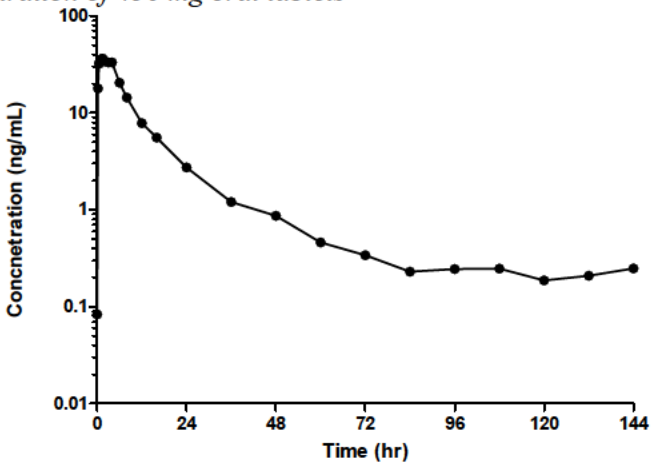
PK characteristics of drug

Following single dose administration of 450 mg MNTX tablet, C_{max} of methylnaltrexone was reached approximately between 1.5-2.5 hours with C_{max} of 43-55 ng/mL. Methylnaltrexone has approximately dose-proportional increase in C_{max} and AUC between the oral doses of 150 mg to 450 mg MNTX. Daily oral dosing of 450 mg does not result in a significant accumulation of methylnaltrexone (10% increase in AUC and 3% decrease in Cmax on Day 7 compared to Day 1). The plasma concentration of methylnaltrexone appears to decline in multi-phasic manner with mean terminal half-life of 14-16 hr. The apparent oral plasma clearance (CL/F) ranged from approximately 1404 to 1744 L/h. Mass balance study was not conducted with the oral formulation. PK variability of methylnaltrexone was relatively high (%CV up to 80% for Cmax and 56% for AUC). The percent of drug recovered in urine as unchanged drug is less than 1.78%. The renal clearance is estimated to be approximately 306.2 mL/min.

Figure 5: Mean Plasma Concentration (ng/mL) Vs. Time Profiles of methylnaltrexone after Single dose Administration of 450 mg oral tablets



The AUC ratio of metabolites to the parent drug at steady-state on Day 7 were 79%, 38.5% and 21.4% for methylnaltrexone-sulfate (M2), methyl-6α nalthrexol (M4), and methyl-6β nalthrexol (M5), respectively. Based on the cross-study comparison with SC administration, it appears that the systemic exposure to methylnaltrexone metabolites is greater after oral administration of 450 mg dose than SC administration of 12 mg dose where AUC_{0-24} ratios of metabolites to methylnaltrexone at steady-state after 12 mg SC daily dosing was 30%, 19%, and 9% for methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol, respectively. Based on the comparison Cmax at steady state to the ki for human mu-opioid receptor of MNTX and its major metabolites, (ki for human mu opioid receptors were 10 ng/mL, 9603 ng/mL,10.7 ng/mL and 21.5 ng/mL for methylnaltrexone, methylnaltrexone-sulfate, methyl-6α nalthrexol, and methyl-6β nalthrexol, respectively), although overall clinical efficacy of oral MNTX 450 mg dose is probably primary driven by the parent drug MNTX, the active metabolite methyl-6α-naltrexol (M4) may still have some contribution to the efficacy to a certain extent.

Figure 6: Mean Plasma Concentration (ng/mL)-Time Profiles of methylnaltrexone and its metabolites after Single dose Administration of 450 mg oral tablets

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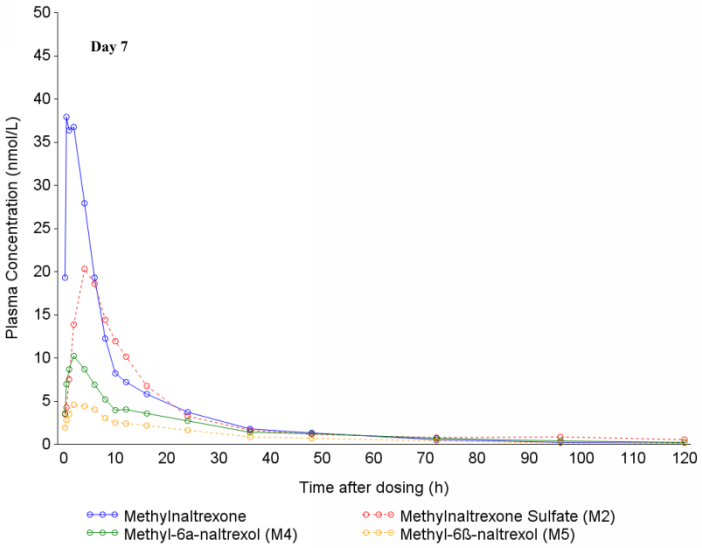

2.5.1 What are the single dose and multiple dose PK parameters of the parent drug and its relevant metabolites in healthy subjects?

Single dose PK of MNTX and its metabolites following oral administration of 450 mg MNTX was evaluated in multiple studies with multiple different oral formulations of MNTX tablets. PK parameters of MNTX and its metabolites following single dose administration appear to be consistent across different studies and across different oral formulations. Multiple-dose PK of MNTX and its metabolites following oral administration of 450 mg MNTX was evaluated only in one study (MNPK1118).

Table 9: Mean (SD) Pharmacokinetic Parameters of 450 mg MNTX oral tablet in Healthy Subjects under Fasting Condition

Study #	Formulation[b]	C_{max} (ng/ml)	T_{max} (h)[a]	AUC_t (ng*h/ml)	AUC_{inf} (ng*h/ml)	$T_{1/2}$ (h)	CL/F (L/h)	PK sampling (hr)
Single Dose PK								
MNEF1001 (n=32)	TBM	42.53 (32.36)	2.0 (0.25, 4.0)	302.69 (147.71)	305.91 (147.93)	14.17 (3.25)	1744.35 (657.17)	96
MNPK1004 (n=6)	TBM	54.70 (34.39)	2.5 (0.5 - 6.0)	435.13 (215.34)	437.53 (215.54)	16.14 (4.70)	1228 (519)	96
MNPK1001 (n=30)	TBM	48.14 (41)	1.50 (0.25, 8.00)	378.19 (191.740)	382.19 (193.411)	15.18 (5.808)	1521.43 (842.09)	144
MNPK1001 (n=178)	Phase 3	46.10 (35.825)	1.79 (0.50, 6.03)	349.71 (190.139)	353.09 (190.992)	14.79 (5.38)	1404.45 (825.13)	144
MNPK1118 (n=16)	Phase 3	35.6 (19.68)	1.0 (0.5-6.00)	278.57 (73.89)	284.84 (75.19)	16.04 (4.3)	1706.88 (549.3)	120
MNPK1118 (n=15)	Interim Stage 2	32.66 (22.19)	2.00 (0.5-6.00)	291.17 (95.44)	293.96 (95.34)	19.33 (7.4)	1696.29 (597.01)	120
MNPK1117 (n=45)	interim	39.89 (32.11)	2.00 (0.50, 6.00)	366.67 (205.71)	373.32 (207.36)	16.57 (4.42)	1664.00 (1035.93)	168
MNPK1118 (n=16)	Interim Stage 1	47.05 (25.88)	2.00 (0.50-4.03)	280.16 (125.35)	320.19 (137.46)	8.81* (2.24)	1653.45 (671.45)	24
Multiple dose PK on Day 7 following QD dosing								
MNPK1118 (n=16)	Interim Stage 1	45.50 (23.58)	2.00 (0.50-4.01)	308.89 (102.34)		19.22 (4.98)	1594.44 (482.46)	120

[a] Median time to maximum concentration (range)
[b] Formulation Definitions:

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- TBM formulation is film-coated tablet with (b) (4)
- Phase 3 formulation is uncoated tablet with (b) (4)
- Interim formulation is film-coated tablet with (b) (4) what is similar to phase 3 formulation with the exception of film-coat on outer layer

*Half-life is shorter with PK sampling of only 24 hour post-dose.

Table 10: Mean (SD) Pharmacokinetic Parameters of MNTX metabolites following oral administration of 450 mg MNTX oral tablet in Healthy Subjects under Fasting Condition

Metabolite	Study #	Formulation[b]	Day	Cmax (ng/ml)	T_{max} (h)[a]	AUCt (ng*h/ml)	AUCinf (ng*h/ml)	T1/2 (h)	PK sampling (hr)
MNTX Sulfate	MNPK1004 (n=6)	TBM	1	22.04 (9.92)	7.0 (3.0 - 8.0)	265.71 (117.05)	272.40 (130.61)	7.04 (1.66)	96
	MNPK1118 (n=16)	Interim	1	17.15 (8.09)	4.0 (4.00, 16.00)	188.18 (85.58)	216.44 (100.76)	7.19 (1.62)	24
	MNPK1118 (n=16)	Interim	7	21.00 (11.50)	4.0 (4.00, 8.00)	243.68 (137.56)		13.87 (6.92)	24
Methyl-6α-naltrexol	MNPK1004 (n=6)	TBM	1	12.00 (7.30)	4.3 (4.0 - 8.0)	143.10 (44.07)	145.41 (43.57)	16.48 (1.60)	96
	MNPK1118 (n=16)	Interim	1	9.01 (5.74)	2.00 (1.00, 4.03)	79.73 (39.06)	157.29 (113.56)	17.48 (7.21)	120
	MNPK1118 (n=16)	Interim	7	10.77 (5.22)	2.00 (1.00, 4.01)	119.61 (57.43)		31.85 (5.04)	24
Methyl-6β-naltrexol	MNPK1004 (n=6)	TBM	1	5.39 (3.15)	4.3 (4.0 - 8.0)	69.44 (23.19)	71.72 (23.17)	16.54 (5.07)	96
	MNPK1118 (n=16)	Interim	1	3.48 (2.10)	4.00 (2.00, 4.03)	40.82 (19.33)	73.74 (n=1)	18.40 (6.94)	24
	MNPK1118 (n=16)	Interim	7	4.89 (2.30)	2.00 (1.00,4.01)	66.33 (31.05)		28.65 (5.52)	24

[a] Median time to maximum concentration (range)

[b]Formulation Definitions:

- TBM formulation is film-coated tablet with (b) (4)
- Phase 3 formulation is uncoated tablet with (b) (4)
- Interim formulation is film-coated tablet with (b) (4) what is similar to phase 3 formulation with the exception of film-coat on outer layer

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

Both Cmax and AUC of methylnatrexone are approximately 27% lower in OIC patient population compared to healthy subjects. As the duration of PK sampling in patients population (24 hour post-dose) is different than the PK sampling in majority of the studies in healthy subjects (96-144 hours post-dose), AUC in OIC patients values were only compared with the healthy subject study with a comparable 24 hour PK sampling (study MNPK1118, please note that this study used Interim formulation which was shown to be bioequivalent to Phase 3 formulation in regards to AUC).

Exposure of the most abundant metabolite methylnaltrexone sulfate (M2), which has the least pharmacological activity, is similar between healthy subjects and OIC patients. Exposure (both AUC and Cmax) of methyl-6α-naltrexol (M4) and methyl-6β-naltrexol (M5), are approximately 20%-50% lower in OIC patients compared to healthy subjects.

Table 11: Mean (SD) PK parameter comparison of Methylnaltrexone and its metabolites in healthy subjects and patients population following single oral dose administration of 450 mg MNTX tablet

Population	Patient	Healthy	Patient	Healthy	Healthy	Heathy

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	(N=36)	(N=178)	(N=37)	(N=30)	(N=6)	(N=16)
Formulation*	Phase 3	Phase 3	TBM	TBM	TBM	Interim
Study	MNOC1111	MNPK1001	MNOC1111	MNPK1001	MNPK1004	MNPK1118
PK sampling time	24 hr	144	24 hr	144	96	24
Methylnaltrexone						
Cmax (ng/mL)	35.35 (28.399)	46.10 (35.825)	34.58 (27.702)	48.14 (41.000)	54.70 (34.39)	47.05 (25.88)
Tmax a (hours)	2.00 (0.50 – 4.18)	1.79 (0.50, 6.03)	1.53 (0.43 –6.23)	1.50 (0.25, 8.00)	2.5 (0.5 - 6.0)	2.00 (0.50, 4.03)
AUC0-t (ng h/mL)	204.86 (136.109)	349.71 (190.139)	183.50 (97.882)	378.19 (191.740)	435.13 (215.34)	280.16 (125.35)
AUC0-∞ (ng h/mL)	228.65 (150.498)	353.09 (190.992	203.53 (104.617)	382.19 (193.411)	437.53 (215.54)	320.19 (137.46)b
CL/F (L/h)	2865.03 (1985.385)	1404.45 (825.133)	3324.13 (3109.837)	1521.43 (842.098)	1228 (519)	1653.45b (67 1.45)
Half-life (hours)	7.20 (2.301)	14.79 (5.383)	6.63 (2.352)	15.18 (5.808)	16.14 (4.70)	8.81 (2.24)
Methylnaltrexone sulfate (M2)						
Cmax (ng/mL)	19.35 (14.970)		17.72 (15.323)		22.04 (9.92)	17.15 (8.09)
Tmax a (hours)	6.00 (2.0, 12.2)		5.95 (0.50, 8.23)		7.0 (3.0 - 8.0)	4.0 (4.00,16.00)
AUC0-t (ng h/mL)	242.56 (200.623)		218.35 (221.862)		265.71 (117.05)	188.18 (85.58)
Methyl-6α-naltrexol (M4)						
Cmax (ng/mL)	6.54 (7.373)		4.62 (2.818)		12.00 (7.30)	9.01 (5.74)
Tmax a (hours)	3.92 (0.70, 6.23)		2.02 (0.50, 7.85)		4.3 (4.0 - 8.0)	2.00 (1.00, 4.03)
AUC0-t (ng h/mL)	64.13 (70.785)		44.95 (25.353)		143.10 (44.07)	79.73 (39.06)
Methyl-6β-naltrexol (M5)						
Cmax (ng/mL)	2.56 (2.818)		1.95 (1.150)		5.39 (3.15)	3.48 (2.10)
Tmax a (hours)	4.0 (0.75, 8.1)		4.00 (0.5, 8.2)		4.3 (4.0 - 8.0)	4.00 (2.00, 4.03)
AUC0-t (ng h/mL)	31.22 (34.252)		23.90 (13.798)		69.44 (23.19)	40.82 (19.33)

[a] Median time to maximum concentration (range)

*Formulation Definitions:

- TBM formulation is film-coated tablet with (b) (4)
- Phase 3 formulation is uncoated tablet with (b) (4)
- Interim formulation is film-coated tablet with (b) (4) what is similar to phase 3 formulation with the exception of film-coat on outer layer

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

In healthy subjects, peak concentration (C_{max}) and the total exposure (AUC) of methylnaltrexone increased in approximately dose-proportional manner between oral doses of 150 mg and 450 mg (study MNPK1117).

Table 12: Mean (SD) Pharmacokinetic Parameters of Methylnaltrexone after a Single Dose Administration of Oral MNTX (150, 300, and 450 mg) tablets in Healthy Subjects

Mean (SD)	MNTX 150 mg Tablet (N = 16)	MNTX 300 mg Tablet (N = 16)	MNTX 450 mg Tablet (N = 16)
C_{max} (ng/mL)[a]	13.22 (15.17)	26.22 (18.40)	39.89 (32.11)

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Dose proportionality ratio (90% CI)[b]	--	1.13 (0.76, 1.68)	1.11 (0.75, 1.65)
$AUC_{0-\infty}$ (ng.h/mL)[a]	106.87 (64.77)	231.24 (115.98)	373.32 (207.36)
Dose proportionality ratio (90% CI)[b]	--	1.10 (0.82 1.49)	1.13 (0.83, 1.52)
AUC_{0-t} (ng.h/mL)[a]	104.65 (64.66)	229.37 (116.27)	366.67 (205.71)
Dose proportionality ratio (90% CI)[b]	--	1.12 (0.83, 1.52)	1.13 (0.84, 1.54)

a Mean values are arithmetic means unless otherwise specified.

b Ratios of dose-normalized geometric means for parameters from 300 mg and 450 mg doses relative to the 150 mg dose.

Note: Although this study MNPK1117 to assess the dose-proportionality was conducted with an interim formulation (film-coated tablet with (b) (4) that is not phase 3 or TBM formulation, we can conclude that MNTX oral tablet (TBM or phase 3 formulation) will have approximately dose-proportional PK as this formulation was BE in AUC to phase 3 formulation and only has 10% lower Cmax compared to phase 3 formulation (study MNPK1118).

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.*)

Following chronic once daily dosing of 450 mg MNTX oral tablet for 7 days, methylnaltrexone PK paremters does not change with chronic dosing (10% increased in AUC and 3% decrease in Cmax on Day 7 compared to Day 1). Based on the visual inspection of trough-concentration, steady state is approximately reached by Day 5- Day 7.

Study MNPK1118: This was a phase 1, open-label, 2-stage study in healthy subjects. The first stage of the study was designed to evaluate the PK of MNTX and its primary metabolites (methylnaltrexone sulfate [M2], methyl-6α-naltrexol [M4], and methyl-6β-naltrexol [M5]) after a single dose followed by multiple daily oral doses of MNTX film-coated tablets for 7 days. Subjects received once-daily dose of 450 mg (3 x 150 mg tablets) MNTX film-coated tablets with (b) (4) for 7 days in the morning following overnight fasting of at least 10 hours.

Figure 7: Mean Plasma Concentration (ng/mL) vs. Time Profiles on Day 1 and Day 7 after Oral once daily administration of 450 mg oral tablets

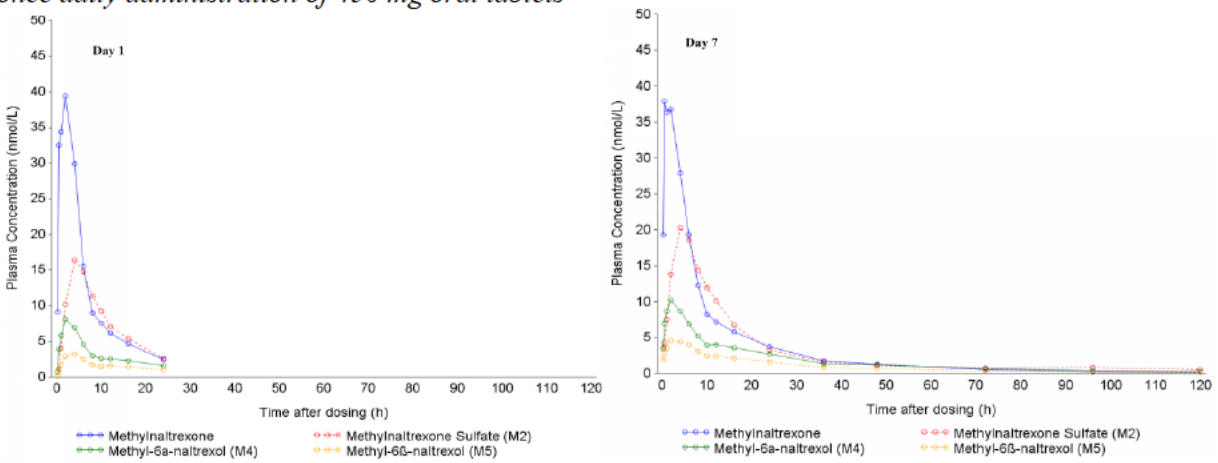

Table 13: PK Parameters of MNTX and Metabolites on after a Single-Dose and Multiple-Dose Administration of MNTX 450 mg Once Daily for 7 Days in Healthy Subjects

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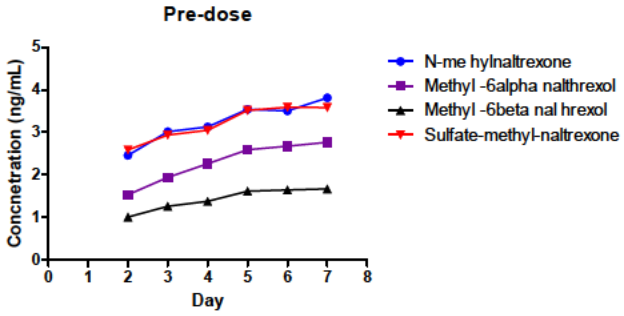
Pharmacokinetic Parameter	Methylnaltrexone (N=16)		Methylnaltrexone Sulfate (M2, N=16)		Methyl-6α-Naltrexol (M4, N=16)		Methyl-6β-Naltrexol (M5, N=16)	
	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7	Day 1	Day 7
C_{max} (ng/mL) Mean (SD)	47.05 (25.88)	45.50 (23.58)	17.15 (8.09)	21.00 (11.50)	9.01 (5.74)	10.77 (5.22)	3.48 (2.10)	4.89 (2.30)
$AUC_{0-\infty}$[a] (ng.h/mL) Mean (SD)	320.19 (137.46)[b]	NR[f]	216.44 (100.76)[b]	NR[f]	157.29 (113.56, 201.22)[c] (n=3)	NR[f]	73.74 (n=1)	NR[f]
AUC_{tau} (ng.h/mL) Mean (SD)	280.16 (125.35)	308.89 (102.34)	188.18 (85.58)	243.68 (137.56)	79.73 (39.06)	119.61 (57.43)	40.82 (19.33)	66.33 (31.05)
T_{max} (hours) Median (min, max)	2.00 (0.50, 4.03)	2.00 (0.50, 4.01)	4.0 (4.00, 16.00)	4.0 (4.00, 8.00)	2.00 (1.00, 4.03)	2.00 (1.00, 4.01)	4.00 (2.00, 4.03)	2.00 (1.00, 4.01)
CL/F (mL/h) Mean (SD)	1653.45[b] (67 1.45)	1594.44 (482.46)	NA	NA	NA	NA	NA	NA
$t_{½}$[d] (hours) Mean (SD)	8.81 (2.24)	19.22 (4.98)	7.19[b] (1.62)	13.87 (6.92)	17.48 (7.21)	31.85 (5.04)	18.40 (6.94)	28.65 (5.52)
AUC_{tau} Ratio[e] Mean (SD)	NA	NA	0.725 (0.29)	0.791 (0.39)	0.297 (0.10)	0.385 (0.12)	0.151 (0.06)	0.214 (0.07)

Table 14: Trough Mean plasma concentration of MNTX and metabolites at pre-dose on Day 2 through Day 7 to assess steady state

Plasma concentration (ng/mL)	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
N-methylnaltrexone	2.455	3.014	3.126	3.540	3.502	3.812
Methyl -6α nalthrexol	1.532	1.939	2.260	2.590	2.672	2.763
Methyl -6 β nalthrexol	1.006	1.259	1.375	1.614	1.644	1.670
Sulfate-methyl-naltrexone	2.584	2.937	3.046	3.513	3.588	3.580

Figure 8: Trough mean plasma concentration of MNTX and metabolite at pre-dose on Day 2 through Day 7 to assess steady state



Reviewer's Comment:

- Based on the visual inspection of trough concentrations vs. Days plot of MNTX and its metabolites, trough concentration does not appear to increase significantly for MNTX and its metabolites between Day 5 through 7 suggesting that steady state was reached by 7 for MNTX and all its metabolites at 450 mg MNTX dose levels.
- There is a minimal accumulation of MNTX following daily dosing of 450 mg MNTX tablet (film coated with (b) (4) for 7 days. The PK profile and MNTX and its metabolites on Day 7 appear to be similar to Day 1. Cmax of MNTX is similar on Day 7 to that of Day 1. AUC of MNTX increased by approximately 10% on Day 7 compared to Day 1. However, there is some accumulation for the metabolites following multiple dosing. Compare to Day 1, Cmax and AUC increased by 22% and 29% for methylnaltrexone sulfate, 20% and 50% for methyl-6α-naltrexol and by 41% and 62.5% for methyl-6β-naltrexol on Day 7, respectively. Although this study for assessing the accumulation following multiple dosing was conducted with a formulation (film coated tablet with (b) (4) that is slightly different from the Phase 3 formulation (uncoated tablet with (b) (4) and from the TBM formulation (coated tablet with (b) (4) this information of lack of accumulation of MNTX following multiple dosing can still be extrapolated to TMB

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formulation as accumulation potential is formulation independent among oral route of administration. Based on the comparison Cmax at steady state to the ki for human mu-opioid receptor of MNTX and its major metabolites, although overall clinical efficacy of oral MNTX 450 mg dose is probably primary driven by the parent drug MNTX, the active metabolite methyl-6α-naltrexol (M4) may still have some contribution to the efficacy to a certain extent. Please see section 2.4.8.4.2 for detailed discussion.

2.4.5 What is the variability of PK parameters of the drug and its relevant metabolites?

High variability of PK parameters of methylnaltrexone and its metabolites were observed with % CV of 23-80% for most of PK parameters in healthy subjects across studies. Cmax was associated with higher variability with %CV of 55-85% where CV% for AUC was around 27%-56%. Half-life estimation had the lowest variability with %CV of 23%-38%.

Table 15: %CV of PK Parameters of 450 mg MNTX oral tablet in Healthy Subjects under Fasting Condition

Study #	Formulation[a]	C_{max}	AUC_t	AUC_{inf}	$T_{1/2}$	CL/F
Single Dose PK on Day 1						
MNEF1001 (n=32)	TBM	76%	49%	48%	23%	38%
MNPK1004 (n=6)	TBM	63%	49%	49%	29%	42%
MNPK1001 (n=30)	TBM	85%	51%	51%	38%	55%
MNPK1001 (n=178)	Phase 3	78%	54%	54%	36%	59%
MNPK1118 (n=16)	Phase 3	55%	27%	26%	27%	32%
MNPK1118 (n=15)	Interim, Stage 2	68%	33%	32%	38%	35%
MNPK1117(n=45)	interim	80%	56%	55%	27%	62%
MNPK1118 (n=16)	Interim, Stage 1	55%	45%	43%	25%	41%
Multiple Dose PK on Day 7 following QD dosing						
MNPK1118 (n=16)	Interim, Stage 1	52%	33%		26%	30%

[a]Formulation Definitions:

- TBM formulation is film-coated tablet with (b) (4)
- Phase 3 formulation is uncoated tablet with (b) (4)
- Interim formulation is film-coated tablet with (b) (4) what is similar to phase 3 formulation with the exception of film-coat on outer layer

Table 16: %CV of Pharmacokinetic Parameters MNTX metabolites following single dose administration of of 450 mg MNTX oral tablet in Healthy Subjects under Fasting Condition

Metabolites	Study Fomrulations[a]	MNPK1004 TBM (n=6)	MNPK1118 Interm (n=16)
MNTX Sulfate	C_{max}	45%	47%
	AUC_{0-24}	44%	46%
	AUC_t	44%	
	AUC_{inf}	48%	47%
	$T_{1/2}$	24%	23%
Methyl-6α-naltrexol	C_{max}	61%	64%
	AUC_{0-24}	43%	49%
	AUC_t	31%	
	AUC_{inf}	30%	72%
	$T_{1/2}$	10%	41%
Methyl-6β-naltrexol	C_{max}	58%	60%
	AUC_{0-24}	47%	47%
	AUC_t	33%	
	AUC_{inf}	32%	
	$T_{1/2}$	30%	38%

[a]Formulation Definitions:

- TBM formulation is film-coated tablet with (b) (4)

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- Phase 3 formulation is uncoated tablet with (b) (4)
- Interim formulation is film-coated tablet with (b) (4) what is similar to phase 3 formulation with the exception of film-coat on outer layer

2.4.6 Is there evidence for a circadian rhythm of the PK parameters?

The proposed dose is 450 mg (3X150 mg) oral dose once daily. The effect of circadian rhythm was not evaluated.

2.4.7 What is the relative bioavailability of this proposed oral formulation compared to the existing product SC formulation?

Currently, methylnaltrexone is available as subcutaneous product as Relistor SC injection with recommended dose of 12 mg SC once daily for OIC in adult patients with chronic non-cancer pain. The sponsor is proposing 450 mg oral dose for the same indication in this submission. Based on the single dose PK comparison in study MNPK1117, it appears that 450 mg oral dose have approximately 5-fold lower Cmax but 23% higher AUC compared to 12 mg SC formulation. After correcting for the dose difference, the dose adjusted relative bioavailability of oral route of administration of MNTX is approximately 3-4% of SC route of administration. Tmax of the oral MNTX is longer than the tmax of SC administration (1.5-2 hours for oral vs. 0.25 hr for SC).

Study MNPK1117: This was a phase 1, randomized, open-label, single-dose, crossover study in 48 healthy subjects consisting of 6 dosing sequences, each with 2 dosing periods; the dosing periods were separated by 7 days. The doses were administered under fasting condition. In each dosing periods, the subjects received either a single oral dose of MNTX tablets (150, 300, or 450 mg administered as 150-mg MNTX film-coated tablet with (b) (4) or a single SC injection of MNTX (12 mg). Subjects were randomized (1:1:1:1:1:1) to 1 of 6 dosing sequences:

Sequence	Period 1 (Day 1 w single dose)	Period 2 (Day 8 w a single dose)
1	150 mg (1 x 150 mg) MNTX tablets	SC injection of 12 mg MNTX
2	SC injection of 12 mg MNTX	150 mg (1 x 150 mg) MNTX tablets
3	300 mg (2 x 150 mg) MNTX tablets	SC injection of 12 mg MNTX
4	SC injection of 12 mg MNTX	300 mg (2 x 150 mg) MNTX tablets
5	450 mg (3 x 150 mg) MNTX tablets	SC injection of 12 mg MNTX
6	SC injection of 12 mg MNTX	450 mg (3 x 150 mg) MNTX tablets

Figure 9: Mean Plasma MNTX Concentration-Time Profiles following Single Oral 150 mg, 300 mg, or 450 mg Tablet Doses and a Single Subcutaneous 12 mg Injection Dose of MNTX

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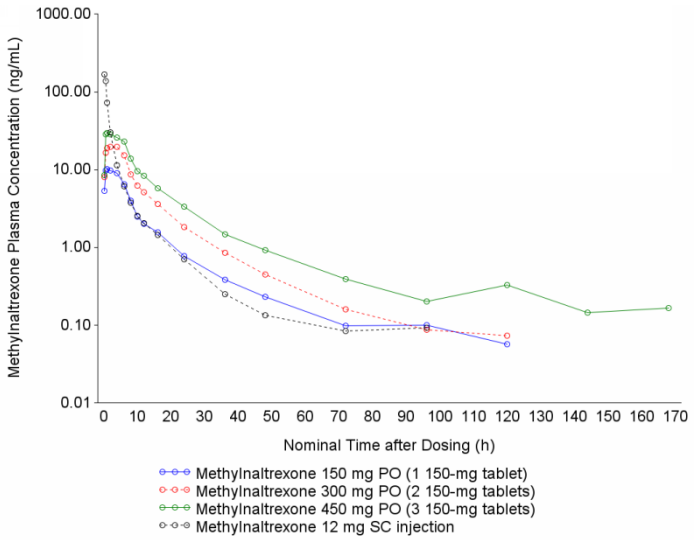

Table 17: Single-Dose Pharmacokinetic Parameters for Oral MNTX (150, 300, and 450 mg) and Subcutaneous MNTX (12 mg)

	MNTX 150 mg Tablet (N = 16)	**MNTX 300 mg Tablet (N = 16)**	**MNTX 450 mg Tablet (N = 16)**	**MNTX 12 mg SC Injection (N = 48)**
C_{max} (ng/mL)[a]				
Mean (SD)	13.22 (15.17)	26.22 (18.40)	39.89 (32.11)	174.01 (61.42)
Dose proportionality ratio (90% CI)[b]	--	1.13 (0.76, 1.68)	1.11 (0.75, 1.65)	--
$AUC_{0-\infty}$ (ng.h/mL)[a]				
Mean (SD)	106.87 (64.77)	231.24 (115.98)	373.32 (207.36)	269.09 (45.14)
Dose proportionality ratio (90% CI)[b]	--	1.10 (0.82 1.49)	1.13 (0.83, 1.52)	--
AUC_{0-t} (ng.h/mL)[a]				
Mean (SD)	104.65 (64.66)	229.37 (116.27)	366.67 (205.71)	267.87 (44.94)
Dose proportionality ratio (90% CI)[b]	--	1.12 (0.83, 1.52)	1.13 (0.84, 1.54)	--
T_{max} (h)				
Median (minimum, maximum)	2.00 (0.45, 6.00)	1.51 (0.50, 6.00)	2.00 (0.50, 6.00)	0.25 (0.25, 0.68)
CL/F (L/h)[a]				
Mean (SD)	1735.47 (683.441)	1564.64 (627.270)	1664.00 (1035.943)	45.699 (6.903)
$t_{½}$ (h)[c]				
Mean (SD)	13.95 (5.51)	14.16 (4.71)	16.57 (4.42)	9.16 (2.03)
Relative bioavailability (%)[d]				
Mean (SD)	3.17 (1.440)	3.38 (1.846)	3.69 (1.749)	--

[a] Mean values are arithmetic means unless otherwise specified.
[b] Ratios of dose-normalized geometric means for parameters from 300 mg and 450 mg doses relative to the 150 mg dose.
[c] Expressed as harmonic means and pseudo standard deviation based on jackknife variance.
[d] Bioavailability of oral MNTX relative to SC MNTX.

Table 18: Comparison of Systemic Exposure Parameters (Cmax and AUC): Geometric Mean Ratios of Parameters for Oral MNTX to SC MNTX and 90% Confidence Intervals

				90% CI for GMR	
Parameter	**Treatment**	**Geometric LSM**	**GMR (%)**	**Lower (%)**	**Upper (%)**
C_{max} (ng/mL)	MNTX 150 mg Tablet	9.47	5.78	4.34	7.69
	MNTX 300 mg Tablet	21.77	13.29	9.99	17.68
	MNTX 450 mg Tablet	32.70	19.96	15.00	26.56
	MNTX 12 mg SC Injection	163.81			
AUC_{0-t} (ng.h/mL)	MNTX 150 mg Tablet	94.20	35.61	29.04	43.68

	MNTX 300 mg Tablet	197.66	74.73	60.93	91.66
	MNTX 450 mg Tablet	321.19	121.44	99.00	148.95
	MNTX 12 mg SC Injection	264.49			
$AUC_{0-\infty}$ (ng.h/mL)	MNTX 150 mg Tablet	96.73	36.41	29.76	44.54
	MNTX 300 mg Tablet	199.77	75.18	61.46	91.98
	MNTX 450 mg Tablet	327.35	123.20	100.71	150.72
	MNTX 12 mg SC Injection	265.71			

Reviewer's Comment:

- Following a single oral dose administration of MNTX tablet, Cmax is reached slower than SC formulation where the tmax was around 1.5 -2.0 hour for oral route of administration and was 15 minutes for SC route of administration. Mean MNTX t½ was shorter for SC administration versus oral administration at all dose levels (9 hour for SC vs. 14-16h hour for oral).
- This study was conducted with a coated MNTX tablet with (b) (4) formulation that is slightly different than the formulation was used in phase 3 study. It is also different than the TBM formulation. Therefore, the results of this study should be interpreted with caution.
- Cmax after oral route of administration at all dose levels were lower than SC administration. Cmax of 150 mg, 300 mg and 450 mg oral doses were 6%, 13% and 20% of Cmax of 12 mg SC MNTX administration, respectively. AUC0-∞ after oral MNTX of 150 mg and 300 mg was 36.4% and 75.2%, respectively, of the AUC0-∞ after SC MNTX 12 mg. AUC0-∞ after oral administration of 450 mg MNTX was 1.2-fold greater than AUC0-∞ after SC MNTX 12 mg. The relative bioavailability of oral MNTX to SC MNTX 12 mg injection after correcting for dose was 3.2%, 3.4%, and 3.7% for oral MNTX 150 mg, 300 mg, and 450 mg, respectively. As this formulation in this study with film coated tablet with (b) (4) has equivalent AUC and 10% lower Cmax compared to the uncoated tablet with (b) (4) used in phase 3 study according to study MNPK1118, this relative bioavailability data with coated oral tablet with (b) (4) compared to SC formulation can be approximately extrapolated to the phase 3 formulation (uncoated tablet with (b) (4)
- Concentration-time profile and PK parameter estimation analysis were repeated and consisted with sponsor's results.
- In study MNOC1111, 12 mg SC was administered followed by administration of either 450 mg oral MNTX TBM formulation or 450 mg MNTX phase 3 oral formulation in OIC patient population. The PK for SC was only collected up to 4 hour post-dose. Therefore, AUC cannot be compared between the SC and oral formulation. However, Cmax of SC formulation appears to be approximately 3.8-fold greater than that of oral formulation in patient population, which is in consistent with what was observed in study MNPK1118 in healthy subject.

2.4.8 What are the ADME characteristics of the drug?

2.4.8.1 What are the characteristics of drug absorption?

Following a single dose administration of 450 mg MNTX tablet, C_{max} was reached approximately between 1.5-2.5 hours with C_{max} of 43-55 ng/mL. Absolute bioavailability of oral MNTX was not evaluated. Compared to SC route of administration, the dose corrected bioavailability of oral MNTX was 3-4%. Methylnaltrexone is not a substrate for P-gp or BCRP.

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2.4.8.2 What are the characteristics of drug distribution?

Plasma protein binding of the parent drug methylnaltrexone ranges 11.0% to 15.3%, as determined previously in NDA 21964 for SC formulation.

In this submission, the sponsor also evaluated the protein binding of the major metabolites by equilibrium dialysis at 37°C in healthy human plasma in study XS-0641. Like the parent drug methylnaltrexone, metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol have relatively low protein binding. The mean *in-vitro* human plasma protein binding at final concentrations of 1, 10, and 50 ng/mL were 17.7%, 17.3% and 28.9% for methylnaltrexone sulfate, 29.4%, 20.8% and 24.3% for methyl-6α-naltrexol, and 41.3%, 30.3% and 31.4% for methyl-6β-naltrexol, respectively. The tested concentration range of 1-50 ng/mL was appropriate as the Cmax of these metabolites were in similar range in healthy subjects and OIC patients, 17-22 ng/mL for methylnaltrexone sulfate, 5-12 ng/mL for methyl-6α-naltrexol and 2-5 ng/mL for methyl-6β-naltrexol.

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

According to the clinical pharmacology review for NDA 21964 (Relistor SC) Supplement 10 by Dr. Insook Kim dated 3/30/2012, methylnaltrexone is mainly metabolized by sulfotransferase SULT1E1 and SULT2A1 isoforms to form methylnaltrexone sulfate and by aldoketo reductase 1C enzymes form methyl-6-naltrexol isomers. The role of CYP enzymes in metabolizing MNTX is not substantial *in-vivo* based on the mass balance study and *in-vitro* metabolism studies.

No significant metabolism was observed when methylnaltrexone incubated in human liver microsome for up to 60 min (Study RPT-63756). Only trace amount of methylnaltrexone glucuronide were observed when methylnaltrexone incubated in human liver microsome for up to 60 min (Study RPT-63601). No metabolism was observed when methylnaltrexone incubated with recombinant isozymes cDNA-expressed human CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4 and a mixed enzyme pool to further demonstrate that the metabolism of methylnaltrexone is not cytochrome P450 mediated metabolism (Study RPT-63643).

2.4.8.4 What are the characteristics of drug excretion?

2.4.8.4.1 What are the results from the mass balance study?

No mass balance study with oral route of administration was conducted.

2.4.8.4.2 What are the major metabolites in urine and/or plasma as presented (*In-vivo*)? Are they different from those measured *In-vitro*? If so, why?

Major metabolites methylnaltrexone-Sulfate (M2), methyl-6α nalthrexol (M4), and methyl-6 β nalthrexol (M5) were measured in study MNPK1118 and MNPK1004 in plasma of healthy subjects. Metabolites found in plasma were consistent with in-vitro data. However, metabolites were not analyzed in urines samples in these studies although urine samples were collected, which were only used for analyzing the parent drug MNTX. Methylnaltrexone sulfate (M2) was the most abundant metabolite observed in plasma, followed by methyl-6α-naltrexol (M4) then methyl-6β-naltrexol (M5) on both Day 1 and Day 7. The AUC ratio of metabolites to the parent drug at steady-state on Day 7 were 79%, 38.5% and 21.4% for methylnaltrexone-sulfate (M2), methyl-6α nalthrexol (M4), and methyl-6β nalthrexol (M5), respectively. Based on the cross-study comparison with SC administration, it appears that the systemic exposure to methylnaltrexone metabolites is greater after oral administration of 450 mg dose than SC administration of 12 mg dose where AUC_{0-24} ratio of metabolites to methylnaltrexone at steady-

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state after 12 mg SC daily dosing was 30%, 19%, and 9% for methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol, respectively.

Although methylnaltrexone-sulfate (M2) is the most abundant metabolite in plasma, it has the least pharmacological activity as mu-opioid receptor antagonist (ki of 9603 ng/mL). Methyl -6α nalthrexol (M4), the second abundant metabolite, has similar potency as the parent drug as a mu-opioid receptor antagonist. Based on the comparison of the Cmax of MNTX and its major metabolites at steady state to their respective ki for human mu-opioid receptor, although overall clinical efficacy of oral MNTX 450 mg dose is probably primary driven by the parent drug MNTX, the active metabolite methyl-6α-naltrexol (M4) may still have some contribution to the efficacy to certain extent.

Table 19: AUC ratio of metabolites to parent drug and comparison of Cmax and Ki for mu-opioid receptor for MNTX and its metabolites

Analytes	[a]Study ID	Day	Cmax (ng/ml)	AUCt (ng*h/ml)	[b]AUCt Ratio	[c]Ki for human mu opioid-receptor
Methylnaltrexone	MNPK1004	1	54.70 (34.39)	435.13 (215.34)		28 nM
	MNPK1118	1	47.05 (25.88)	280.16 (125.35)		
	MNPK1118	7	45.50 (23.58)	308.89 (102.34)		10 ng/mL
MNTX Sulfate (M2)	MNPK1004	1	22.04 (9.92)	265.71 (117.05)	0.609	2200 nM
	MNPK1118	1	17.15 (8.09)	188.18 (85.58)	0.725 (0.29)	
	MNPK1118	7	21.00 (11.50)	243.68 (137.56)	0.791 (0.39)	9603 ng/mL
Methyl-6α-naltrexol (M4)	MNPK1004	1	12.00 (7.30)	143.10 (44.07)	0.33	30 nM
	MNPK1118	1	9.01 (5.74)	79.73 (39.06)	0.297 (0.10)	
	MNPK1118	7	10.77 (5.22)	119.61 (57.43)	0.385 (0.12)	10.7 ng/mL
Methyl-6β-naltrexol (M5)	MNPK1004	1	5.39 (3.15)	69.44 (23.19)	0.160	60 nM
	MNPK1118	1	3.48 (2.10)	40.82 (19.33)	0.151 (0.06)	
	MNPK1118	7	4.89 (2.30)	66.33 (31.05)	0.214 (0.07)	21.5 ng/mL

a: Study MNPK1004 (n=6) was conducted with TBM formulation (film-coated tablet with (b) (4) and study MNPK1118 (n=16) was conducted with interim formulation (film-coated tablet with (b) (4)

b: Metabolite to parent ratio of AUC_{tau}

c: in vitro ki for human mu-opioid receptor were obtained from study 1026730 for the parent drug and from study RPO-70910 for the metabolites

- The mean Cmax for parent drug MNTX (45.5 ng/mL) is about 4.5-fold higher than its *vitro* Ki for the human μ-opioid receptor (10 ng/mL) suggesting a significant contribution of parent drug MNTX to the overall efficacy.
- Although MNTX sulfate (M2) is the most abundant metabolite in plasma after oral administration of MNTX, it is the least potent antagonist of the μ-opioid receptor. The mean Cmax (21.00 ng/mL) was 457-fold lower than its *vitro* Ki for the human μ-opioid receptor (9603 ng/mL) suggesting almost no contribution of MNTX sulfate to the overall efficacy.
- The mean Cmax of methyl-6α-naltrexol (M4) (10.77 ng/mL) is similar to its *vitro* Ki for the human μ-opioid receptor (10.1 ng/mL). Therefore, methyl-6α-naltrexol may contribute to the overall efficacy.
- The mean Cmax of methyl-6β -naltrexol (M5) (4.89 ng/mL) is 5-fold lower than its *vitro* Ki for the human μ-opioid receptor (21.5 ng/mL), suggesting very little contribution of methyl-6β -naltrexol (M4) to the overall efficacy.

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2.4.8.4.3 What is the major route of elimination?

As no mass balance study with oral route of administration was conducted and the relative bioavailability of oral tablet compared to IV formulation was not evaluated to link oral formulation to the results of the mass balance study with the IV formulation, the contribution of renal clearance vs. hepatobiliary clearance cannot be estimated for the for the oral formulation of methylnaltrexone .

After intravenous administration of methylnaltrexone, approximately half of the dose was excreted in the urine (53.6%) and 17.3% of administered dose was excreted in the feces up to 168 hours post-dose. Methylnaltrexone is excreted primarily as the unchanged drug in the urine and feces. However, radiolabeled recovery in this study was only 70.9 % after 7 days. Active renal secretion of methylnaltrexone is suggested by renal clearance of methylnaltrexone that is approximately 4-5 folds higher than creatinine clearance.

2.4.8.4.4 What are the characteristics of drug excretion in urine?

The percent of drug recovered in urine as unchanged drug is very low following oral administration of 450 mg MNTX tablet, less than 1.78%. The renal clearance is estimated to be approximately 306.2 mL/min.

Table 20: Mean (SD) Urine PK Parameters of Unchanged Parent Drug MNTX in Healthy Subjects following oral administration of 450 mg MNTX tablet

Study ID	Formulation	Urine collection	Day	Total amount excreted (mg)	%Fe	Renal Clearance (mL/min)
MNPK1118 (n=16)	Interim	Up to 24 hr	1	5.78 (3.42)	1.31 (0.76)	~344
MNPK1118 (n=16)	Interim	UP to 120 hr	7	7.01 (2.74)	1.56 (0.61)	~378
MNPK1004 (n=6)	TBM	Up to 96 hr	1	8.07 (3.88)	1.78	306.2 (52.3)

%Fe = Fraction recovered as unchanged drug in urine

Methylnaltrexone and its metabolites methyl-6α-naltrexol, and methyl-6β-naltrexol are substrate for OCT1 and OCT2. In addition, methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol appear to be substrate for MATE1 and MATE2-K. In a previously conducted in vivo clinical drug interaction study with cimetidine, an inhibitor of OCT1, OCT2 , MATE1 and MATE2-K, Cmax and AUC of methylnaltrexone increased by 10% when 24 mg methylnaltrexone as an IV infusion over 20 minutes was administered before and after multiple doses of cimetidine 400 mg every 6 hours. The lack of significant clinical interaction with cimetidine demonstrates the inhibition of OCT1, OCT2, MATE1 and MATE2-K had no clinical meaningful effect on systemic disposition of methylnaltrexone.

2.4.8.4.5 Is there evidence for excretion of parent drug and/or metabolites into bile?

There are no human data to assess the potential excretion of methylnaltrexone or its metabolites into bile.
Methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol are not substrates for OATP1B1, OATP1B3, P-gp, and MRP2 based on in-vitro data.

2.4.8.4.6 Is there evidence for enterohepatic recirculation for parent and/or metabolites?

As the mean plasma concentration profiles and majority of the individual plasma concentration profiles do not display clear double peak plasma concentration profiles for the parent drug and all the metabolites, there is no strong evidence suggesting enterohepatic recirculation for parent drug and all the metabolites.

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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 full waiver request for all ages.

Hepatic impairment

- Following a single oral dose administration of 3 x 150 mg MNTX (b) (4) tablet, the exposure (both AUC and Cmax) of MNTX in subjects with hepatic impairment are significantly higher than the exposure in subjects with normal hepatic function. The mean MNTX Cmax in subjects with Child-Pugh A, B and C hepatic impairment was 1.7-, 4.8- and 3.7-fold higher than that of subjects with normal hepatic function, respectively. AUC of MNTX was similar between healthy subjects and Child-Pugh A subjects where the AUC of subjects with Child-Pugh B and C hepatic impairment were approximately 2.5- and 2.2- fold higher than the healthy subjects, respectively.
- For MNTX metabolites methyl-6α-naltrexol and methyl-6β-naltrexol, the exposures were lower in subjects with hepatic impairment than in healthy subjects, demonstrating lower metabolism in subjects with hepatic impairment. However, for metabolite MNTX sulfate, the exposure did not have a clear relationship with the extent of hepatic impairment. Based on the comparison of Cmax of MNTX and its major metabolites to their respective ki for human mu-opioid receptor, it appears that the overall clinical efficacy of oral MNTX 450 mg dose is primary driven by the parent drug MNTX with some contribution from the metabolite methyl-6α-naltrexol.
- The sponsor is proposing no dose adjustment in patients with Child-Pugh Class A and proposing 150 mg tablet (reducing the dose by 3-fold) in patients with Child-Pugh Class B and C. The sponsor's proposal is acceptable for the following reasons:
  - Safety profile of 1.7-fold higher Cmax in patients with Child-Pugh Class A is adequately covered by the safety profile of currently marketed SC formulation which has approximately 5-fold higher Cmax compared to oral 450 mg MNTX product.
  - MNTX has linear PK between 150 mg to 450 mg oral dose
  - Efficacy is primarily driven by the parent drug, with some possible contribution of metabolite methyl-6α-naltrexol.

In the hepatic impairment study with SC administration in healthy subjects and patients with mild and moderate hepatic impairment (n=8 per group), the Cmax and AUC in patients with mild and moderate hepatic impairment were similar to those of healthy subjects. Therefore, no dose adjustment was recommended in patients with mild and moderate hepatic impairment for SC formulating. The effect of severe hepatic impairment on the PK of SC methylnaltrexone was not evaluated. In this submission, the sponsor is proposing to monitor for methylnaltrexone-related adverse reactions in patients with severe hepatic impairment for SC formulation and if considering dose adjustment, reduce the SC dose by half based on the weight of the patients. The sponsor's proposal appears to be reasonable based on the available data from the hepatic impairment study with oral formulation in which moderate and severe hepatic impairment have similar degree of effect on the PK of methylnaltrexone. Nonetheless, efficacy of SC cannot be assured at lower dose as efficacy of SC for OIC was only evaluated and established at 12 mg.

Study MNPK1004: This was a phase 1, open-label single-dose study conducted in subjects with hepatic impairment and in healthy subjects (6 subjects/cohort). Hepatic impairment was assessed

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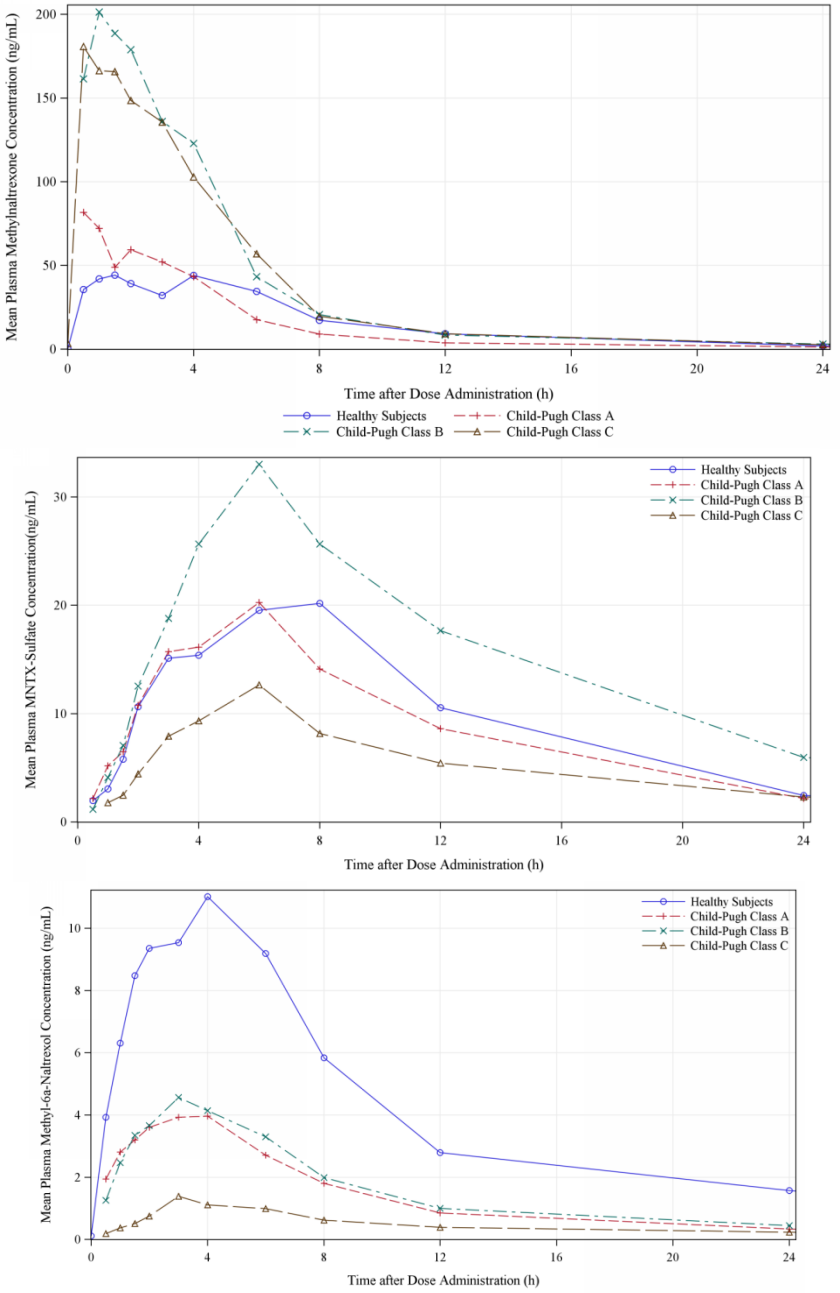
based on Child-Pugh Classification per FDA guidelines. Subjects received a single 450-mg oral dose of MNTX (3 x 150 mg) film-coated tablet with [redacted] (b) (4) (TBM) under fasting condition. PK blood samples were collected for up to 96 hours post-dose to determine the plasma concentrations of methylnaltrexone and its metabolites and urine samples were also collected up to 96 hours post-dose to determine the amount of parent drug recovered in urine.

Figure 10: Mean Plasma Concentration-Time Profiles for MNTX and its metabolites Following a Single oral dose of 450 mg (3 X 150 mg Tablets) in Healthy Subjects and Subjects with varying degree of Hepatic Impairment



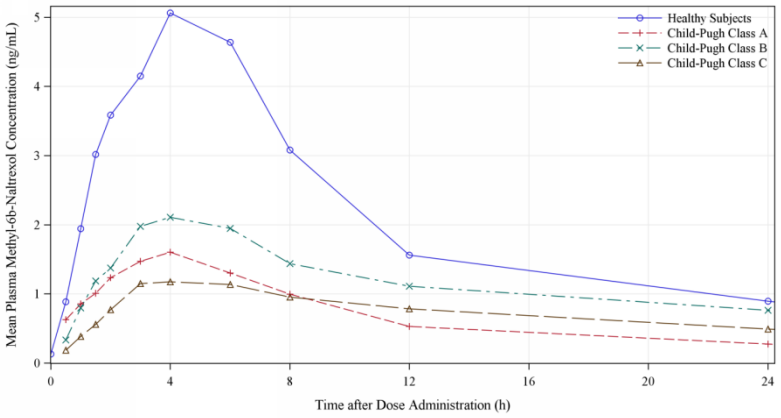


Table 21: Mean (± SD) Plasma PK Parameters for MNTX and its Metabolites Following a Single 450-mg Oral Dose in Healthy Subjects and Subjects with Hepatic Impairment

Parameters	Healthy Subjects (N=6)	Child-Pugh A (N=6)	Child-Pugh B (N=6)	Child-Pugh C (N=6)
MNTX				
C_{max} (ng/mL)	54.70 (34.39)	96.17 (67.42)	260.35 (144.40)	200.45 (125.16)
T_{max} [a] (h)	2.5 (0.5 - 6.0)	1.3 (0.5 - 1.2)	1.0 (0.6 - 4.0)	1.0 (0.5 - 6.0)
T_{lag} (h)	0	0	0	0
AUC_{0-24} (ng.h/mL)	395.74 (218.14)	365.52 (229.25)	966.66 (658.86)	920.38 (634.60)
AUC_{0-t} (ng.h/mL)	435.13 (215.34)	390.63 (236.16)	1056.71 (758.66)	979.58 (666.51)
$AUC_{0-\infty}$ (ng.h/mL)	437.53 (215.54)	392.80 (236.44)	1092.41 (820.07)	983.17 (668.25)
λ_z (h^{-1})	0.043 (0.012)	0.036 (0.006)	0.047 (0.031)	0.041 (0.009)
$t_{1/2}$ [b] (h)	16.14 (4.70)	19.40 (3.18)	14.92 (12.01)	17.05 (3.57)
V/F (L)	29561 (13295)	44447 (24208)	14369 (4622)	16257 (10241)
CL/F (L/h)	1228 (519)	1564 (856)	700 (580)	661 (444)
MNTX Sulfate				
C_{max} (ng/mL)	22.04 (9.92)	20.57 (18.45)	34.83 (26.80)	12.66 (11.63)
T_{max} [a] (h)	7.0 (3.0 - 8.0)	5.0 (4.0 - 6.0)	6.0 (3.0 - 8.0)	6.0 (6.0 - 6.0)
T_{lag} (h)	0.31 (0.25)	0.08 (0.20)	0.26 (0.29)	0.68 (0.29)
AUC_{0-24} (ng.h/mL)	247.40 (109.95)	220.03 (176.25)	392.18 (356.20)	151.638 (122.19)
AUC_{0-t} (ng.h/mL)	265.71 (117.05)	244.59 (179.37)	457.33 (492.36)	166.16 (151.59)
$AUC_{0-\infty}$ (ng.h/mL)	272.40 (130.61)	255.93 (174.97)	466.70(492.71)	222.01 (152.46)
λ_z (h^{-1})	0.099 (0.023)	0.116 (0.048)	0.131 (0.048)	0.086 (0.062)
$t_{1/2}$ [b] (h)	7.04 (1.66)	6.00 (2.35)	5.31 (1.90)	8.06 (6.85)
Methyl-6α-naltrexol				
C_{max} (ng/mL)	12.00 (7.30)	4.36 (2.60)	5.05 (2.52)	1.46 (0.95)
T_{max} [a] (h)	4.3 (4.0 - 8.0)	2.3 (0.5 - 4.1)	3.0 (1.5 - 48.0)	3.5 (3.0 - 6.0)
T_{lag} (h)	0	0	0	0.08 (0.20)
AUC_{0-24} (ng.h/mL)	109.77 (46.74)	36.34 (24.17)	40.10 (26.30)	12.62 (6.67)
AUC_{0-t} (ng.h/mL)	143.10 (44.07)	41.52 (25.19)	51.37 (36.03)	19.35 (10.64)
$AUC_{0-\infty}$ (ng.h/mL)	145.41 (43.57)	42.61 (24.94)	56.98 (41.30)	21.41 (2.36)
λ_z (h^{-1})	0.042 (0.00)	0.077 (0.03)	0.082 (0.05)	0.079 (0.05)
$t_{1/2}$ [b] (h)	16.48 (1.60)	9.00 (3.14)	8.50 (6.13)	8.81 (5.59)
Methyl-6β-naltrexol				
C_{max} (ng/mL)	5.39 (3.15)	1.67 (1.09)	2.31 (1.14)	1.26 (0.79)
T_{max} [a] (h)	4.3 (4.0 - 8.0)	4.1 (0.5 - 6.0)	3.5 (3.0 - 48.0)	5.0 (3.0 - 48.0)
T_{lag} (h)	0	0.08 (0.20)	0	0.08 (0.20)
AUC_{0-24} (ng.h/mL)	53.42 (24.86)	17.57 (11.27)	28.93 (20.18)	18.40 (15.21)
AUC_{0-t} (ng.h/mL)	69.44 (23.19)	22.30 (13.86)	53.39 (42.17)	33.93 (24.94)
$AUC_{0-\infty}$ (ng.h/mL)	71.72 (23.17)	25.83(14.43)	60.07(56.04)	49.27 (24.21)
λ_z (h^{-1})	0.042 (0.01)	0.047 (0.02)	0.036 (0.04)	0.021 (0.01)
$t_{1/2}$ [b] (h)	16.54 (5.07)	14.66 (7.48)	19.47 (30.08)	33.17 (8.04)

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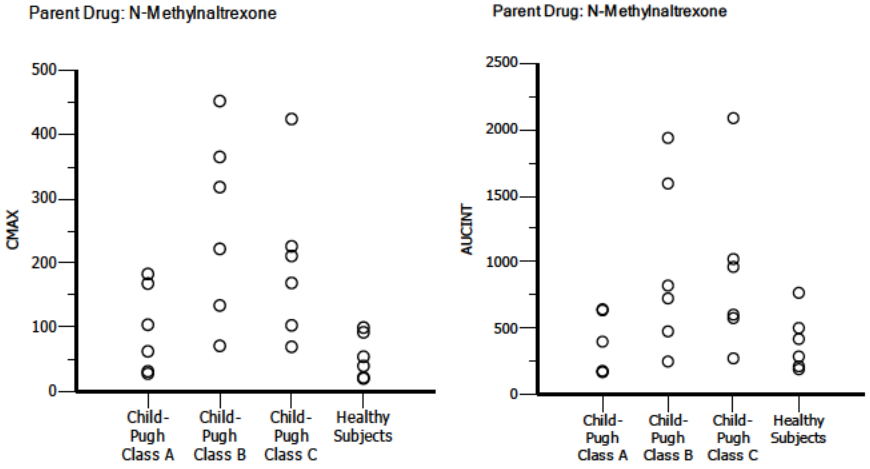
Table 22: Geometric LS Means and GMRs (Hepatic Impairment / Healthy) and 90% CIs for MNTX Cmax and AUC after Single Doses of MNTX 450 mg

Parameters	Geometric LS Mean	Geometric Mean Ratios[a]	90% CI for Geometric Mean Ratios	
			Lower[a]	Upper[a]
MNTX				
C_{max} (ng/mL)				
Healthy Subjects	45.46			
Child-Pugh A	74.85	1.646	0.811	3.342
Child-Pugh B	219.21	4.822	2.376	9.786
Child-Pugh C	170.46	3.749	1.847	7.610
AUC_{0-24} (ng.h/mL)				
Healthy Subjects	350.37			
Child-Pugh A	306.62	0.875	0.451	1.697
Child-Pugh B	775.05	2.212	1.141	4.289
Child-Pugh C	760.68	2.171	1.120	4.210
AUC_{0-t} (ng.h/mL)				
Healthy Subjects	396.10			
Child-Pugh A	332.24	0.839	0.438	1.605
Child-Pugh B	829.88	2.095	1.095	4.010
Child-Pugh C	813.86	2.055	1.074	3.932
$AUC_{0-\infty}$ (ng.h/mL)				
Healthy Subjects	398.68			
Child-Pugh A	334.55	0.839	0.436	1.614
Child-Pugh B	844.81	2.119	1.102	4.075
Child-Pugh C	817.48	2.050	1.066	3.943

Figure 11: Individual AUC and Cmax of MNTX and its metabolites in healthy subjects and in patients with various degrees of hepatic impairment functions (reviewer's analysis)



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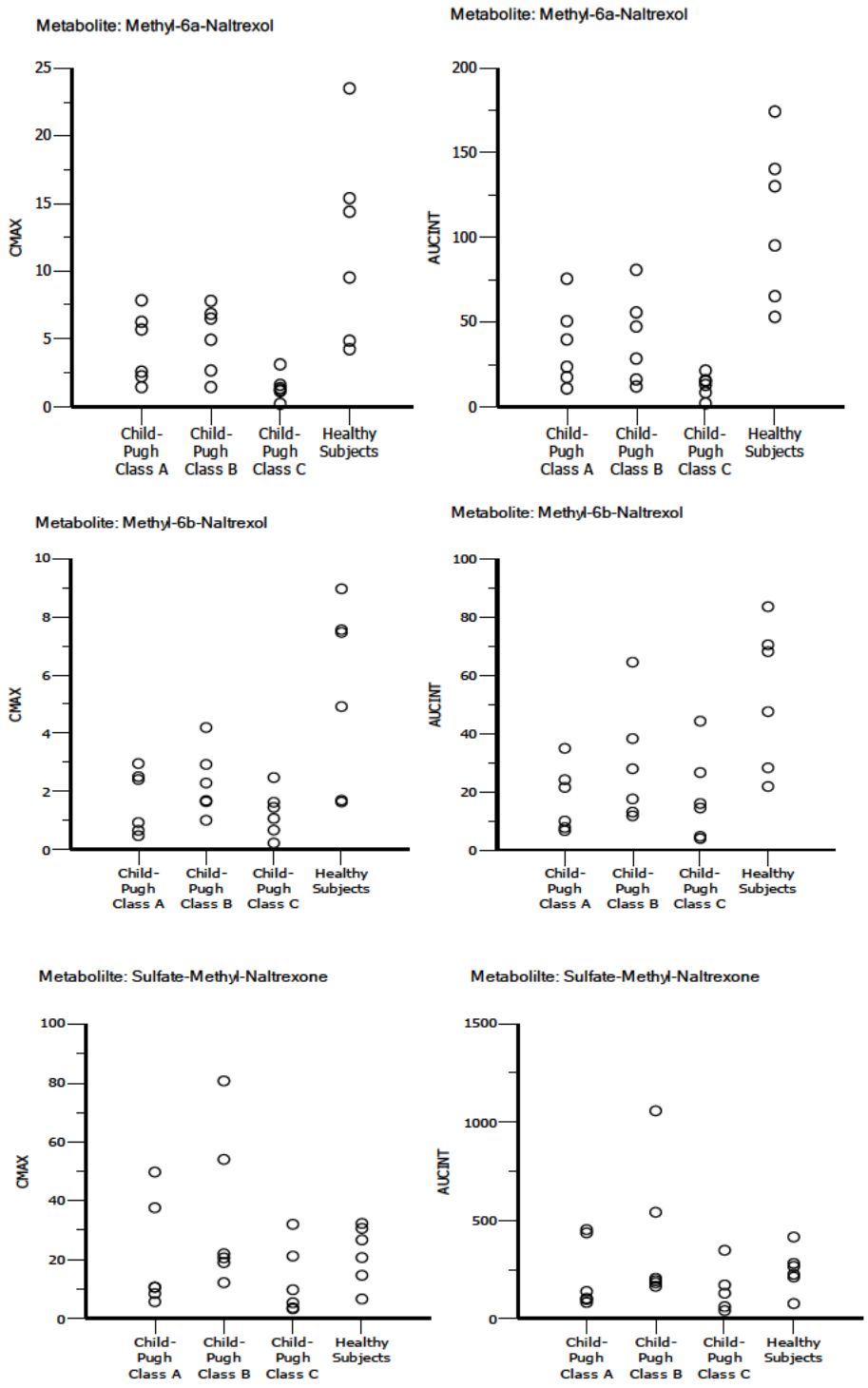

Mean amount of parent drug excreted in urine was comparable between healthy subjects and patients with Child-Pugh A hepatic impairment, but was higher in patient with Child-Pugh B and C. This demonstrates lower metabolism of MNTX in patients with hepatic impairment and thus higher recovery of parent drug MNTX in urine in patients with hepatic impairment. Moderate hepatic impairment has the lowest renal clearance. Nonetheless, renal function appears to be

comparable across the healthy subjects and subjects with various degree of hepatic impairment, based on comparison of creatinine clearance in different cohorts.

Table 23: Summary of Urine Concentrations of MNTX

Parameters (Mean [SD])	**Healthy Subjects (N=6)**	**Child-Pugh A (N=6)**	**Child-Pugh B (N=6)**	**Child-Pugh C (N=6)**
Amount excreted in urine (μg)	8066 (3883)	6606 (4727)	12648 (7211)	16879 (8638)
Renal clearance (mL/min)	306.2 (52.3)	289.2 (88.3)	244.1 (131.8)	314.8 (69.9)

Table 24: Cmax and Ki for Mu-opioid receptor of MNTX and its metabolites

	Cmax (ng/mL)				*In-vitro Ki for human Mu Opioid-receptor
	Healthy	CP-A	CP-B	CP-C	(ng/mL)
Parent drug MNTX	54.70 (34.39)	96.17 (67.42)	260.35 (144.40)	200.45 (125.16)	10
methylnaltrexone sulfate (M2)	22.04 (9.92)	20.57 (18.45)	34.83 (26.80)	12.66 (11.63)	9603
methyl-6α-naltrexol (M4)	12.00 (7.30)	4.36 (2.60)	5.05 (2.52)	1.46 (0.95)	10.7
methyl-6β-naltrexol (M5)	5.39 (3.15)	1.67 (1.09)	2.31 (1.14)	1.26 (0.79)	21.5

*: in vitro ki for human mu-opioid receptor were obtained from study 1026730 for the parent drug and from study RPO-70910 for the metabolites

Renal impairment

A dedicated renal impairment (RI) study was not conducted in this submission with oral tablet formulation.

The effects of renal impairment on the PK of 0.30 mg/kg dose SC MNTX were evaluated in subjects with mild, moderate and severe renal impairment (8 subjects/cohort). Cmax did not change significantly as a function of renal function while AUC increased as a function of renal impairment. AUC increased by 30%, 75% and 90% in patients with mild, moderate and severe renal impairment compared to subjects with normal renal function. The following Table-24 and Figure-12 were taken the Clinical Pharmacology review for NDA 21964 by Dr. Insook Kim dated 12/12/2007. The current label for SC formulation recommends halving the dose for SC administration for patients with severe renal impairment only.

Table 25: PK of MNTX in subjects with various degree of renal impairment following single dose administration a 0.30mg/kg dose SC

Study MNTX 1105 Mean Data %CV

	AUCt	AUCinf	Cmax	Fr%
Normal	427(15%)	433 (21%)	257 (36%)	45.2 (30%)
Mild	560 (12%)	566 (13%)	208 (23%)	31 (47%)
Moderate	752 (19%)	754 (19%)	231 (18%)	17 (49%)
Severe	819 (9%)	822 (9%)	304 (41%)	10 (64%)

Figure 12: AUC and Cmax of MNTX in subjects with various degree of renal impairment following single dose administration a 0.30mg/kg dose SC

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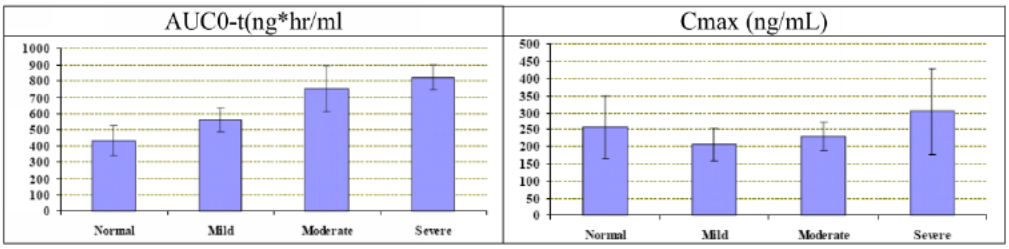

(b) (4)

the agency recommends 150 mg dose in patients with severe renal impairment as the expected exposure from this dose (236 ng.h/ml) is comparable to the exposure from 300 mg dose in subjects with normal renal function (231 ng/h/ml). Please note that 300 mg dose had demonstrated statistically significant efficacy over placebo in phase 3 trials. In addition, since the extent of increase in AUC in patients with moderate renal impairment (75%) is similar to that of severe renal impairment (90%), we recommend the same dose adjustment, 150 mg, for the patients with moderate renal impairment.

Table 26: Predicted AUC in patients with moderate and severe renal impairment with 150 mg and 300 mg oral MNTX dose

Source of data	Renal Function	Dose	$AUC_{0-\infty}$ (ng h/mL)
Observed mean (SD) AUC in study MNPK1117	Normal	150 mg PO (N = 16)	106.87 (64.77)
		300 mg PO (N = 16)	231.24 (115.98)
		450 mg PO (N = 16)	373.32 (207.36)
		12 mg SC (N = 48)	269.09 (45.14)
Predicted	Moderate renal impairment	150 mg PO	218
		300 mg PO	435
	Severe renal Impairment	150 mg PO	236
		300 mg PO	473

2.7 Extrinsic Factors

2.7.1 What extrinsic factors (*drugs, herbal products, diet, smoking, and alcohol use*) nfluence 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?

2.7.2 Drug-Drug Interactions

2.7.2.1 Is there an i*n-vitro* basis to suspect *In-vivo* drug-drug interactions?

None

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

Inhibition and induction of CYP enzymes by the parent drug methylnaltrexone was already addressed previously in NDA 21964 for SC formulation. Methylnaltrexone did not significantly inhibit (up to 100 uM) or induce (25 uM) the activity of cytochrome P450 (CYP) isozymes

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CYP1A2, CYP2A6, CYP2B6, CYP2C9, CYP2C19, or CYP3A4. In addition, methylnaltrexone did not induce CYP2E1. As the Cmax of 450 mg oral dose is (32-54 ng/mL = 73-124 nM) is about 200 fold lower than the highest previously tested concentration for inhibition (100 uM) and induction (25 uM), the lack of interaction of methylnaltrexone with CYP enzyme from previous studies is still applicable to the 450 mg oral dose to rule out the potential of methylnaltrexone to inhibit or induce CYP enzymes in the liver.

However, the available data from previous studies is not adequate to rule out the potential of methylnaltrexone to inhibit CYP3A4 in the gut when methylnaltrexone is administered as 450 mg oral tablet. Potential of methylnaltrexone to inhibit CYP3A4 was evaluated only up to 100 uM and no inhibition was observed at that concentration (IC50 >100 uM). As Igut is approximated to be 450 mg/250 mL = 4.128 mM = 4128 uM and Igut/IC50 =4128 uM/100 uM = 41.28 > 10, the potential for methylnaltrexone to inhibit CYP3A4 in the gut cannot be completely ruled out with the limited available data when methylnaltrexone is given as 450 mg oral dose. However, there is no strong scientific evidence to suggest the potential of MNTX to inhibit CYP3A4 in gut either for the following reason:

- There is no trend of inhibition of CYP3A4 with increasing concentration of MNTX when MNTX was evaluated as an inhibitor of CYP3A4 across concentration of 0.015 to 100 uM. Therefore, IC50 is much greater than 100uM.

Summary of inhibition of CYP3A4 activity expressed as % Vehicle control

MNTX concentration (uM)	100	33.3	11.1	3.70	1.23	0.41	0.137	0.046	0.015	0 VC)
CYP3A4 inhibition	109	109	103	102	110	110	106	107	101	100

Taken from Clinical pharmacology review for NDA 20964, supplement 10 dated XX by Dr. Kim Insook.

- MNTX has very low permeability across Caco-2 cells suggesting that very little amount of drug is getting into enterocytes where apical to basolateral permeability was less than 7nm/sec) which is less than mannitol permeability (13 nm/sec).

Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol do not induce CYP1A2, CYP2B6 and CYP3A4/5 enzyme at both mRNA level and enzyme activity level in freshly isolated human hepatocytes at up to 60 μM concentration, which is approximately 1000-fold higher than expected Cmax of these metabolites at the clinical dose of 450 mg MNTX oral tablets .

Methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol did not cause direct, time-dependent or metabolism-dependent inhibition of CYP1A2, CYP2B6 ,CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 in human liver microsomes at concentrations up to 4500 ng/mL for methylnaltrexone sulfate, (200 fold higher than Cmax) , 1000 ng/mL for methyl-6α-naltrexol (80-fold higher than Cmax) and 500 ng/mL for methyl-6β-naltrexol (100-fold higher than Cmax), which is approximately 80-200-fold higher than expected Cmax of these metabolites at the clinical dose of 450 mg MNTX oral tablets.

Induction (Study XT123038):
Cultured human hepatocytes (from 3 donors) were treated once daily for three consecutive days with DMSO (0.1% v/v, vehicle control), one of five concentrations of Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol (0.6, 3, 6, 30 or 60 μM) or one of three known human CYP enzyme inducers, namely, omeprazole (50 μM), phenobarbital (750 μM) and rifampin (10 μM) as positive controls. After treatment, the cells were harvested to isolate

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microsomes for the analysis of several human CYP enzymes. The activity of target enzyme CYP1A2, CYP2B6 and CYP3A4 was assessed by incubating the microsome with model probe substrate phenacetin O-dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6), and testosterone 6β-hydroxylation (marker for CYP3A4/5) for each target enzyme for 10 or 30 minutes and measuring the appearance rate of their respective metabolites by LC/MS/MS. Additional hepatocytes from the same treatment groups were harvested with TRIzol to isolate RNA, which was analyzed by qRT-PCR to assess the effect of Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol on CYP1A2, CYP2B6 and CYP3A4 mRNA levels.

Table 27: CYP activity percent positive control: The effects of treating cultured human hepatocytes with Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol or prototypical inducers on microsomal cytochrome P450 (CYP) enzyme activity

Treatment	Concentration	Percent positive control [a]		
		Phenacetin *O*-dealkylation (CYP1A2)	Bupropion hydroxylation (CYP2B6)	Testosterone 6β-hydroxylation (CYP3A4/5)
Dimethyl sulfoxide	0.1% (v/v)	0 ± 0	0 ± 0	0 ± 0
Methylnaltrexone sulfate	0.6 µM	−0.340 ± 1.912	1.17 ± 2.07	−0.884 ± 3.064
Methylnaltrexone sulfate	3 µM	−2.31 ± 1.93	−2.37 ± 2.49	−4.89 ± 4.34
Methylnaltrexone sulfate	6 µM	−1.13 ± 0.50	−1.14 ± 1.33	−4.87 ± 3.13
Methylnaltrexone sulfate	30 µM	0.310 ± 0.989	−0.787 ± 1.460	−4.25 ± 3.73
Methylnaltrexone sulfate	60 µM	−0.864 ± 1.071	−1.28 ± 1.65	−2.38 ± 2.84
Methyl-6α-naltrexol	0.6 µM	−2.41 ± 0.88	−2.08 ± 2.09	−4.74 ± 4.56
Methyl-6α-naltrexol	3 µM	−2.29 ± 1.64	−2.04 ± 3.67	−3.39 ± 6.59
Methyl-6α-naltrexol	6 µM	−5.24 ± 7.58	−1.12 (n = 2)	−2.20 (n = 2)
Methyl-6α-naltrexol	30 µM	0.364 ± 2.414	2.21 ± 8.23	2.43 ± 10.41
Methyl-6α-naltrexol	60 µM	−0.123 ± 3.310	2.59 ± 7.58	0.0667 ± 15.5549
Methyl-6β-naltrexol	0.6 µM	0.323 ± 1.421	0.847 ± 0.775	2.92 ± 4.57
Methyl-6β-naltrexol	3 µM	−0.122 ± 1.853	−2.23 ± 1.53	0.224 ± 5.689
Methyl-6β-naltrexol	6 µM	−0.803 ± 1.915	−0.0837 ± 4.5410	−2.41 ± 6.70
Methyl-6β-naltrexol	30 µM	0.512 ± 0.798	0.176 ± 2.334	3.05 ± 6.58
Methyl-6β-naltrexol	60 µM	−0.652 ± 3.150	−1.95 ± 5.63	1.22 ± 10.35
Flumazenil	50 µM	NA	NA	NA
Omeprazole	50 µM	100 ± 0	NA	NA
Phenobarbital	750 µM	NA	100 ± 0	NA
Rifampin	10 µM	NA	NA	100 ± 0

Table 28: mRNA percent positive control: The effects of treating cultured human hepatocytes with Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol or prototypical inducers on microsomal cytochrome P450 (CYP) mRNA levels as determined by qRT-PCR

Treatment	Concentration	Percent positive control [a]		
		CYP1A2	CYP2B6	CYP3A4
Dimethyl sulfoxide	0.1% (v/v)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Methylnaltrexone sulfate	0.6 µM	−0.199 ± 0.388	−2.33 ± 4.23	−3.13 ± 4.78
Methylnaltrexone sulfate	3 µM	0.158 ± 0.325	1.77 ± 3.11	2.84 ± 4.44
Methylnaltrexone sulfate	6 µM	0.227 ± 0.143	1.01 ± 2.93	1.89 ± 8.31
Methylnaltrexone sulfate	30 µM	0.0419 ± 0.2263	−3.34 ± 4.35	−0.570 ± 4.543
Methylnaltrexone sulfate	60 µM	0.320 ± 0.228	2.28 ± 2.07	1.19 ± 10.58
Methyl-6α-naltrexol	0.6 µM	0.117 ± 0.108	3.27 ± 3.08	2.49 ± 4.69
Methyl-6α-naltrexol	3 µM	−0.0950 ± 0.1830	1.68 ± 2.25	3.96 ± 5.04
Methyl-6α-naltrexol	6 µM	0.441 ± 1.338	0.460 ± 4.560	0.654 ± 2.951
Methyl-6α-naltrexol	30 µM	0.0100 ± 0.1532	2.92 ± 5.65	2.62 ± 3.45
Methyl-6α-naltrexol	60 µM	−0.0713 ± 0.1653	4.12 ± 4.50	4.44 ± 4.96
Methyl-6β-naltrexol	0.6 µM	0.0653 ± 0.0463	1.55 ± 1.68	2.54 ± 3.55
Methyl-6β-naltrexol	3 µM	−0.263 ± 0.597	−1.79 ± 4.62	2.49 ± 6.41
Methyl-6β-naltrexol	6 µM	−0.0460 ± 0.3267	−0.493 ± 2.542	0.327 ± 6.731
Methyl-6β-naltrexol	30 µM	0.0370 ± 0.1212	2.38 ± 7.54	4.79 ± 4.55
Methyl-6β-naltrexol	60 µM	−0.322 ± 0.367	−2.64 ± 4.19	2.59 ± 6.14
Flumazenil	50 µM	NA	NA	NA
Omeprazole	50 µM	100 ± 0.00	NA	NA
Phenobarbital	750 µM	NA	100 ± 0.00	NA
Rifampin	10 µM	NA	NA	100 ± 0.00

Reviewer's Comment:

- *The tested concentration of, methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol at 0.6, 3, 6, 30 or 60 µM are acceptable as they approximately cover the Cmax and*

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10 times Cmax values to be expected in healthy human subjects or OIC patients taking 450 mg of MNTX oral tablet

- *Cmax was around 17-22 ng/mL (~ 39-50 nM) for methylnaltrexone sulfate, 5-12 ng/mL (14-33 nM) for methyl-6α-naltrexol and 2-5 ng/mL (6-14 nM) for methyl-6β-naltrexol in healthy subject and OIC patients.*

- *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 CYP1A2, CYP2B6, and CYP3A4 activities as the treatment of human hepatocytes with positive controls CYP inducer caused anticipated and appropriate in CYP activity and mRNA levels.*
- *Methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol) up to 60 µM did not induce CYP1A2, 2B6 and 3A4 activities as the percent of change in target enzyme activity is not greater than 40% of the positive control with known inducer. They also did not induces CYP1A2, 2B6 or 3A4 mRNA.*

Inhibition (Study XT125033):

Human liver microsomal from a pool of sixteen individual was incubated with corresponding selective model substrates in presence and absence of the test compound in duplicate at 37°C. Marker substrate reactions were initiated by the addition of an aliquot of an NADPH-generating system and were automatically terminated at approximately 5 minutes by the addition of the appropriate internal standard and stop reagent (b) (4). To distinguish between time dependent and metabolism-dependent inhibition, each test article was pre-incubated with human liver microsomes for 30 minutes without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrate. This pre-incubation with NADPH generating system (for metabolism-dependent inhibition) allowed for the generation of intermediates that could inhibit human CYP enzymes. The pre-incubation without NADPH generating system (for time-dependent inhibition) allowed assessment of whether any potential increase in inhibition was dependent upon NADPH (e.g., potentially CYP mediated). Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments, as applicable. Solvent controls in absence of test compounds were used as the negative control. 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.

Test Item and Concentration:

- Methylnaltrexone Sulfate: 0, 4.5, 15, 45, 150, 450, 1500, 4500 ng/mL
- Methyl-6α- Naltrexol: 0, 1, 3, 10, 30, 100, 300, 1000 ng/mL
- Methyl-6β- Naltrexol: 0, 0.5, 1.5, 5, 15, 50, 150, 500 ng/mL

Table 29: Incubation Concentrations of Probe Substrates and Positive Controls

CYP	Model Substrate	Metabolites	Positive control Direct Inhibition	Positive-Controls Metabolism-dependent inhibition
1A2	Phenacetin (40 uM)	acetaminophen	α-Naphthoflavone (0.5 uM)	Furafylline (1.0 uM)
2B6	Efavirenz (3 uM)	8-Hydroxyefavirenz	Orphenadrine (750 uM)	Phencyclidine (30 uM)
2C8	Amodiaquine (1.5 uM)	*N*-Desethylamodiaquine	Montelukast (0.05 uM)	Gemfibrozil glucuronide (5.0 uM)
2C9	Diclofenac (6 uM)	4'-hydroxydiclofenac	Sulphaphenazole (2.0 uM)	Tienilic acid (0.25 uM)
2C19	S-Mephenytoin (40 uM)	4-hydroxymephenytoin	Modafinil (250 uM)	*S*-Fluoxetine (20 uM)
2D6	Dextromethorphan (7.5 uM)	Dextrorphan	Quinidine (0.5 uM)	Paroxetine (0.3 uM)
3A4	Testosterone (70 uM)	6β-hydroxytestosterone	Ketoconazole (0.15 uM)	Troleandomycin (25 uM)
3A4	Midazolam (4 uM)	1'-hydroxymidazolam	Ketoconazole (0.075 uM)	Troleandomycin (7.5 uM)

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Table 30: In vitro evaluation of methylnaltrexone sulfate as an inhibitor of human CYP enzymes

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (ng/mL) [a]	Inhibition observed at 4500 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 4500 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 4500 ng/mL (%) [b]	
CYP1A2	Phenacetin *O*-dealkylation	> 4500	NA	> 4500	5.3%	> 4500	0.6%	No
CYP2B6	Efavirenz 8-hydroxylation	> 4500	NA	> 4500	NA	> 4500	NA	No
CYP2C8	Amodiaquine *N*-dealkylation	> 4500	NA	> 4500	2.3%	> 4500	NA	No
CYP2C9	Diclofenac 4′-hydroxylation	> 4500	0.9%	> 4500	0%	> 4500	NA	No
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	> 4500	4.4%	> 4500	6.6%	> 4500	4.9%	No
CYP2D6	Dextromethorphan *O*-demethylation	> 4500	NA	> 4500	3.2%	> 4500	8.1%	No
CYP3A4/5	Testosterone 6β-hydroxylation	> 4500	NA	> 4500	NA	> 4500	NA	No
CYP3A4/5	Midazolam 1′-hydroxylation	> 4500	NA	> 4500	NA	> 4500	NA	No

Table 31: In vitro evaluation of methyl-6α-naltrexol as an inhibitor of human CYP enzymes

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (ng/mL) [a]	Inhibition observed at 1000 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 1000 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 1000 ng/mL (%) [b]	
CYP1A2	Phenacetin *O*-dealkylation	> 1000	NA	> 1000	NA	> 1000	NA	No
CYP2B6	Efavirenz 8-hydroxylation	> 1000	0%	> 1000	0.7%	> 1000	2.2%	No
CYP2C8	Amodiaquine *N*-dealkylation	> 1000	1.7%	> 1000	NA	> 1000	6.2%	No
CYP2C9	Diclofenac 4′-hydroxylation	> 1000	7.3%	> 1000	6.0%	> 1000	3.3%	No
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	> 1000	NA	> 1000	NA	> 1000	NA	No
CYP2D6	Dextromethorphan *O*-demethylation	> 1000	NA	> 1000	NA	> 1000	NA	No
CYP3A4/5	Testosterone 6β-hydroxylation	> 1000	3.6%	> 1000	NA	> 1000	2.2%	No
CYP3A4/5	Midazolam 1′-hydroxylation	> 1000	1.2%	> 1000	2.9%	> 1000	7.6%	No

Table 32: In vitro evaluation of methyl-6β-naltrexol as an inhibitor of human CYP enzymes

Enzyme	Enzyme reaction	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation		30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (ng/mL) [a]	Inhibition observed at 500 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 500 ng/mL (%) [b]	IC_{50} (ng/mL) [a]	Inhibition observed at 500 ng/mL (%) [b]	
CYP1A2	Phenacetin *O*-dealkylation	> 500	13%	> 500	3.7%	> 500	14%	No
CYP2B6	Efavirenz 8-hydroxylation	> 500	7.8%	> 500	9.6%	> 500	7.6%	No
CYP2C8	Amodiaquine *N*-dealkylation	> 500	4.5%	> 500	6.7%	> 500	9.3%	No
CYP2C9	Diclofenac 4′-hydroxylation	>500	NA	>500	1.1%	>500	3.1%	No
CYP2C19	*S*-Mephenytoin 4′-hydroxylation	>500	2.1%	>500	6.0%	>500	9.0%	No
CYP2D6	Dextromethorphan *O*-demethylation	> 500	0%	> 500	NA	> 500	6.9%	No
CYP3A4/5	Testosterone 6β-hydroxylation	> 500	3.6%	> 500	6.1%	> 500	4.1%	No
CYP3A4/5	Midazolam 1′-hydroxylation	> 500	0%	> 500	3.1%	> 500	2.5%	No

Reviewer's Comment:

- *The test system of human liver microsomes were well characterized in respect to various CYP enzymes. The observed Km for CYP enzymes were within previously reported range.*
- *The choices of CYP-specific model substrates and their respective concentrations to evaluate the inhibitory potential test compound on each CYP isoforms were acceptable as those model substrate concentrations are approximately below the respective Km value.*
- *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.*
- *The tested concentration methylnaltrexone sulfate up to 4500 ng/mL, Methyl-6α-naltrexol up to 1000 ng/mL and Methyl-6β-naltrexol up to 500 ng/mL are acceptable as they approximately cover the Cmax and 10 times Cmax values to be expected in healthy human subjects or OIC patients taking t 450 mg of MNTX oral tablet*

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- *Cmax was around 17-22 ng/mL (~ 39-50 nM) for methylnaltrexone sulfate, 5-12 ng/mL (14-33 nM) for methyl-6α-naltrexol and 2-5 ng/mL (6-14 nM) for methyl-6β-naltrexol in healthy subject and OIC patients.*

- *Methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol did not cause direct, time-dependent or metabolism-dependent inhibition of any CYP enzyme activity investigated, CYP1A2, CYP2B6,CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5, at concentrations up to 4500, 1000 or 500 ng/mL, respectively.*

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

In previous studies with NDA 21964 for SC formulation, it was already demonstrated that methylnaltrexone was a substrate of OCT1 but not a substrate of OAT1or P-gp.

In this submission, it was shown that methylnaltrexone and its metabolites methyl-6α-naltrexol, and methyl-6β-naltrexol are substrates for OCT1 and OCT2. In addition, methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol appears to be substrates for MATE1 and MATE2-K. In a previously conducted in vivo clinical drug interaction study with cimetidine, an inhibitor of OCT1, OCT2 , MATE1 and MATE2-K, Cmax and AUC of methylnaltrexone increased by 10% when 24 mg methylnaltrexone as an IV infusion over 20 minutes was administered before and after multiple doses of cimetidine 400 mg every 6 hours. The lack of significant clinical interaction with cimetidine from the previous studies alleviates the need for further in-vivo study to address the potential interaction of methylnaltrexone and its metabolites with OCT2, MATE1 and MATE2-K.
Metabolites Methylnaltrexone sulfate and methyl-6α-naltrexol could potentially be substrate for BRCP. However, efflux ratio of methylnaltrexone sulfate and methyl-6α-naltrexol across MDCKII-BCRP cells and their respective uptake inhibition by BCRP model inhibitor are not consistent across different concentration (0.3 uM, 1 uM and 10 uM).

Table 33: Integrated Summary of Methylnaltrexone and its metabolites for Potential Substrate for Transporters

Transporter	Methylnaltrexone	Methylnaltrexone sulfate	Methyl-6α-naltrexol	Methyl-6β-naltrexol
	Substrate results: Is the compound a potential substrate of the transporter (Yes, No)			
P-gp	No	No	No	No
BCRP	No	Yes	Yes	No
MRP2	No	No	No	No
OATP1B1	No	No	No	No
OATP1B3	No	No	No	No
OCT1	Yes	No	Yes	Yes
OCT2	Yes	No	Yes	Yes
OAT1	No	No	No	No
OAT3	No	No	No	No
MATE1	Yes	No	Yes	Yes
MATE2-K	Yes	Yes	Yes	Yes

Methylnaltrexone did not inhibit P-gp and BCRP up to 500 uM and MRP2 up to 100 uM. OCT2 was inhibited and IC50 was estimated to be greater than 100 uM. Methylnaltrexone did not inhibit OATP1B1 and OTAP1B3 significantly up to 1800 uM. Estimated IC50 was greater than 10 uM for OCT1, OCT2, OAT1, OAT3, MATE1 and MATE2-K. For all metabolites, the IC50

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values are estimated to be greater than highest tested concentration of 10 uM. Based on Cmax values and estimated IC50 values for all transporter, it is unlikely that there will be any in-vivo inhibition of transporters by methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol.

Table 34: Integrated summary of likelihood of in-vivo inhibition of transporters by Methylnaltrexone and its metabolites

Test cmpd	Trans-porter	Study Number	IC_{50} (µM)	[I]/IC_{50} Ratio	[I_{gut}]/IC_{50} Ratio	Unbound C_{max}/IC_{50} Ratio	Likely Interaction (Y/N)
MNTX	P-gp	XT128007	NI at 500 µM	<0.001	<8.256		N
	BCRP	XT128007	NI at 500 µM	<0.001	<8.256		N
Cmax =	MRP2	XT128007	NI at 100 µM				N
32-54 ng/mL	OATP1B1	XT148071	>1800	<0.001			N
= 73-124 nM	OATP1B3	XT148071	>1800	<0.001			N
	OCT1	RPT-66294 /300777817	>25			<0.005	N
	OCT2	XT128007	>100			<0.001	N
Igut =	OCT2	XT148071	>10			<0.011	N
450mg/250mL = 4128 uM	OAT1	XT148071	>10			<0.011	N
	OAT3	XT148071	>10			<0.011	N
	MATE1	XT148071	>10			<0.011	N
	MATE2-K	XT148071	>10			<0.011	N
MNTX Sulfate	P-gp	XT148071	>10	< 0.005			N
	BCRP	XT148071	>10	< 0.005			N
	MRP2	XT148071	>10				N
	OATP1B1	XT148071	>10	< 0.005			N
Cmax =	OATP1B3	XT148071	>10	<0.005			N
17-22 ng/mL	OCT1	XT148071	>10			<0.004	N
=39-50 nM	OCT2	XT148071	>10			<0.004	N
	OAT1	XT148071	>10			<0.004	N
	OAT3	XT148071	>10			<0.004	N
	MATE1	XT148071	>10			<0.004	N
	MATE2-K	XT148071	>10			<0.004	N
Methyl-6α-naltrexol	P-gp	XT148071	>10	<0.003		<0.002	N
	BCRP	XT148071				<0.002	
	MRP2	XT148071	>10	<0.003		<0.002	N
Cmax = 5-12 ng/mL	OATP1B1	XT148071	>10			<0.002	N
=14-33nM	OATP1B3	XT148071	>10	<0.003		<0.002	N
	OCT1	XT148071	>10	<0.003		<0.002	N
	OCT2	XT148071	>10				N
	OAT1	XT148071	>10				N
	OAT3	XT148071	>10				N
	MATE1	XT148071	>10				N
	MATE2-K	XT148071	>10				N
			>10				N

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Methyl-6β-naltrexol Cmax = 2-5 ng/mL =6-14 nM	P-gp	XT148071	>10	<0.001		<0.001	N
	BCRP	XT148071				<0.001	
	MRP2	XT148071	>10	<0.001		<0.001	N
	OATP1B1	XT148071	>10			<0.001	N
	OATP1B3	XT148071	>10	<0.001		<0.001	N
	OCT1	XT148071	>10	<0.001		<0.001	N
	OCT2	XT148071	>10				N
	OAT1	XT148071	>10				N
	OAT3	XT148071	>10				N
	MATE1	XT148071	>10				N
	MATE2-K	XT148071	>10				N
			>10				N

Abbreviations: [I] = maximum plasma concentration of putative inhibitor; [I2] = estimated maximal intestinal concentration of putative inhibitor; NI = no inhibition

Substrate:

Study XT128007: This study evaluated the potential of methylnaltrexone to be a substrate of BCRP, MRP2, OATP1B1, OAT1P1B3, OAT1 and OAT3 in vitro- at 37 °C:

The test systems were appropriately validated with positive controls with model substrates and known model inhibitors. The tested concentrations were appropriate as it cover expected Cmax and 10 times the Cmax in patient population and healthy subjects with the clinical dose of 450 mg oral dose (Cmax of methylnaltrexone with clinical dose of 450 mg oral dose is approximately 32-54 ng/mL). Methylnaltrexone is not a substrate for BCRP, MRP2, OATP1B1, OATP1B3, OAT1 and OAT3.

Table 35: Evaluation of Methylnaltrexone for being potential Substrate for various transporters

Transporter	Test system	Positive Control substrate	Positive Control inhibitor	Methylnaltrexone concentration	Incubation Time (min)	Experimental design
BCRP	MDCKII-BCRP	Prazosin (1 μM)	Ko143 (1 μM)	1, 10 and 100 μM	15, 30, 60, 120	Substrate determination in MDCKII cells by measuring the bidirectional permeability across transporter expressing and control cells (n=3)
MRP2	Vesicles	Estradiol 17-β-D-glucuronide (50 uM)	Benzbromarone (100 μM)	1 and 10 uM	2 and 20	Substrate determination in vesicles, HEK293 and S2 cells by measuring the accumulation of the test article in the transporter expressing and control vesicles or cells
OATP1B1	HEK293	Estradiol glucuronide (50 nM)	Rifampin (10 μM)	0.5 and 5 μM		
OATP1B3	HEK293					
OAT1	S2	p-Aminohippurate (5 μM)	Probenecid (100 μM)			
OAT3	S2	Estrone sulfate (50 nM)				

* For inhibition studies of uptake transporters by model inhibitors, cells were pre-incubated with inhibitors for 15 minutes before incubating the cells with model substrates and model inhibitors.

Table 36: Bidirectional permeability of Methylnaltrexone across MDCKII-BCRP cells

Substrate (μM)	Control Cells Papp (× 10−6 cm/sec)			BCRP-expressing cells Papp (× 10−6 cm/sec)			Net ER
	A-B	B-A	ER	A-B	B-A	ER	
1 uM Methylnaltrexone	2.98±0.28	1.45±0.11	0.486	0.898±0.472	0.393±TFR	0.438	0.901
10 uM Methylnaltrexone	1.19±0.32	0.863±0.028	0.727	0.610±0.060	0.350±0.040	0.574	0.789

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100 uM Methylnaltrexone	1.49±0.28	0.588±0.028	0.394	0.481±0.038	0.379±0.034	0.788	2.00
1 uM Prazosin	40.9 ± 2.8	34.8 ± 5.5	0.851	10.7 ± 0.9	86.0 ± 5.4	8.07	9.49
1 uM Prazosin+1 uM Ko143	37.1 ± 2.0	45.5 ± 1.5	1.23	34.6 ± 3.1	53.3 ± 1.6	1.54	1.26

Table37: Uptake of Methylnaltrexone into MRP2-expressing vesicles

Substrate	Time (min)	Rate of uptake into Vesicles (pmol/ mg protein/ min)					
		MRP2			Control		
		+ATP	+AMP	ATP/AMP Ratio	+ATP	+AMP	ATP/AMP Ratio
1 uM Methylnaltrexone	2	NA	NA	NC	NA	NA	NA
	20	0.556 ± TFR	NA	NC	NA	NA	NA
10 uM Methylnaltrexone	2	11.5 ± 1.3	10.6 ± 1.2	1.14	10.1 ± 2.2	9.06 ± 1.56	NA
	20	1.32 ± 0.15	1.12 ± 0.15	0.89	1.48 ± 0.26	1.10 ± 0.08	NA
		Cleared volume (µL/mg protein)					
50 uM [3H]-Estradiol 17β-glucuronide		27.9 ± 3.4	2.29 ± 0.22	25.6	4.33 ± 0.13	3.22 ± 0.18	1.3
50 uM [3H]-Estradiol 17β-glucuronide + 100 uM Benzbromarone		7.49 ± 3.30	2.20 ± 0.27	3.4	3.52 ± 0.33	2.83 ± 0.35	1.2

Table 38: Uptake of Methylnaltrexone in the update transporter expressing cells

Uptake Transporter	Methylnaltrexone Concentration (µM)	Incubation time (min)	Uptake rate (pmol/mg/min)		Uptake ratio Transporter/Control
			Control cells	Transporter expressing cells	
OATP1B1	0.5	2	NC	NC	NC
		20	0.0663 ± TFR	NC	NC
	5	2	1.27 ± 0.39	1.34 ± 0.62	1.06
		20	0.271 ± 0.084	0.290 ± 0.065	1.07
OATP1B3	0.5	2	NC	NC	NC
		20	NC	NC	NC
	5	2	1.27 ± 0.39	1.13 ± 0.03	0.891
		20	0.271 ± 0.099	0.290 ± 0.058	1.07
OAT1	0.5	2	NC	NC	NC
		20	NC	0.096 ± TFR	NC
	5	2	0.968 ± TFR	0.961 ± TFR	0.992
		20	0.37±0.04	0.49 ± 0.06	1.34
OAT3	0.5	2	NC	NC	NC
		20	0.0912 ± TFR	NC	NC
	5	2	0.912 ± TFR	1.17 ± TFR	1.29
		20	0.562 ± 0.047	0.523 ± 0.011	0.929

Table 39: Positive Controls for Uptake transporters

Transporter	Model Substrate	Model Inhibitor	[Inhibitor] (µM)	Cleared volume (µL/ mg protein)		Percent of control (%)
				Control cells	Transporter-expressing cells	
OAPT1B1	[3H]-Estradiol 17-β-D-glucuronide (50 nM)	Rifampin	0	1.62 ± 0.41	38.7 ± 2.6	100
			10	0.667 ± TFR	1.88 ± 0.61	3.3
OAPT1B3	[3H]-Estradiol 17-β-D-glucuronide (50 nM)	Rifampin	0	0.743 ± 0.152	19.5 ± 1.5	100
			10	1.68 ± 0.62	1.18 ± 0.43	−2.7
OAT1	[14C]-p-Aminohippuric acid (5 uM)	Probenecid	0	1.68 ± 0.14	17.1 ± 2.8	100
			100	1.78 ± 0.45	3.13 ± 0.90	8.8
OAT3	[3H]-Estrone 3-Sulfate (50 uM)	Probenecid	0	0.354 ± 0.344	28.1 ± 4.4	100
			100	0.620 ± 0.298	2.45 ± 0.47	6.6

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Study XT148071:
This in-vitro study evaluated the potential of methylnaltrexone being a substrate of OCT2, MATE1 and MATE2-K and potential of methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol as being substrates of P-gp, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT1, OCT2, MATE1 and MATE2-K.

- The tested concentration up to 10 uM for methylnaltrexone, methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol are acceptable as they approximately cover the respective expected Cmax and 10 times Cmax values in healthy human subjects or OIC patients taking 450 mg of MNTX oral tablet.
    - Cmax was around 32-54 ng/mL (~ 73-124 nM) for methylnaltrexone, 17-22 ng/mL (~ 39-50 nM) for methylnaltrexone sulfate, 5-12 ng/mL (14-33 nM) for methyl-6α-naltrexol and 2-5 ng/mL (6-14 nM) for methyl-6β-naltrexol in healthy subject and OIC patients with clinical dose of 450 mg oral dose.

- All of the test systems appear to be valid as positive controls with model substrates has significant accumulation in uptake transporter and efflux in efflux transporter and the uptake or efflux were significantly inhibited in presence of model inhibitors (positive controls).

Table 40: Experimental conditions to evaluate the substrate potential of MNTX and its metabolites for efflux transporters

	P-gp	BCRP	MRP2
Test-System	MDCKII-MDR1	MDCKII-BCRP	Human MRP2 vesicles
[Methylnaltrexone sulfate] (µM)	0.3, 1 and 10	0.3, 1 and 10	0.3, 1, and 10
[Methyl-6α-naltrexol] (µM)	0.3, 1 and 10	0.3, 1 and 10	0.3, 1, and 10
[Methyl-6β-naltrexol] (µM)	0.3, 1 and 10	0.3, 1 and 10	0.3, 1, and 10
Positive control substrate	Digoxin (10 µM)	Prazosin (1 µM)	Estradiol-17β-glucuronide
Positive control inhibitor 1	Valspodar (10 µM)	Ko143 (1 µM)	Benzbromarone (100 µM)
Positive control inhibitor 2	NA	NA	
Permeability control	Lucifer yellow (40 µg/mL)	Lucifer yellow (40 µg/mL)	
Start reagent			MgATP or MgAMP
Nominal cell number per well	0.3 to 0.4 × 106	0.3 to 0.4 × 106	
Volume per well (µL)	A: 200; B: 980	A: 200; B: 980	50
Preincubation time (min)	30 to 60	30 to 60	15
Test article sampling time (min)	D: 0, 120 R: 0, 15, 30, 120	D: 0, 120 R: 0, 15, 30, 120	1, 3, 5 and 10
Positive control substrate sampling time (min)	D: 0, 120 R: 120	D: 0, 120 R: 120	
Incubation temperature (°C)	37 ± 2	37 ± 2	37 ± 2
Number of replicates	3	3	3
Analysis method	LC-MS/MS	LC-MS/MS	LSC
Experimental Design:	Bidirectional permeability of test article in transporter expressing cells and control cells		Accumulation in the presence and absence of ATP

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 41: Experimental conditions to evaluate the substrate potential of MNTX and its metabolites for uptake transporters

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	OATP1B1/ OATP1B3	OCT1	OCT2	OAT1	OAT3	MATE1	MATE2-K
Test System	HEK293	HEK293	HEK293	HEK293	HEK293	HEK293	HEK293
[Methylnaltrexone]	Not applicable	Not applicable	0.3, 1, 10 µM	Not applicable	Not applicable	0.3, 1, and 10 µM	
[Methylnaltrexon e sulfate]	0.3, 1, and 10 µM						
[Methyl-6α- naltrexol]	0.3, 1, and 10 µM						
[Methyl-6β-naltrexol]	0.3, 1, and 10 µM						
Positive control substrate	Estradiol-17β-glucuronide (0.05 µM)	Tetraethylammonium bromide (5 µM)	Metformin (10 µM)	p-Aminohippurate (1 µM)	Estrone-3-sulfate (0.05 µM)	[14C]Metformin (10 uM)	
Positive control inhibitor	Rifampin (10 µM)	Quinidine (100 µM)	Quinidine (300 µM)	Probenecid (100 µM)	Probenecid (100 µM)	Cimetidine (10 µM)	Cimetidine (100 µM)
Volume per well (µL)	300	300	300	300	300	300	500
Preincubation time (min)	15						
Incubation time (min)	1, 2, 3, and 10	1, 3, 10, and 15	1, 2, 3, and 10	1, 3, and 10	1, 2, 3, and 10	1, 3 and 10	1, 3 and 10
Incubation temperature (°C)	37 ± 2						
Number of replicates	3						
Analysis method (Test article)	LC-MS/MS						
Analysis method (positive control substrate)	LSC						
Experimental Design:	Accumulation of the test article in the transporter expressing and control cells						

Control cells were included with each transporter experiment under identical conditions.

Table 42: Transporter Mediated Transport of Methylnaltrexone (MNTX)

Transporters	**Positive control (probe substrate)**	**Positive control + inhibitor**	**0.3 uM test**	**0.3 uM test + inhibitor**	**1 uM test**	**1 uM test + inhibitor**	**10 uM test**	**10 uM test + inhibitor**
Ratio of Uptake in transporter overexpressing cell vs. control cells								
OCT2	20.8	6.3	240	4.05	213	7.16	149	6.87
MATE1	29.8	9.8	3.3	NA	2.5	1.3	1.2	0.7
MATE2-K	14.5	8.3	NA	NA	NA	8.4	71.2	9.4

Table 43: Transporter Mediated Transport of methylnaltrexone sulfate

Transporters	**Positive control (probe substrate)**	**Positive control + inhibitor**	**0.3 uM test**	**0.3 uM test + inhibitor**	**1 uM test**	**1 uM test + inhibitor**	**10 uM test**	**10 uM test+ inhibitor**
Ratio of Uptake in transporter overexpressing cell vs. control cells								
OCT1	20	6	0.452	0.633	0.567	0.872	0.310	0.485
OCT2	22	3.2	0.512	1.72	0.794	1.48	2.70	3.06
OAT1	542	16.7	2.34	4.43	2.59	9.12	3.32	3.54
OAT3	40	4.8	1.15	NC	1.13	1.55	1.07	1.20
OATP1B1	305	9.05	1.13	2.04	0.516	3.34	0.419	3.36
OATP1B3	28	1.6	NC	NC	NC	NC	0.131	0.111
MATE1	29.8	9.8	NA	NA	NA	NA	0.8	0.5
MATE2-K	14.5	8.3	NA	NA	1.4	1.4	1.8	0.9
Efflux Ratio (P_{B-A}/P_{A-B})								
P-gp	6.28	**0.825**	0.515	0.477	0.265	0.329	0.916	1.48
BCRP	7.89	**1.15**	3.88	1.04	1.18	0.791	1.65	1.54
Ratio of Vesicular Accumulation in ATP / AMP								
MRP2	35.6	3.49	1.02	0.845	1.02	0.827	0.999	1.07

NA: Not applicable

Reference ID: 3942818

Table 44: Transporter Mediated Transport of methyl-6α-naltrexol

Transporters	Positive control (probe substrate)	Positive control + inhibitor	0.3 uM test	0.3 uM test + inhibitor	1 uM test	1 uM test + inhibitor	10 uM test	10 uM test + inhibitor
Ratio of Uptake in transporter overexpressing cell vs. control cells								
OCT1	42	9	81.7	NC	126	16.8	105	19.1
OCT2	55	1.7	122	NC	150	1.10	86.4	1.43
OAT1	117	6.6	NC	NC	0.950	1.15	1.04	1.18
OAT3	48	2.8	NC	NC	1.04	0.702	1.18	0.590
OATP1B1	160	6.9	NC	NC	NC	1.37	0.668	1.50
OATP1B3	24	1.6	NC	5.68	3.64	3.65	7.17	1.52
MATE1	29.8	9.8	NA	NA	8.3	NA	7.0	1.6
MATE2-K	14.5	8.3	NA	NA	NA	5.2	31.1	4.0
Efflux Ratio (PB-A/PA-B)								
P-gp	4.69	0.634	0.729	0.398	0.501	0.353	0.968	1.03
BCRP	7.89	1.15	0.945	0.375	0.702	0.289	2.31	0.426
Ratio of Vesicular Accumulation in ATP / AMP								
MRP2	39.7	2.18	NC	0.954	0.599	0.788	0.649	0.771

NA: Not applicable
NC Not calculated

Table 45: Transporter Mediated Transport of Methyl-6β-naltrexol

Transporters	Positive control (probe substrate)	Positive control + inhibitor	0.3 uM test	0.3 uM test + inhibitor	1 uM test	1 uM test + inhibitor	10 uM test	10 uM test + inhibitor
Ratio of Uptake in transporter overexpressing cell vs. control cells								
OCT1	20	5.9	30.2	3.85	35.6	4.00	19.0	5.56
OCT2	83.8	15.8	16.1	1.93	22.0	2.23	30.4	1.42
OAT1	542	16.7	1.56	1.21	1.14	1.25	1.39	0.899
OAT3	40	4.8	2.03	2.24	2.10	2.90	1.85	2.07
OATP1B1	50	0.8	0.817	0.321	0.494	0.470	0.596	0.339
OATP1B3	18	1.78	3.23	6.21	2.23	8.43	3.08	3.57
MATE1	29.8	9.8	NA	NA	5.6	1.1	5.6	2.4
MATE2-K	14.5	8.3	NA	1.0	75.6	1.9	49.9	0.6
Efflux Ratio (PB-A/PA-B)								
P-gp	6.29	0.395	1.73	1.76	1.17	1.08	2.26	2.04
BCRP	15.1	1.24	2.55	2.72	1.49	1.21	1.85	0.773
Ratio of Vesicular Accumulation in ATP / AMP								
MRP2	35.3	0.851	1.17	1.99	0.888	1.06	1.07	1.24

NA: Not applicable

Table 46: Potential of Substrates for Transporters:

Transporter	Methylnaltrexone	Methylnaltrexone sulfate	Methyl-6α-	Methyl-6β-naltrexol
	Substrate results: Is the compound a potential substrate of the transporter (Yes, No)			
P-gp	NT	No	No	No
BCRP	NT	Yes	Yes	No
MRP2	NT	No	No	No
OATP1B1	NT	No	No	No
OATP1B3	NT	No	No	No
OCT1	NT	No	Yes	Yes
OCT2	Yes	No	Yes	Yes
OAT1	NT	No	No	No
OAT3	NT	No	No	No
MATE1	Yes	No	Yes	Yes

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MATE2-K	Yes	Yes	Yes	Yes

- Methylnaltrexone is good substrate for OCT2 as uptake ratio in OCT2 expressing cell vs. control cell varies 92-374 for Methylnaltrexone and this uptake ratio was reduced in presence of know OCT2 inhibitor quinidine to 3-7.
- Methylnaltrexone sulfate appears to be a potential substrate for BCRP. The efflux ratio for BCRP transporter is 3.88 at 0.3 uM concentration and efflux ratio was reduced to 1.04 in presence of known BCRP inhibitor Ko143. However, efflux ratio was less than 2 at concentration 1 uM and 10 uM.
- Methyl-6α-naltrexol appears to be a potential substrate for BCRP. The efflux ratio is for BCRP transporter is 2.31 at highest tested concentration of 10 uM and efflux ratio was reduced to 0.43 in presence of known BCRP inhibitor Ko143. However, the efflux ratio for BCRP less than 2 at concentration 0.3 and 1 uM. In addition, methyl-6α-naltrexol is good substrate for OCT1 and OCT2 as uptake ratio for transporter expressing cells vs control cell were 32-105 for OCT1 and 25-122 for OCT2 and the uptake ratios were reduced to 1-19 in presence of know OCT1/2 inhibitor quinidine.
- Methyl-6β-naltrexol appears to be a substrate for OCT1 and OCT2 as uptake ratio for transporter expressing cells vs control cell were 5-30 for OCT1 and OCT2 and the uptake ratios were reduced to 1-5 in presence of know OCT1/2 inhibitor quinidine.
- Methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol appears to be substrate for MATE1 and MATE2-K as uptake ratio of uptake in transfected cells was greater than two and reduced to below two in the presence of the positive control inhibitor.
- In-vivo implication: Methylnaltrexone and its metabolites methyl-6α-naltrexol, and methyl-6β-naltrexol shown to be substrate for OCT2. In addition, Methylnaltrexone and its metabolites methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol appears to be substrate for MATE1 and MATE2-K. In a previously conducted in vivo clinical drug interaction study with cimetidine, an inhibitor of OCT2 , MATE1 and MATE2-K, Cmax and AUC of methylnaltrexone increased by 10% when 24 mg methylnaltrexone as an IV infusion over 20 minutes was administered before and after multiple doses of cimetidine 400 mg every 6 hours. Result of this previous study with cimetidine alleviates the need for further in-vivo study to address the potential interaction of methylnaltrexone and its metabolites with OCT2, MATE1 and MATE2-K.

Inhibition:

Study XT128007:
Methylnaltrexone was evaluated for its ability to inhibit human P-gp, BCRP, MRP2 and OCT2 transporters as outlined in the following table.

Table 47: Evaluation of Methylnaltrexone for being potential Inhibitor of various transporters

Transporter	Test system	Probe substrate	Positive Controls	Methylnaltrexone concentration	**Experimental design**
P-gp	Caco-2	Digoxin (10 uM)	valspodar (1 µM)	0, 5, 15, 50, 150 and 500 µM	Inhibition of the bidirectional transport of the probe substrate across Caco-2 cells (P-gp) or MDCKII-BCRP and control MDCKII cells (BCRP) in triplicate
BCRP	MDCKII-BCRP	Prazosin (1 uM)	Ko143 (1 µM)	0, 5, 15, 50, 150 and 500 µM	
MRP2	Vesicles	Estradiol 17-β-D-glucuronide (50 uM)	Benzbromarone (100 µM)	0,0.1, 0.3, 1, 3, 10, 30 and 100 µM	Inhibition of the uptake of the probe substrate into transporter-expressing and control vesicles (MRP2) or cells (OCT2)
OCT2	HEK293	Metformin (10 uM)	Quinidine (300 uM)	0, 0.1, 0.3, 1, 3, 10, nd 100 µM	

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Table 48: P-gp Inhibition: Bidirectional permeability of digoxin (10 μM) across Caco-2 cells in the presence of Methylnaltrexone

Substrate	Inhibitor	[Inhibitor] (μM)	Papp (× 10−6 cm/sec)		ER	Relative transport (% of control)
			Apical to basal	Basal to apical		
Digoxin (10 μM)	Methylnaltrexone	0	3.02 ± 0.31	22.4 ± 1.3	7.41	100
		5	4.21 ± 2.38	19.4 ± 1.3	4.62	56.4
		15	3.23 ± 0.46	23.2 ± 2.4	7.16	96.0
		50	2.37 ± 1.13	21.5 ± 1.4	9.10	126
		150	3.30 ± 0.23	24.0 ± 2.5	7.26	97.6
		500	4.40 ± 1.17	25.3 ± 1.1	5.75	74.0
	Valspodar	0	3.02 ± 0.31	22.4 ± 1.3	7.41	100
		1	9.31 ± 0.51	8.98 ± 0.11	0.964	−0.6

Table 49: BCRP Inhibition: Bidirectional permeability of prazosin (1 μM) across MDCKII-BCRP cells in the presence of Methylnaltrexone

Substrate	Inhibitor	[Inhibitor] (μM)	Control cells			BCRP-expressing cells			Net ER	Relative transport (% of control)
			Papp (× 10−6 cm/sec)		ER	Papp (× 10−6 cm/sec)		ER		
			Apical to basal	Basal to apical		Apical to basal	Basal to apical			
Prazosin (1 μM)	Methylnaltrexone	0	29.4 ± 6.4	60.7 ± 11.7	2.07	6.29 ± 3.29	85.1 ± 16.3	13.5	6.55	100
		5	40.5 ± 3.9	54.1 ± 3.5	1.34	10.6 ± 0.7	84.6 ± 7.5	8.02	5.99	91.6
		15	29.1 ± 1.5	49.5 ± 9.9	1.70	6.75 ± 2.54	76.6 ± 21.7	11.3	6.67	102
		50	35.0 ± 2.9	44.1 ± 13.3	1.26	6.46 ± 2.18	75.6 ± 9.0	11.7	9.29	142
		150	26.7 ± 4.5	50.9 ± 18.9	1.91	7.54 ± 2.32	96.6 ± 17.2	12.8	6.73	103
		500	28.8 ± 4.1	38.0 ± 5.6	1.32	6.10 ± 0.77	70.3 ± 20.2	11.5	8.74	134
	Ko143	0	29.4 ± 6.4	60.7 ± 11.7	2.07	6.29 ± 3.29	85.1 ± 16.3	13.5	6.55	100
		1	36.3 ± 12.9	54.8 ± 10.6	1.51	31.3 ± 12.7	44.1 ± 9.4	1.41	0.931	14.2

- *The tested concentration of 5-500 uM of methylnaltrexone was adequate to assess the potential inhibition of P-gp and BCRP in systemic level as it cover the expected plasma Cmax and 10 times the Cmax value for methylnaltrexone in healthy human subjects or OIC patients taking clinical dose of 450 mg of MNTX oral tablet (Cmax of methylnaltrexone with clinical dose of 450 mg oral dose is approximately 32-54 ng/mL = 73-124 nM)).*
- *However, the tested concentration of 5-500 uM of methylnaltrexone was not adequate to assess the potential inhibition of methylnaltrexone to inhibit P-gp or BCRP in gut level as the expected concentration of methylnaltrexone in gut is about 10 fold higher than the highest tested concentration (Igut is estimated to be 450 mg/250 mL = 4.128 mM=4128 uM)). Nonetheless, based on available data, IC50 can be estimated to be higher than 500 uM for both P-gp and BCRP if there is any potential inhibition beyond tested concentration (IC50 >500 uM). Since Igut/IC50 = 4128/500 = 8.256 <10, an in-vivo interaction of methylnaltrexone with P-gp and /or BCRP in gut is unlikely.*

Table 50: MRP2 Inhibition: Uptake of [3H]-Estradiol 17β-glucuronide into MRP2-expressing vesicles in the presence of Methylnaltrexone

Substrate	Inhibitor	[Inhibitor] (μM)	Cleared volume (μL/mg protein)			Percent of control (%)	+ATP / −ATP ratio	IC50 (μM)
			+ATP	+AMP	ATP dependent			
50 uM [3 H]-Estradiol 17β-glucuronide in MRP2 vesicles	Methylnaltrexone	0	65.6	4.58	61.0	100	14.3	NC
		0.1	70.1	4.00	66.1	108	17.6	

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		0.3	67.4	3.59	63.8	105	18.8	
		1	67.6	3.73	63.8	105	18.1	
		3	71.4	2.57	68.9	113	27.8	
		10	68.6	4.54	64.1	105	15.1	
		30	67.5	4.03	63.5	104	16.8	
		100	72.4	3.65	68.7	113	19.8	
50 uM [3H]-Estradiol 17β-glucuronide in Control vesicles	Methylnaltrexone	0	5.48	4.24	1.24	NA	1.30	
		100	5.28	4.21	1.07	NA	1.30	
50 uM [3H]-Estradiol 17β-glucuronide in MRP2 vesicles	Benzbromarone	0	65.6	4.58	61.0	100	14.3	
		100	6.57	3.73	2.84	4.7	1.80	NA
50 uM [3H]-Estradiol 17β-glucuronide in Control vesicles	Benzbromarone	0	5.48	4.24	1.24	NA	1.30	
		100	3.83	3.61	0.224	NA	1.10	

NA Not applicable
NC Not calculated

Table 51: OCT2 Inhibition: Uptake of metformin into OCT2-expressing cells in the presence of Methylnaltrexone

Substrate	Inhibitor	[Inhibitor] (μM)	Cleared volume (μL/ mg protein)		Uptake ratio Transporter/Control	Percent of control (%)	IC50
			Control	OCT2-HEK293			
[14C]-Metformin (10 μM)	Methylnaltrexone	0	0.516 ± 0.061	15.9 ± TFR	30.6	100	>100
		0.1	0.765 ± 0.111	15.7 ± 3.7	20.5	96.8	
		0.3	0.630 ± 0.054	14.8 ± 1.4	23.5	92.1	
		1	0.585 ± 0.224	16.1 ± 3.3	27.5	101	
		3	0.412 ± 0.112	16.5 ± 3.2	40	104	
		10	0.347 ± 0.043	18.3 ± 3.6	52.7	116	
		30	0.190 ± 0.129	13.2 ± 2.2	69.5	84.7	
		100	0.060 ± TFR	8.02 ± 2.52	133.7	51.6	
	Quinidine	300	0.241 ± TFR	0.466 ± 0.155	1.9	1.5	

All The test systems were appropriately validated with positive controls with model substrates and known model inhibitors. The tested concentrations were appropriate as it cover expected Cmax and 10 times the Cmax in patient population and healthy subjects with the clinical dose of 450 mg oral dose.
Methylnaltrexone did not inhibit P-gp and BCRP up to 500 uM and MRP2 up to 100 uM. OCT2 was inhibited and IC50 was estimated to be greater than 100 uM.

Study XT148071:
This in-vitro study evaluated the ability of methylnaltrexone, methylnaltrexone sulfate, methyl-6α-naltrexol and methyl-6β-naltrexol were evaluated for their ability to inhibit human efflux and uptake transporters as outlined in the following table.

- The tested concentration up to 10 uM for methylnaltrexone, methylnaltrexone sulfate, Methyl-6α-naltrexol and Methyl-6β-naltrexol are acceptable as they approximately cover the respective expected Cmax and 10 times Cmax values in healthy human subjects or OIC patients taking 450 mg of MNTX oral tablet.
    - Cmax was around 32-54 ng/mL (~ 73-124 nM) for methylnaltrexone, 17-22 ng/mL (~ 39-50 nM) for methylnaltrexone sulfate, 5-12 ng/mL (14-33 nM) for methyl-6α-naltrexol and 2-5 ng/mL (6-14 nM) for methyl-6β-naltrexol in healthy subject and OIC patients with clinical dose of 450 mg oral dose.

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- All of the test systems appear to be valid as positive controls with model substrates has significant accumulation in uptake transporter and efflux in efflux transporter and the uptake or efflux were significantly inhibited in presence of model inhibitors (positive controls).

Table 52: Experimental conditions to evaluate the inhibition potential of MNTX and its metabolites for efflux transporters

	P-gp	BCRP	MRP2
Test-System	Caco-2	MDCKII-BCRP	Human MRP2 vesicles
[Methylnaltrexone sulfate] (µM)	0, 0.03, 0.1, 0.3, 1, 3, 10		
[Methyl-6α-naltrexol] (µM)	0, 0.03, 0.1, 0.3, 1, 3, 10		
[Methyl-6β-naltrexol] (µM)	0, 0.03, 0.1, 0.3, 1, 3, 10		
Positive control substrate	Digoxin (10 µM)	Prazosin (1 µM)	Estradiol-17β-glucuronide (50
Positive control inhibitor 1	Valspodar (10 µM)	Ko143 (1 µM)	Benzbromarone (100 µM)
Positive control inhibitor 2	Verapamil (60 µM)	Ritonavir (50 µM)	
Permeability control	Lucifer yellow (40	Lucifer yellow (40 µg/mL)	
Start reagent			MgATP or MgAMP
Nominal cell number per well	0.3 to 0.4 × 10^6	0.3 to 0.4 × 10^6	
Volume per well (µL)	A: 200; B: 980	A: 200; B: 980	50
Preincubation time (min)	30 to 60	30 to 60	15
Incubation time (min)	D: 0, 120; R: 120	D: 0, 120; R: 120	5
Incubation temperature (°C)	37 ± 2	37 ± 2	37 ± 2
Number of replicates	3	3	2
Analysis method	LC-MS/MS	LC-MS/MS	LSC
Experimental design	Bidirectional transport of the probe substrate across Caco-2 cells	Bidirectional transport of the probe substrate across MDCKII-BCRP and control MDCKII cells	Uptake of the probe substrate into MRP2 vesicles in the presence and absence of ATP

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 53: Experimental conditions to evaluate the inhibition potential of MNTX and its metabolites for uptake transporters

	OATP1B1/ OATP1B3	OCT1	OCT2	OAT1	OAT3	MATE1	MATE2-K
Test system	HEK293	HEK293	HEK293	HEK293	HEK293	HEK293	HEK293
[Methylnaltrexone] (µM)	3, 10, 30, 100, 300, 1000, 1800	NA	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM				
[Methylnaltrexone sulfate])	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM						
[Methyl-6α- naltrexol]	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM						
[Methyl-6β- naltrexol]	0.01, 0.03, 0.1, 0.3, 1, 3, 10 µM						
Experimental design	Uptake of the probe substrate into transporter expressing cells and control cells						
Substrate	Estradiol-17β-glucuronide (0.05 µM)	Tetraethylammonium bromide (5 µM)	Metformin (10 µM)	p-Aminohippurate (1 µM)	Estrone-3-sulfate (0.05 µM)	[^{14}C]Metformin (10 uM)	
Positive control inhibitor 1	Rifampin (10 µM)	Quinidine (100 µM)	Quinidine (300 µM)	Probenecid (100 µM)	Probenecid (100 µM)	Cimetidine (10 µM)	Cimetidine (100 µM)
Positive control inhibitor 2	Cyclosporine (1 µM)	Verapamil (10 µM)	Cimetidine (1000 µM)	Novobiocin (300 µM)	Ibuprofen (100 µM)	Pyrimethamine (03 uM)	
Volume per well (µL)	300	300	300	300	300	300	300
Preincubation time (min)	15	15	15	15	15	15	15
Incubation time (min)	2	15	2	1	2	5	5
Incubation temperature (°C)	37 ± 2	37 ± 2	37 ± 2	37 ± 2	37 ± 2	37 ± 2	37 ± 2
Number of replicates	3	3	3	3	3	3	3

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Analysis method	LSC	LSC	LSC	LSC	LSC	LSC	LSC

Control cells were included with each transporter experiment under identical conditions.

Potential of Inhibitors of Transporters:

Table 54: IC50 and maximum % inhibition of transporters by MNTX and its metabolites

Transporter	**Methylnaltrexone**	**Methylnaltrexone sulfate**	**Methyl-6α-naltrexol**	**Methyl-6β-naltrexol**
	Inhibition results: IC50 (μM)			
P-gp	NT	> 10	> 10	> 10
BCRP	NT	> 10	> 10	> 10
MRP2	NT	> 10	> 10	> 10
OATP1B1	> 1800	> 10	> 10	> 10
OATP1B3	> 1800	> 10	> 10	> 10
OCT1	NT	> 10	> 10	> 10
OCT2	> 10	> 10	> 10	> 10
OAT1	> 10	> 10	> 10	> 10
OAT3	> 10	> 10	> 10	> 10
MATE1	> 10	> 10	> 10	> 10
MATE2-K	> 10	> 10	> 10	> 10
Transporter	**Methylnaltrexone**	**Methylnaltrexone sulfate**	**Methyl-6α-naltrexol**	**Methyl-6β-naltrexol**
	Maximum percent inhibition (%)			
P-gp	NT	<15%	<15%	<15%
BCRP	NT	<15%	<15%	**29%**
MRP2	NT	<15%	**23%**	<15%
OATP1B1	**16**	**17%**	**23%**	<15%
OATP1B3	<15%	<15%	<15%	<15%
OCT1	NT	<15%	**19%**	**48%**
OCT2	<15%	<15%	**30%**	≤15%
OAT1	**48**	<15%	**24%**	<15%
OAT3	<15%	**34%**	<15%	<15%
MATE1	**43**	<15%	**16%**	**33%**
MATE2-K	<15%	<15%	<15%	<15%

- Methylnaltrexone, up 10 uM concentration, does not inhibit OCT2, OAT3 and MATE2-K greater than 15%. Therefore IC50 could not be calculated. IC50 can be estimated to be greater than the highest tested concentration 10 uM. Methylnaltrexone does inhibit OAT1 and MATE1 to certain extent. However, inhibit does not exceed 50% at the highest tested concentration of 10 uM. Therefore, IC50 can be estimated to be greater than 10 uM. Methylnaltrexone, up 1800 uM concentration, does not inhibit OATP1B1 and OATP1B5 greater than 16% and IC50 can be estimated to be greater than the highest tested concentration 1800 uM for OATP1B1 and OATP1B3.
- Methylnaltrexone sulfate up 10 uM concentration, does not inhibit P-gp, BCRP, MRP2, OATP1B3, OCT1, OCT2, OAT1, MATE1 and MATE2-K greater than 15%. Therefore IC50 could not be calculated. IC50 can be estimated to be greater than the highest tested concentration 10 uM. Methylnaltrexone sulfate does inhibit OATP1B1 and OAT3 to certain extent. However, inhibit does not exceed 50% at the highest tested concentration of 10 uM. Therefore, IC50 can be estimated to be greater than 10 uM for these transporters too.

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- Methyl-6α-naltrexol, up 10 uM concentration, does not inhibit P-gp, BCRP, OATP1B3, OAT3 and MATE2-K greater than 15%. Therefore IC50 could not be calculated. IC50 can be estimated to be greater than the highest tested concentration 10 uM. Methyl-6α-naltrexol does inhibit MRP2, OATP1B1, OCT1, OCT2, OAT1 and MATE1 to certain extent. However, inhibit does not exceed 50% at the highest tested concentration of 10 uM. Therefore, IC50 can be estimated to be greater than 10 uM for theses transporters as well.
- Methyl-6β-naltrexol, up 10 uM concentration, does not inhibit P-gp, MRP2, OATP1B1, OATP1B3, OCT2, OAT1, OAT3 and MATE2-K greater than 15%. Therefore IC50 could not be calculated. IC50 can be estimated to be greater than the highest tested concentration 10 uM. Methyl-6β-naltrexol does inhibit BCRP, OCT1 and MATE1 to certain extent. However, inhibit does not exceed 50% at the highest tested concentration of 10 uM. Therefore, IC50 can be estimated to be greater than 10 uM for theses transporters as well.

Induction:

Potential of methylnaltrexone and its metabolites to induce transporters were not evaluated in this NDA submission. Potential of methylnaltrexone and its metabolites to induce P-gp transporter can be ruled out based on absence of induction of CYP3A4 them in *in-vitro* study.

2.7.2.4 What In-vivo drug interaction studies were conducted based on *in-vitro* findings?

The sponsor did not conduct any in-vivo drug-drug interaction in this submission. The results of in-vitro studies in this submission did not suggest need for any in-vivo drug-drug interaction study.

2.7.2.5 Does the label specify co-administration of another drug and, if so, has the interaction potential between these drugs been evaluated?

No, the label does not specify co-administration of methylnaltrexone with another drug.

2.7.2.6 Are there any other *In-vivo* drug-drug interaction studies indicating that exposure alone and/or E-R relationships are different when drugs are co-administered?

No.

2.7.2.7 Has modeling and simulation been used to project drug interactions?

No

2.7.2.8 What is the effect of other extrinsic factors (herbal products, diet, smoking, and alcohol use) on exposure and safety?

There were no specific studies or analyses designed to evaluate the effects of extrinsic factors such as herbal products, diet (other than high-fat meal), smoking or alcohol use on the exposure or safety of methylnaltrexone. The effect of a high fat meal is discussed in Section 2.8.6.

2.7.2.9 Is there a known mechanistic basis for pharmacodynamic drug-drug interactions, if any?

Methylnaltrexone, by having mu opioid antagonistic effect, is designed to speed up the gastrointestinal transit time to resolve constipation. As a result, this may shorten the absorption time for other concomitant medications leading to decreased absorption and exposure.

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2.7.2.10 Were *In-vivo* PD drug interaction studies conducted?

No

2.7.2.11 Are there any unresolved questions related to metabolism, active metabolites, metabolic drug interactions?

No.

2.7.3 Are there any issues related to dose, dosing regimens, or administration with respect to extrinsic factors that are unresolved and/or represent significant omissions??

No

2.8 General Biopharmaceutics

2.8.1 What are the solubility and the permeability of eluxadoline?

Methylnaltrexone Bromide is soluble in water. The solubility profile of methylnaltrexone bromide over a pH range of 3.4 to 12.0 in aqueous phase is summarized in Table 22.

Table 55: Solubility Profile of Methylnaltrexone Bromide

Characteristics	Mallinckrodt (b) (4) (Lot P00882)	Mallinckrodt (b) (4) (Lot P05890)	(b) (4) (Lot 11P1136)
pH Solubility		Mean Concentration (mg/mL)	
pH 1.2	56.44445	67.25464	65.38089
pH 4.5	52.81723	66.99995	69.38750
pH 6.8	66.77189	71.26030	72.05630
pH 7.2	66.43003	71.00134	72.26866
pH 8.4	19.53545	15.65701	16.10815

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?

Methylnaltrexone Bromide is developed only at one strength as 150 mg oral tablet.

Table 56: Composition of To-Be Marketed (TMB) Formulation of Methylnaltrexone Bromide Tablets, 150 mg

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Ingredient	Quality Standard	Function	Theoretical Quantity	
			mg/tablet	% w/w [a]
[redacted]				
Methylnaltrexone bromide [b]	In house	Active	150.00	[redacted]
Microcrystalline cellulose [c]	NF	[redacted]	[redacted]	
Crospovidone	NF			
Edetate calcium disodium	USP			
Sodium lauryl sulfate	NF			
Colloidal silicon dioxide	NF			
Croscarmellose sodium	NF			
Poloxamer 407 [redacted]	NF			
Silicified microcrystalline cellulose	NF			
Stearic acid (vegetable source)	NF			
[redacted]				
Total Theoretical Weight:	---		533.59	100.0

Abbreviations: DMF = drug master file, NF = National Formulary, USP = United States Pharmacopeia, w/w = weight/weight.

[a] Based on coated tablet weight

[b] [redacted]

[c] [redacted]

[d] [redacted]

[e] [redacted]

2.8.3 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 pivotal clinical trial NMTX 3201 was conducted with an uncoated tablet formulation that was produced by a [redacted] process. [redacted] To bridge the uncoated and coated formulations with [redacted] the sponsor had conducted a BE study MNPK1118 (failed BE in Cmax by [redacted]%). Simultaneously, the sponsor had also conducted a food effect study (MNPK 1114) and relative BA to SC formulation study (MNPK 1117) with coated tablet with [redacted] process. [redacted]

To address these formulation issues with [redacted] process, the sponsor had conducted a further formulation exploration study MNPK 1001 to test 6 different formulations and have chosen formulation C (coated tablet with [redacted] process) as the final TBM based on BE criteria to the uncoated tablet with [redacted] process used in phase 3 study as the reference product. Relative BA of these two formulations (TBM vs. phase 3 formulations) was also compared in patient population in parallel study design in study MNOC1111.

With this TBM formulation, the sponsor conducted another food effect study (MNFE1001) and hepatic impairment study (MNPK1004).

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Table 57: Comparison of Phase 3 and Proposed Commercial Formulations

	PHASE 3 FORMULATION		PROPOSED COMMERCIAL FORMULATION	
Clinical Use [a]	**MNTX 3201 (Phase 3 Study) MNPK 1118, MNPK1001, and MNOC111 (Bridging Studies)**		**MNPK1001 and MNOC1111 (Bridging Studies)**	
Manufacturing Process	(b) (4)		(b) (4)	
Components (Listed by Functionality)	**Theoretical Quantity**		**Theoretical Quantity**	
	mg/tablet	**% w/w**	**mg/tablet**	**% w/w**
Active Ingredient				
Methylnaltrexone bromide (b) (4)	150.00	(b) (4)	150.00	(b) (4)
Silicified microcrystalline cellulose	(b) (4)			
Microcrystalline cellulose (b) (4) (b) (4)				
Croscarmellose sodium				
Crospovidone (b) (4) (b) (4)				
Sodium lauryl sulfate (b) (4)				
Poloxamer 407 (b) (4) (b) (4)				
Edetate calcium disodium (b) (4)				
Talc				
Colloidal silicon dioxide (b) (4) (b) (4)				
Stearic acid (b) (4)				
Total Theoretical Weight	533.59 [b]	100.0	533.59	100.0

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

The to-be-marketed (TBM) formulation was not used in the phase III pivotal clinical study MNTX3201. However, it was used in the food effect study (MNFE1001) and hepatic impairment study MNPK1004.

2.8.5 What is the relative bioavailability of the proposed to-be-marketed formulation to the pivotal clinical trial?

The proposed to-be-marketed (TBM) formulation (coated tablet with (b) (4) process) is bioequivalent to the formulation used in pivotal clinical study MNTX 3201 (uncoated tablet with (b) (4) process). Their bioequivalency was demonstrated in study MNPK1001 in healthy subjects in crossover study design. In addition, comparable exposures between these two products were also shown in patient population in parallel group study design in study MNOC1111.

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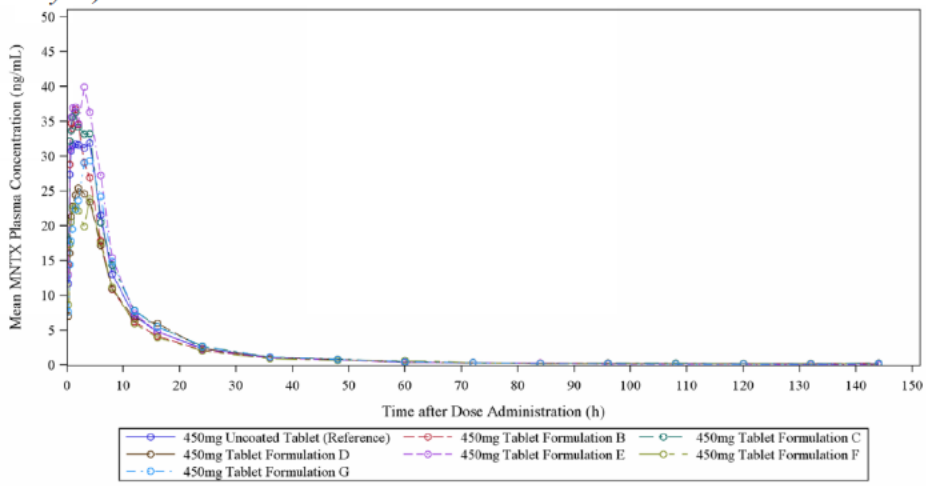
An inspection was requested for pivotal bioequivalence (BE) study MNPK1001 for both clinical site (PPD Development, LLC, Austin, TX) and bioanalytical site ([redacted]) on 08/19/2015. The Division of New Drug Bioequivalence Evaluation (DNDBE) within the Office of Study Integrity and Surveillance (OSIS) conducted the inspection of the bioanalytical site and recommended the analytical data from study MNPK1001 be accepted for Agency review on [redacted] The final classification for this analytical site inspection was voluntary action indicated (VAI). For the clinical site inspection, DNDBE within OSIS recommended accepting data without on-site inspection on 09/02/2016 since OSIS recently inspected the requested clinical site and the inspectional outcome from the inspection was classified as No Action Indicated (NAI).

Study MNPK1001: This study was an open-label, phase 1, randomized, single-center, single - dose, 2-way crossover study in healthy subjects to evaluate the bioequivalence of a single oral 450 mg dose of MNTX (3 x150 mg tablets) from each of 6 test formulations compared with a single oral 450 mg dose of the reference MNTX (3x150 mg) uncoated tablet formulation used in phase 3 Study 3201 (Formulation A in this study). All 6 test formulations (B through G) of methylnaltrexone bromide 450 mg (3 x 150 mg tablets) had [redacted] film coating. Formulations A (reference), B, D, and E were manufactured using a [redacted] process, and Formulations C, F, and G were manufactured with a [redacted] method. A total of 180 healthy subjects were randomized into 1 of 6 cohorts (30 subjects per cohort). Within each cohort, subjects were randomized into 1 of 2 study sequences. Each sequence had 2 study periods, and two periods were separated by a 7-day washout period. The dose was administered following an overnight of fast of at least 10 hour and no food was allowed for at least 4 additional hours post-dose on Day 1. PK blood samples were collected for up to 144 hours post-dose to determine the plasma concentrations of methylnaltrexone bromide following both treatments.

Figure 13: MNTX Mean Plasma Concentration vs. Time profile by Treatment Group (Sponsor's Analysis)



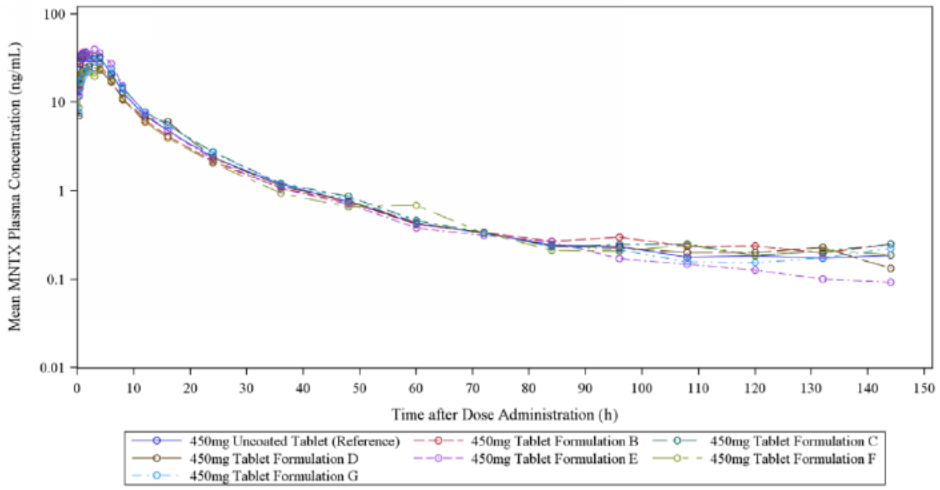
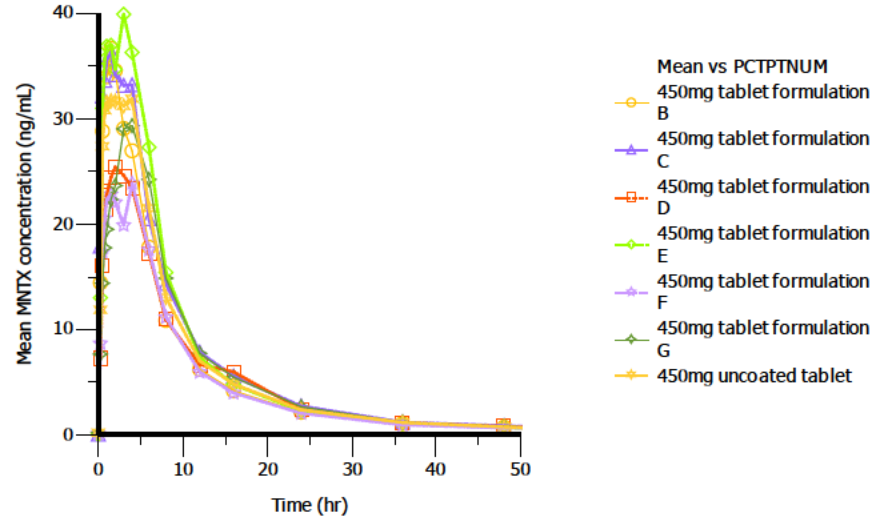
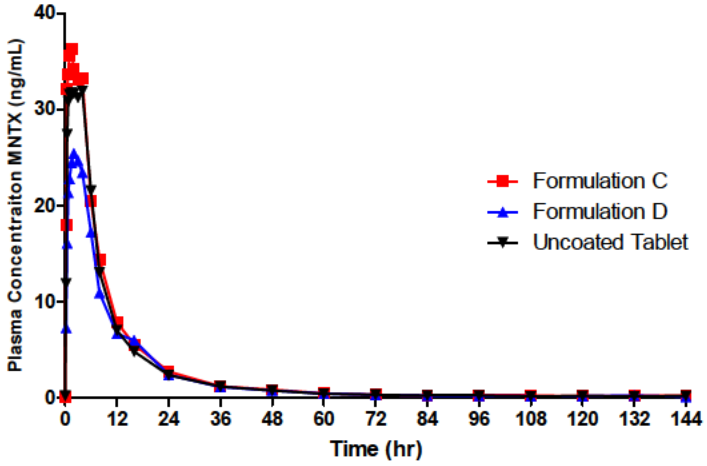


Figure 14: MNTX Mean Plasma Concentration vs. Time Profile by Treatment Group (Reviewer's Analysis)





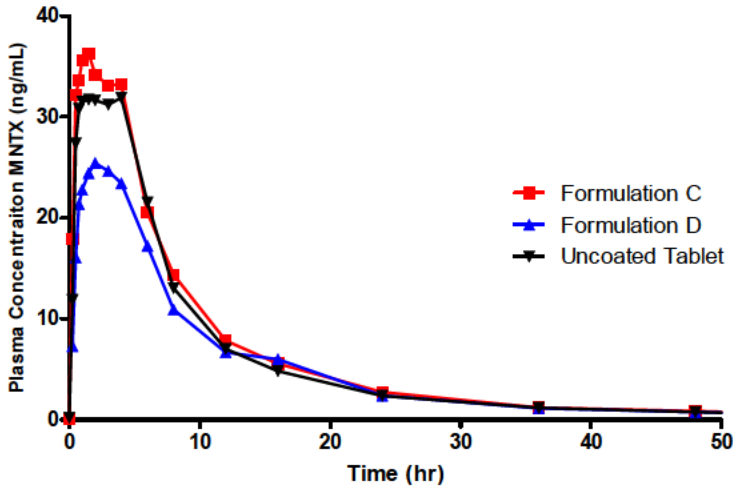


Table 58: Mean (± SD) Plasma PK Parameters: MNTX 450 mg Reference Formulation and Test Formulations (Sponsor's Analysis)

Parameters	450 mg Reference Tablet (Formulation A) (N=178)	450 mg Tablet Formulation B (N=30)	450 mg Tablet Formulation C (N=30)	450 mg Tablet Formulation D (N=29)	450 mg Tablet Formulation E (N=30)	450 mg Tablet Formulation F (N=30)	450 mg Tablet Formulation G (N=29)
C_{max} (ng/mL)	46.10 (35.825)	50.38 (48.309)	48.14 (41.000)	33.46 (17.463)	54.17 (39.778)	31.08 (19.761)	37.70 (32.447)
T_{max} (h) [a]	1.79 (0.50, 6.03)	1.50 (0.25, 6.00)	1.50 (0.25, 8.00)	2.00 (0.25, 16.00)	1.50 (0.25, 6.00)	2.00 (0.27, 8.00)	4.00 (0.25, 8.15)
AUC_{0-t} (ng.h/mL)	349.71 (190.139)	324.19 (183.371)	378.19 (191.740)	304.26 (133.761)	389.02 (211.957)	278.10 (116.122)	341.66 (242.302)
$AUC_{0-\infty}$ (ng.h/mL)	353.09 (190.992)	328.21 (186.630)	382.19 (193.411)	306.90 (134.433)	391.18 (212.360)	283.12 (117.468)	344.56 (244.391)
CL/F (L/h)	1404.45 (825.133)	1842.74 (966.249)	1521.43 (842.098)	1803.10 (919.835)	1425.35 (630.584)	1061.11 (505.598)	989.64 (464.031)
λ_z (h^{-1})	0.05 (0.017)	0.05 (0.017)	0.05 (0.017)	0.05 (0.016)	0.04 (0.012)	0.04 (0.019)	0.05 (0.018)
$t_{½}$ (h) [b]	14.79 (5.383)	14.75 (5.4721)	15.18 (5.808)	15.11 (5.370)	15.62 (4.366)	15.60 (6.584)	14.65 (5.521)

Table 59: Mean (± SD) Plasma PK Parameters: MNTX 450 mg Reference Formulation and Test Formulations (Reviewer's Analysis)

		450 mg tablet formulation B	450 mg tablet formulation C	450 mg tablet formulation D	450 mg tablet formulation E	450 mg tablet formulation F	450 mg tablet formulation G	450 mg uncoated tablet
AUCall (ng.h/mL)	Mean	323.90	378.24	302.71	388.71	278.10	341.44	349.49
	SD	182.70	191.64	131.58	211.11	116.33	242.16	189.52
AUC inf (ng.h/mL)	Mean	327.92	382.24	305.31	390.87	283.13	344.34	352.86
	SD	185.95	193.31	132.26	211.52	117.67	244.24	190.37
Cmax (ng/mL)	Mean	50.38	48.14	33.03	54.17	31.08	37.70	46.03
	SD	48.31	41.00	17.32	39.78	19.76	32.45	35.74
Half life (hr)	Mean	16.94	18.01	17.14	17.43	21.24	17.22	18.37
	SD	7.21	8.53	6.47	7.25	18.72	8.12	17.75
Lamda (hr-1)	Mean	0.05	0.05	0.05	0.04	0.04	0.05	0.05
	SD	0.02	0.02	0.02	0.01	0.02	0.02	0.02
Tmax (hr)	Mean	1.93	2.07	2.48	2.16	2.64	3.41	2.18
	SD	1.44	1.76	2.82	1.44	1.89	1.92	1.61

Table 60: Geometric Mean Ratios and 90% CIs for MNTX Cmax and AUC: Oral MNTX 450 mg of Test Formulations versus Reference Formulation (Sponsor's Analysis)

Reference ID: 3942818

Comparison Groups	Parameter	GMR of Treatment[a]	90 % CI for GMR	
			Lower	Upper
Formulation B vs Reference (A) (Nonbioequivalent)	C_{max} (ng/mL)	90.69	75.84	108.44
	AUC_{0-t} (ng.h/mL)	94.67	82.90	108.11
	$AUC_{0-\infty}$ (ng.h/mL)	94.75	83.02	108.12
Formulation C vs Reference (A) (Bioequivalent)	C_{max} (ng/mL)	102.03	87.33	119.20
	AUC_{0-t} (ng.h/mL)	103.70	91.41	117.63
	$AUC_{0-\infty}$ (ng.h/mL)	103.59	91.34	117.47
Formulation D vs Reference (A)* (Bioequivalent)	C_{max} (ng/mL)	97.57	82.34	115.61
	AUC_{0-t} (ng.h/mL)	96.83	84.17	111.41
	$AUC_{0-\infty}$ (ng.h/mL)	96.58	83.94	111.13
Formulation E vs Reference (A) (Nonbioequivalent)	C_{max} (ng/mL)	118.53	99.22	141.59
	AUC_{0-t} (ng.h/m)	116.16	102.54	131.58
	$AUC_{0-\infty}$ (ng.h/mL)	115.54	102.17	130.67
Formulation F vs Reference (A) (Nonbioequivalent)	C_{max} (ng mL)	67.93	58.86	78.39
	AUC_{0-t} (ng.h/mL)	81.16	73.89	89.15
	$AUC_{0-\infty}$ (ng.h/mL)	82.03	74.62	90.17
Formulation G vs Reference (A)* (Nonbioequivalent)	C_{max} (ng/mL)	77.38	67.74	88.38
	AUC_{0-t} (ng.h/mL)	84.09	74.64	94.73
	$AUC_{0-\infty}$ (ng.h/mL	84.04	74.58	94.71

Table 61: Geometric Mean Ratios and 90% CIs for MNTX Cmax and AUC: Oral MNTX 450 mg of Test Formulations versus Reference Formulation (Reviewer's Analysis)

	Parameter	GMR	Lower 90% CI	Upper 90% CIs
Formulation C vs. Reference Formulation A	Cmax	102.03	87.33	119.20
	AUC(0-t)	103.69	91.41	117.63
	AUC(0-inf)	103.58	91.34	117.47
Formulation D vs. Reference Formulation A	Cmax	97.10	82.08	114.88
	AUC(0-t)	96.88	84.31	111.32
	AUC(0-inf)	96.62	84.08	111.04

Reviewer's Comment:

- Based on the PK profile in log-scale, MNTX concentration appears to decline in biphasic manner.
- Formulations B, E, F, and G were not bioequivalent to the reference Formulation A.
- Formulations C and D were bioequivalent to the reference Formulation A used in phase 3 Study 3201 (uncoated tablet with (b) (4) process). Each of these formulations met the accepted bioequivalence criteria: specifically, the 90% CIs for the GMRs relative to the reference formulation were within the 80% to 125% range for all 3 parameters, C_{max}, AUC_{0-t}, or $AUC_{0-\infty}$.
- Formulation C was manufactured by a (b) (4) process and Formulation D was manufactured by (b) (4) (b) (4) the sponsor has chosen Formulation C as the to-be-marketed (TBM) formulation for further development.
- Sponsor's PK plots, PK analysis and BE analysis were repeated and were consistent with sponsor's result.

Study MNOC1111: This was a phase 1b, randomized, double-blind, placebo-controlled, parallel group, single-dose study evaluate the laxation efficacy, PK and safety of single oral dose of 450 mg (3x150 mg) MNTX of phase 3 formulation (uncoated tablet with (b) (4) vs. single

oral dose of 450 mg (3X150 mg) MNTX of TBM formulation (coated tablet with [redacted] in OIC patient population with non-cancer pain. Subjects fasted for at least 8 hours prior to study drug administration. PK samples were collected up to 24 hours post oral dose to determine the plasma concentrations of methylnaltrexone bromide and its metabolites.

Figure 15: Mean Plasma Concentration-Time Profiles for Methylnaltrexone (MNTX) Following Single-Dose MNTX 12 mg SC and Single-Dose Oral MNTX 450 mg from the [redacted] or [redacted] Tablet Formulations

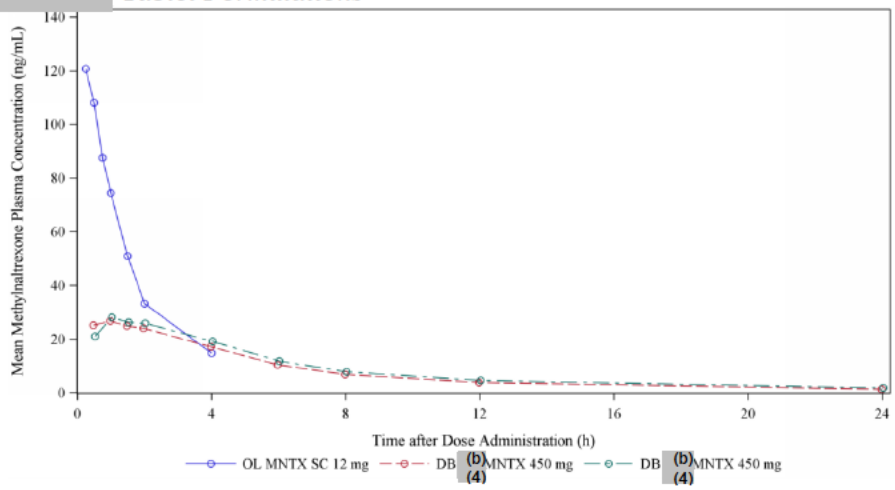


Table 62: Mean (± SD) Plasma MNTX PK Parameters Following Single MNTX 450 mg Oral Tablet Doses with the [redacted] or [redacted] Formulations

Methylnaltrexone Parameters	[redacted] MNTX 450 mg (N=37)	[redacted] MNTX 450 mg (N=36)
C_{max} (ng/mL)	34.58 (27.702)	35.35 (28.399)
T_{max} [a] (hours, median & range)	1.53 (0.43 – 6.23)	2.00 (0.50 – 4.18)
AUC_{0-t} (ng h/mL)	183.50 (97.882)	204.86 (136.109)
AUC_{0-4} (ng h/mL)	84.95 (58.538)	89.19 (70.364)
$AUC_{0-\infty}$ (ng h/mL)	203.53 (104.617)	228.65 (150.498)
CL/F (L/h)	3324.13 (3109.837)	2865.03 (1985.385)
Half-life (hours)	6.63 (2.352)	7.20 (2.301)

Reviewer's comment:

- TBM formulation (coated tablet with [redacted] and phase 3 formulation (uncoated tablet with [redacted] appear to have similar PK profiles and PK parameters in OIC patient population with non-cancer pain from 0 to 24 hours post dose.
- The reported half-life of 6-7 hours in this study with 24 hours of post-dose PK sampling is little shorter than the half-life of 14-16 hours reported in healthy subjects with 144 hours post-dose PK sampling in study MNPK1001. As MNTX appears to have biphasic decline in concentration, the reported half-life of 6-7 hour may only reflect the 1st phase of concentration decline rather than the terminal half-life.
- TBM formulation (coated tablet with [redacted] appears to have worse safety profile compare to phase 3 formulation (uncoated tablet with [redacted] 31.6% subject with TEAE in TBM formulation vs. 16.2% subjects with TEAE in phase 3

formulation. Please see Medical Reviewer Dr. Dina Zand's review for detailed safety comparison of these two formulations.

2.8.6 Were various drug products used in clinical studies comparable in terms of PK exposure?

Relative bioavailability of film-coated tablet with (b) (4) *(interim formulation that is neither a phase 3 formulation nor a TBM formulation) was compared with uncoated tablet with* (b) (4) *that was used in phase 3 pivotal efficacy study MNTX3201 and have shown that it has bioequivalent AUC but* (b) (4)*% lower Cmax compared to phase 3 formulation.*

Study MNPK1118: This was a phase 1, open-label, 2-stage study in healthy subjects. The second stage of the study was designed to compare the PK of MNTX after a single oral dose of 450 mg (3 x 150 mg) MNTX film-coated tablet with (b) (4) (test) to single oral dose of 450 mg (3 x 150 mg) MNTX uncoated tablets with (b) (4) that was used in Phase 3 study MNTX 3201 (reference) under fasted conditions in randomized crossover design. This test formulation is the similar to the phase 3 formulation, with the exception of a (b) (4) film coating of the MNTX tablets. Both of these two formulations were manufactured with a (b) (4) process. Please note that this test formulation is neither a phase 3 formulation, nor a TBM formulation. PK samples were collected up to 120 hours post-dose to determine the concentration of plasma concentration of N-methylnaltrexone.

Figure 16: Mean Plasma MNTX Concentrations Following a Single Oral Dose of 450 mg MNTX as Film-Coated (test formulation) or Uncoated Tablets (phase 3 formulation)

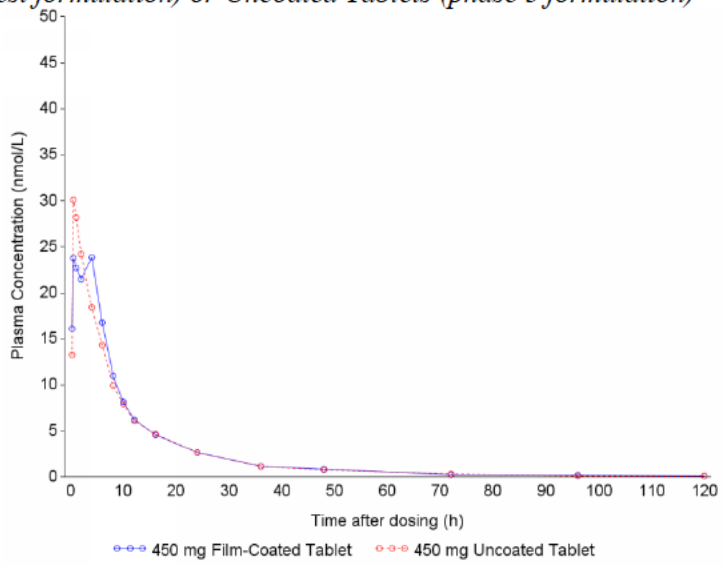


Table 63: Single-Dose PK Parameters for Oral MNTX (450 mg) in Stage 2, Film-Coated and Uncoated Tablets

Reference ID: 3942818

Pharmacokinetic Parameter	Methylnaltrexone	
	450 mg Film-Coated Tablet (N = 15)	450 mg Uncoated Tablet (N = 16)
C_{max} (ng/mL) Mean (SD)	32.66 (22.19)	35.60 (19.68)
$AUC_{0-\infty}$ (ng.h/mL) Mean (SD)	293.96 (95.34)	284.84 (75.19)
AUC_{0-t} (ng.h/mL) Mean (SD)	291.17 (95.44)	278.57 (73.89)
T_{max} (hours) Median (min, max)	2.00 (0.50, 6.00)	1.00 (0.50, 6.00)
CL/F (mL/h) Mean (SD)	1696.29 (597.01)	1706.88 (549.30)
$t_{½}$ (hours) [a] Mean (SD)	17.33 (7.40)	16.04 (4.30)

Table 64: Geometric Mean Ratios and 90% Confidence Intervals for Film-Coated Tablet (Test) to Uncoated Tablet (Reference) MNTX Systemic Exposure Parameters in Stage 2

Pharmacokinetic Parameter	Treatment	Intra-subject CV (%)	Geometric Least-square Means	Ratio of Treatment (%)	90% Confidence Interval (%)
C_{max} (ng/mL)	450 mg Film-Coated Tablet 450 mg Uncoated Tablet	36.12	28.392 31.315	90.67	(72.36, 113.61)
AUC_{0-t} (ng.h/mL)	450 mg Film-Coated Tablet 450 mg Uncoated Tablet	28.75	276.889 268.786	103.01	(86.04, 123.34)
$AUC_{0-\infty}$ (ng.h/mL)	450 mg Film-Coated Tablet 450 mg Uncoated Tablet	28.89	280.455 274.724	102.09	(85.19, 122.34)

***Reviewer's Comment*:**

- Test formulation (film coated MNTX tablet with [redacted] (b) (4) is NOT bioequivalent to the reference formulation, uncoated MNTX tablet with [redacted] (b) (4) that was used in Phase 3 study MNTX 3201. Although AUC met the BE criteria, the lower bound of 90% CI for GMR for Cmax was [redacted] (b) (4) outside the BE range. Cmax from the test formulation was [redacted] (b) (4) % lower than the reference product.

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

Administration of 450 mg (3X150 mg) MNTX tablet with [redacted] (b) (4) (TBM formulation) with high-fat breakfast reduced the AUC by 43% C_{max} by 60% and delayed the tmax by 2 hours compared to fasted state (study MNEF1001). This observed effect of food on TBM was consistent with the effect of food on film-coated tablet with [redacted] (b) (4) (neither a phase 3 nor a TBM formulation) where food had decreased AUC by 50% and Cmax by 64% (study MNPK 1114). In the phase III study MNTX3201, patients were instructed to take 450 mg MNTX tablets (3X150 mg) with water, first thing in the morning on an empty stomach 30 minutes before ingesting any food. The sponsor's proposed label recommends taking the tablet on an empty stomach at least 30 minutes before the first meal of the day. Based on the result of this food effect study and design of phase III study MNTX 3201, the sponsor's proposed labeling in regards to food is acceptable.

Study MNEF1001: This study was an open-label, phase 1, randomized, single-center, single dose-dose, 2-way crossover study in 32 healthy adult subjects to evaluate the effects of a high-fat meal on the PK of 450 mg (3X150 mg) TBM formulation of MNTX tablet with [redacted] (b) (4) tablet. Under fasted condition, following an overnight fasting of at least 10 hours, subject

received a single oral dose of 450 mg MNTX. Under fed condition, following an overnight fasting of at least 10 hours, subject consumed a high-fast breakfast 30 minutes prior to administration of 450 mg MNTX. Under both fed and fasted treatment, subjects were required to fast at least 4 hours following the administration of 450 mg MNTX. PK blood samples were collected for up to 96 hours post-dose to determine the plasma concentrations of methylnaltrexone bromide following both treatments.

An example high-fat breakfast was 2 eggs fried in butter, 2 strips of bacon, 2 slices of toast with butter, 4 ounces of hash brown potatoes and 8 ounces of whole milk. The nutritional content of the standard high-fat breakfast consisted of approximately 800 to 1000 calories with 15% of calories from protein, 25% carbohydrate, and 60% fat, which was consistent with the recommendation in the FDA Guidance for Industry, Food-Effect Bioavailability and Fed Bioequivalence Studies.

In this study, high fat, high-caloric breakfast reduced the AUC by 43% and Cmax by 60%, delayed tmax by 2 hours compared to MNTX administration under fasted conditions

Figure 17: MNTX Mean Plasma Concentration-Time Profiles Following Single Oral 450 mg (3 x 150 mg) Tablet Doses Under Fasted and Fed Conditions (Linear and Semi-log Plots)

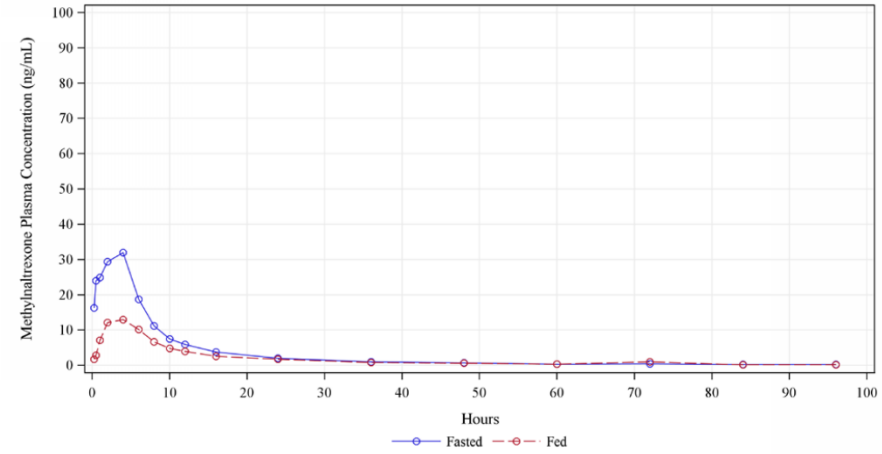


Table 65: Effect of food on Mean (± SD) Plasma PK Parameters of a 450 mg Dose of MNTX:

Parameters	Single-Dose Fasted N = 32	Single-Dose Fed N = 32
C_{max} (ng/mL)	42.53 (32.36)	15.08 (5.76)
T_{max} (h) [a]	2.0 (0.25, 4.0)	3.99 (1.00, 6.04)
AUC_{last} (ng.h/mL)	302.69 (147.71)	161.06 (32.35)
$AUC_{0-\infty}$ (ng.h/mL)	305.91 (147.93)	164.06 (34.56)
CL/F (L/h)	1744.35 (657.17)	2867.44 (628.24)
λ_z (h^{-1})	0.049 (0.011)	0.049 (0.014)
$t_{½}$ (h) [b]	14.17 (3.25)	14.15 (4.04)

Table 66: Geometric Least Squares Mean and Geometric mean Ratios (Fed/Fasted) and 90% CIs for MNTX Cmax and AUC After Single Doses of MNTX 450 mg

Parameters	Geometric Least Squares Mean	Geometric Mean Ratios of Treatment	90% CI for Geometric Mean Ratios	
			Lower[a]	Upper[a]
C_{max} (ng/mL)				
Fasted	35.05	40.32	32.68	49.74
Fed	14.13			
AUC_{last} (ng.h/mL)				
Fasted	275.59	57.27	50.07	65.50
Fed	157.82			
$AUC_{0-\infty}$ (ng.h/mL)				
Fasted	278.92	57.54	50.34	65.78
Fed	160.50			

Table 67: Statistical Comparisons (Geometric mean Ratios of Fed/Fasted and 90% CIs) of PK Parameter Estimates for MNTX Following Single Oral Dose Administration of 3X150 mg MNTX Tablet in the Fed State and the Fasted State in Healthy Subjects: (Reviewer's Analysis)

Parameter	GMR of Fed/Fasted	Lower 90% CI	Upper 90% CIs
Cmax	40.32	32.3	50.30
AUC(0-inf)	57.544	50.34	65.78
AUC (0-last)	57.26	50.07	65.49

MNPK1114: The sponsor had also conducted another food effect study with 450 mg (3X150 mg) MNTX film-coated tablet formulation with (b) (4) In this study, high fat meal had reduced the AUC by 50 % and C_{max} by 64%, delayed t_{max} by 2 hours, which is consistent with the effect of food on TBM formulation in study MNEF1001. Since this study used 450 mg (3X150 mg) MNTX film-coated tablet formulation with (b) (4) that is neither a phase 3 formulation nor a TBM formulation, this study was not reviewed in detail and were only evaluated for the consistency in effect of food on MNTX oral formulation.

2.9 Analytical Section

2.9.1 How are the active moieties identified and measured in the plasma/urine in the clinical pharmacology and biopharmaceutics studies?

Concentration of N-methylnaltrexone in human plasma and urine and its metabolites in plasma samples were identified and measured with validated HPLC-MS/MS methods.

2.9.2 Which metabolites have been selected for analysis and why?

In two studies, hepatic impairment study (MNPK1004) and multiple-dose PK study (MNPK 1118), plasma concentration of N-methylnaltrexone primary metabolites methylnaltrexone sulfate [M2], methyl-6α-naltrexol [M4], and methyl-6β-naltrexol [M5] were measured.

These metabolites were measured because, based on prior knowledge, following 12 mg once daily dosing the mean AUC0-24 ratio of metabolites to methylnaltrexone at steady-state was 30%, 19%, and 9% for methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol, respectively. Methyl-6α-naltrexol, and methyl-6β-naltrexol are active mu-opioid receptor antagonists and methylnaltrexone sulfate is a weak mu-opioid receptor antagonist.
Following once daily dosing of 450 mg oral tablets in this submission, the mean AUC0-tau ratio of metabolites to methylnaltrexone at steady-state was 79%, 39%, and 21% for methylnaltrexone sulfate, methyl-6α-naltrexol, and methyl-6β-naltrexol, respectively.

2.9.3 For all moieties measured, is free, bound, or total measured?

Total drug concentration was measured.

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

The concentrations of parent drug N-methylnaltrexone in human plasma were determined using validated liquid chromatography mass spectrometry (LC/MS/MS) methods validated at (b) (4) (b) (4) (validation report (b) (4) 107-0301, (b) (4) 107-0511, (b) (4) 107-0511 addendum 1, (b) (4) 107-0511 amendment 1, (b) (4) 388-1402, (b) (4) 107-0301 amendment 1)

The concentrations of Metabolites: Methyl-6α-Naltrexol, Methyl-6β-Naltrexol, and Sulfate-Methyl-Naltrexone in human plasma were determined using validated liquid chromatography mass spectrometry (LC/MS/MS) methods validated at (b) (4) (validation report (b) (4) 18-0707, (b) (4) 18-0707 addendum 1, (b) (4) 18-0707 addendum 2, (b) (4) 388-1502, (b) (4) 388-1502 amendment 1).

The concentrations of parent drug N-methylnaltrexone in human urine were determined using validated liquid chromatography mass spectrometry (LC/MS/MS) methods validated at (b) (4) (b) (4) (validation report (b) (4) 107-0308 and (b) (4) 107-0308 addendum 1).

2.9.5 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	Range of Standard Curve	LLOQ	ULOQ
N-methylnaltrexone	Plasma	0.05 to 250 ng/mL (10 levels)	0.05 ng/mL	250 ng/mL
N-methylnaltrexone	Urine	0.05 to 250 ng/mL (10 levels)	0.05 ng/mL	250 ng/mL
Methyl-6α-Naltrexol	Plasma	0.05 to10.0 ng/mL (8 levels)	0.05 ng/mL	10.0 ng/mL
Methyl-6β-Naltrexol	Plasma	0.05 to 10.0 ng/mL (8 levels)	0.05 ng/mL	10.0 ng/mL
Sulfate-Methyl-Naltrexone	Plasma	0.5 to100 ng/mL (8 levels)	0.5 ng/mL	100 ng/mL

For all measurements, concentrations were estimated with linear (1/concentration squared weighted) regression algorithm for all methods.
For plasma methylnaltrexone samples with dilution factor of 100, 500 and 1500 were evaluated and were within acceptable range
For urine methylnaltrexone samples with dilution factor of 100, 500 and 1500 were evaluated and were within acceptable range.
For all metabolites in plasma, 10 fold dilution were evaluated and were within acceptable range.

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

Analyte	Matrix		Intra-assay	Inter-Assay
N-methylnaltrexone	Plasma	Precision (CV%)	1.3% to 11.5%	2.1% to 12.2%
		Accuracy	-7.3% to 0.0%	-7.5% to -1.3%
N-methylnaltrexone	Urine	Precision (CV%)	1.2% to 13.6%	3.8% to 11.2%
		Accuracy	-6.6% to 7.2%	-4.7% to 8.7%
Methyl-6α-Naltrexol	Plasma	Precision (CV%)	1.7% to 6.1%	4.2% to 8.1%
		Accuracy	-2.6% to 8.4%	-3.7% to 7.9%
Methyl-6β-Naltrexol	Plasma	Precision (CV%)	2.9% to 6.4%	3.9% to 10.0%
		Accuracy	-4.5% to 5.4%	-5.7% to 4.1%
Sulfate-Methyl-Naltrexone	Plasma	Precision (CV%)	3.9% to 6.5%	5.5% to 8.1%
		Accuracy	-6.4% to 4.8%	-4.1% to 4.1%

All methods had adequate selectivity:

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- N-methylnaltrexone in plasma: Of the ten individual lots of blank human plasma, the mean interference peak area at the retention time of the peak for N-methylnaltrexone was < 20% LLOQ for analyte; < 5% for IS
- N-methylnaltrexone in Urine: Of the ten individual lots of blank human urine, the mean interference peak area at the retention time of the peak for N-methylnaltrexone was < 20% LLOQ for analyte; < 5% for IS.
- Metabolites in urine: Of the six individual lots of blank human plasma, the mean interference peak areas at the retention times of the peaks for methyl-6α-naltrexol, methyl-6β-naltrexol, and sulfate-methyl-naltrexone was < 20% LLOQ for analytes; < 5% for IS.

2.9.7 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.

Matrix	Analyte	Freeze-thaw (Cycles)		Room temperature (benchtop)	At 4°C (autosampler)	At -20°C	At -70°C
		-20°C	at-70°C				
Plasma	N-Methylnaltrexone	6	5	24 hr	641 hr	365 Days	259 Days
Urine	N-methylnaltrexone		5	24 hr	121 hr	366 Days	613 Days
Plasma	Methyl-6α-Naltrexol	5	6	7 hr	122 hr	222 Days	768 days
Plasma	Methyl-6β-Naltrexol	5	6	7 hr	122 hr	222 Days	768 days
Plasma	Sulfate-Methyl-	5	6	7 hr	122 hr	222 Days	754 days

In addition, stability of N-methylnaltrexone and its all metabolites in whole blood at ambient temperature and in ice bath (benchtop stability) were established for 2 hours.

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

QC samples for methylnaltrexone in human urine were at 0.15, 5, 90, 150, and 225 ng/mL.
QC samples for Methyl-6α-Naltrexol in human plasma were at 0.05, 0.15, 1.50, and 9.00 ng/mL.
QC samples for Methyl- 6β -Naltrexol in human plasma were at 0.05, 0.15, 1.50, and 9.00 ng/mL
QC samples for sulfate-methyl-naltrexone in human plasma were at 0.5, 1.5, 15.0, and 90.0 ng/mL.

At least 8% of the study samples were re-assayed as incurred sample repeats to demonstrate the reproducibility of quantification. The incurred sample repeats met the acceptance criteria of relative percent difference from the original and re-assay values from two-thirds of repeated samples being <20%.

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3 Appendices

3.1 Pharmacometric review

OFFICE OF CLINICAL PHARMACOLOGY: PHARMACOMETRIC REVIEW

1. Summary of Findings

1.1 Key Review Questions

The purpose of this review is to address the following key questions.

1.1.1 Do the dose-response relationships for safety and efficacy support the proposed dose of relistor?

Yes, the proposed dose appears to be acceptable as higher doses are not expected to give greater exposures via oral administration (Table 1) while lower doses would result in lower efficacy compared to 450 mg which is already lower than already approved the efficacy observed with 12 mg SC injection of methylnaltrexone. Additionally the difference in response rate between the 150 mg and 300 mg doses (6.4% for the key secondary endpoint and 3.7% for the applicant's primary endpoint) is greater than between the 300 mg and 450 mg doses (2.8% for the key secondary endpoint and 2.6% for the applicant's primary endpoint) suggesting that increasing the dose above 450 mg will likely yield even less additional benefit. Secondly, lower doses (i.e. 150, 300 mg) appear to be less ideal in that their efficacy is reduced compared to the 450 mg dose (*(Source: Applicant's Summary of Clinical Pharmacology, Studies [1]MNOC1111, [2]MNPK1117, [3]MNTX-2201)* Appears this way on original , ***Table 4***). While acknowledging the concerns related to cross study comparison of efficacy, the efficacy of oral 450 mg relistor is already reduced when compared to the approved SC formulation dose of 12 mg (13% difference from placebo for oral 450 mg compared to a 20% difference from placebo for 12 mg SC dose). While there are exposure-response relationships for the adverse events abdominal pain and diarrhea (***Table***), the AE rates for the high oral dose are lower than the approved SC dose. Details regarding PK exposures, efficacy by dose, and safety by dose are outlined below in more detail.

Methylnaltrexone Pharmacokinetic Exposures by Dose:

Table 1 indicates the AUC and Cmax values as the mean value for the respective dose for various studies. A range indicates the range of mean values for each study with that dose. Presenting the data in this way is intended to illustrate the PK variability where relevant.

Two notable points from an exposure perspective regarding the dose are:

1) There is approximately a 4-fold difference in Cmax between the proposed dose of 450 mg PO compared to the approved 12 mg SC dose (Table).

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2) Above 450 mg, a less than proportional increase in exposure suggests increasing dose may not yield higher exposures. Cmax and AUC do not appear to increase for 17 subjects with 600 mg dose (Table 1).

Table 1. Pharmacokinetic Exposures of Methylnaltrexone Across the Range of Studied Oral Doses in Comparison to the 12 mg SC Dose.

	150 mg PO	**300 mg PO**	**450 mg PO DC**	**600 mg PO**	**12 mg SC**
AUC (ng·hr/mL)	107	231	**105[1] - 373[2]**	105[3]	**186 - 269**
Cmax (ng/mL)	13.2	26.2	**35[1] – 48[3]**	44[3]	**133 - 174**

(Source: Applicant's Summary of Clinical Pharmacology, Studies [1]MNOC1111, [2]MNPK1117, [3]MNTX-2201)

The applicant's primary and key secondary endpoint findings for the phase 3 trial are shown in **Figure 1** and ***Table 4***. The primary efficacy endpoint has not been agreed upon by the agency and recommendations were made during development to change the primary endpoint (See the medical review by Dr. Dina Zand for further details). As such the agency is considering the key secondary endpoint to be more clinically relevant as it is consistent with prior approvals for naloxegol and is currently recommended as the primary endpoint for other products being developed in this class for opioid induced constipation. This is in part due to the more durable nature of the endpoint, considering response over the double blind duration of the trial.

The applicant's primary efficacy endpoint is defined as:

> "The primary efficacy endpoint was the average percentage of dosing days that resulted in RFBMs within 4 hours of dosing during Weeks 1 – 4. A RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement. The weekly number of RFBMs was calculated as 7 × total RFBMs in a week/all nonmissing assessment days."

The applicant's key secondary efficacy endpoint is defined as:

> "Responder endpoint: Proportion of subjects who responded to study drug during Weeks 1 to 4, where a responder is defined as ≥ 3 RFBMs/week, with an increase of ≥ 1 RFBM/week over baseline, for ≥ 3 out of the first 4 weeks of the treatment period."

Figure 1. Applicants Primary Efficacy Endpoint for the phase 3 trial MNTX-3201: Average Percentage of Dosing Days that Resulted in RFBMs within 4 hours of Dosing During Weeks 1-4 (ITT Population).

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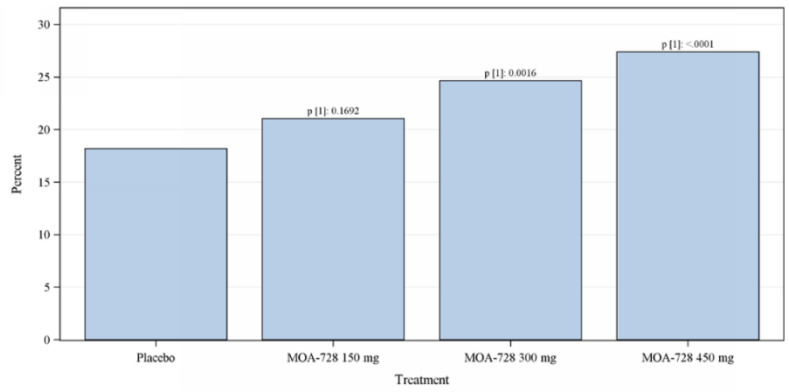

(Source: Applicant's Clinical Study Report, Trial MNTX-3201, Figure 5)

Table 2. Applicant's First Key Secondary Efficacy Endpoint (Endpoint of FDA Focus): Proportion of Subjects Responding to Study Drug during Weeks 1-4 (ITT Population, Study MTX-3201)

Responders[a]	Placebo	MNTX 150 mg	MNTX 300 mg	MNTX 450 mg
N	201	201	201	200
LOCF Approach[b]				
Yes, n (%)	82 (40.8)	91 (45.3)	104 (51.7)	109 (54.5)
Percentage Difference (vs Placebo)	—	4.5	10.9	13.7
95% CI for Percentage Difference (vs Placebo)	—	-5.2, 14.1	1.3, 20.6	4.0, 23.4
Odds Ratio (Active/Placebo)[c]	—	1.23	1.54	1.79
95% CI for Odds Ratio[c]	—	0.82, 1.84	1.03, 2.29	1.20, 2.66
Raw p-value (vs Placebo)[c]	—	0.3076	0.0339	0.0043
Worst Case Approach[d]				
Yes, n (%)	77 (38.3)	86 (42.8)	99 (49.3)	103 (51.5)
Percentage Difference (vs Placebo)	—	4.5	10.9	13.2
95% CI for Percentage Difference (vs Placebo)	—	-5.1, 14.1	1.3, 20.6	3.5 22.8
Odds Ratio (Active/Placebo)[c]	—	1.23	1.55	1.77
95% CI for Odds Ratio[c]	—	0.82, 1.84	1.04, 2.32	1.19, 2.65
Raw p-value (vs Placebo)[c]	—	0.3185	0.0321	0.0052

Source: Table 14.2.2.1a. Abbreviations: CI = confidence interval, ITT = intent-to-treat, LOCF = last observation carried forward; MNTX or MOA-728 = methylnaltrexone, RFBM = rescue-free bowel movement.
a A responder was defined as having ≥ 3 RFBMs/week, with ≥ 1 RFBM/week increase from baseline for ≥ 3 weeks of Weeks 1–4. The weekly number of RFBMs was calculated as 7 × total RFBMs in a week/all nonmissing assessment days in the week. The weekly number of RFBMs was set to missing for any week when the subjects completed < 4 diary days. RFBM was defined as a bowel movement without laxative use within 24 hours prior to bowel movement. If a subject did not receive concomitant daily opioid treatments during a week, the subject was considered no change from baseline for the week.
b LOCF approach: the last nonmissing 4 days with diary data was imputed for the week with < 4 diary days.
c Based on logistic regression model with treatment as effect and analysis region as a covariate.
d Worst case approach: missing weeks were classified as nonresponse for that week.

(Source: Applicant's Clinical Study Report, Trial MNTX-3201, Figure 5)

Furthermore, the safety profile appears reasonable for the 450 mg dose. From a safety perspective, there appears to be no dose-response for the majority of adverse event categories (Table). However, dose-response is evident for abdominal pain and diarrhea which is consistent with the mechanism of action and experience with other products in this class of drugs. Based on the cross trial comparison, the rate of the abdominal pain AEs does not exceed that of SC 12 mg relistor.

Table 3. Treatment-Emergent Adverse Events in ≥ 2% of Subjects Treated with MNTX or Placebo (Safety Population, Trial 3201).

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System Organ Class Preferred Term	Placebo n (%)	MNTX 150 mg n (%)	MNTX300 mg n (%)	MNTX 450 mg n (%)	All MNTX n (%)
Subjects Randomized (N)	**201**	**201**	**201**	**200**	**602**
Any system organ class	127 (63.2)	117 (58.2)	120 (59.7)	118 (59.0)	355 (59.0)
Gastrointestinal disorders					
Abdominal discomfort	4 (2.0)	2 (1.0)	0	1 (0.5)	3 (0.5)
Abdominal distension	6 (3.0)	6 (3.0)	3 (1.5)	7 (3.5)	16 (2.7)
Abdominal pain	17 (8.5)	11 (5.5)	16 (8.0)	21 (10.5)	48 (8.0)
Abdominal pain upper	7 (3.5)	4 (2.0)	6 (3.0)	6 (3.0)	16 (2.7)
Diarrhea	7 (3.5)	7 (3.5)	13 (6.5)	16 (8.0)	36 (6.0)
Flatulence	9 (4.5)	11 (5.5)	7 (3.5)	10 (5.0)	28 (4.7)
Nausea	18 (9.0)	13 (6.5)	16 (8.0)	12 (6.0)	41 (6.8)
Vomiting	9 (4.5)	3 (1.5)	6 (3.0)	7 (3.5)	16 (2.7)
Infections and infestations					
Influenza	5 (2.5)	4 (2.0)	6 (3.0)	2 (1.0)	12 (2.0)
Sinusitis	4 (2.0)	5 (2.5)	7 (3.5)	2 (1.0)	14 (2.3)
Upper respiratory tract infection	9 (4.5)	9 (4.5)	7 (3.5)	8 (4.0)	24 (4.0)
Urinary tract infection	7 (3.5)	7 (3.5)	8 (4.0)	7 (3.5)	22 (3.7)
Injury, poisoning, and procedural complications	9 (4.5)	12 (6.0)	9 (4.5)	11 (5.5)	32 (5.3)
Muscle strain	4 (2.0)	0	2 (1.0)	1 (0.5)	3 (0.5)
Musculoskeletal and connective tissue disorders					
Arthralgia	4 (2.0)	7 (3.5)	5 (2.5)	4 (2.0)	16 (2.7)
Back pain	7 (3.5)	12 (6.0)	6 (3.0)	5 (2.5)	23 (3.8)
Nervous system disorders					
Headache	8 (4.0)	2 (1.0)	8 (4.0)	9 (4.5)	19 (3.2)
Tremor	1 (0.5)	7 (3.5)	4 (2.0)	3 (1.5)	14 (2.3)
Psychiatric disorders	9 (4.5)	11 (5.5)	19 (9.5)	10 (5.0)	40 (6.6)
Anxiety	3 (1.5)	6 (3.0)	9 (4.5)	7 (3.5)	22 (3.7)
Respiratory, thoracic, and mediastinal disorders					
Rhinorrhea	3 (1.5)	5 (2.5)	4 (2.0)	4 (2.0)	13 (2.2)
Skin and subcutaneous tissue disorders					
Hyperhidrosis	4 (2.0)	6 (3.0)	8 (4.0)	6 (3.0)	20 (3.3)
Vascular disorders	6 (3.0)	5 (2.5)	5 (2.5)	5 (2.5)	15 (2.5)
Hot flush	4 (2.0)	2 (1.0)	2 (1.0)	2 (1.0)	6 (1.0)

(Source: Applicant's Clinical Study Report, Trial 3201, Table 24)

Additionally the exposure-response for the applicant's primary endpoint indicates the efficacy with the oral dose appears lower than with the IV or SC formulations (***Figure***).
Several aspects with regards to exposure-response and dose response prevent conclusions being drawn from these analysis which make the interpretation of these analysis difficult:

- Exposure Response was developed on Phase 1 and 2 Studies for only the Primary Endpoint and this included non-relevant patient populations (i.e. methadone maintenance and post-surgery pain). See Table 4 for further details.
- PK data was not collected in Phase 3 trial MNTX-3201 to use this data for exposure-response analyses.
- Exposure response for the key secondary endpoint (FDA's clinically relevant endpoint): The secondary endpoint data was not collected in the Phase 2 trials where the PK information was available, so the applicant simulated PK for the phase 3 study, where the

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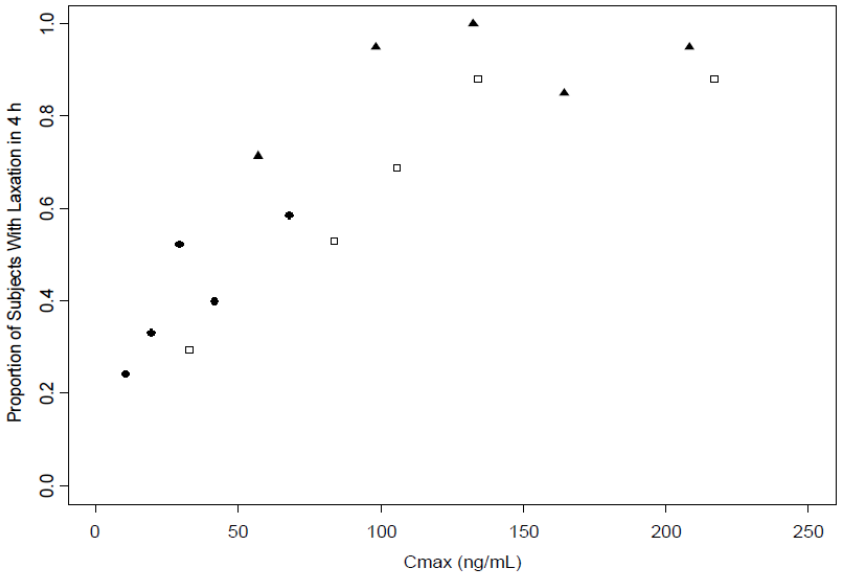
secondary endpoint was available, to conduct this analysis. This approach would be acceptable when there is a good covariate model to explain inter-individual variability and the unexplained variability is small (i.e. the covariates explain majority of the difference in PK between subjects). However, the applicant did not provide sufficient description of their PK model in their response to justify these simulations.

- The exposure response plot for primary endpoint (***Figure***) substantially over predicts the observed response in each dose group for the phase 3 study when considering the expected Cmax exposure for each dose group in ***Table*** .

Figure 2. Observed Proportion of Subjects Who Experienced Laxation Within 4 Hours After Administration of MNTX Versus the Midpoint of Each Cmax Quintile By Route of Administration.



Circles – oral administration
Triangles – subcutaneous administration
Open squares – intravenous administration

(Source: Applicant's Summary of Clinical Pharmacology, Figure 6)

1.1.2 Do Patients with abdominal pain have a higher response rate and can potentially benefit from a lower dose of relistor?

No, patients with abdominal pain AEs do not exhibit a higher response rate. Using the four week secondary responder endpoint (proportion of subjects who responded to study drug during weeks 1 to 4 where a responder is defined as ≥3 RFBMs/week, with an increase of ≥1 RFBM/week over baseline, for ≥3 out of the first 4 weeks of the treatment period), the response rate for patients with abdominal pain was 30.1% compared with 28.6% for subjects without abdominal pain. This analysis was performed based on the hypothesis that abdominal pain was associated with either too much antagonism of the gut opioid receptor or that abdominal pain was reflective of GI motility.

1.2 Recommendations

The Division of Pharmacometric in the Office of Clinical Pharmacology has reviewed this application and found the proposed dose of 450 mg to be acceptable.

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2. Pertinent regulatory background

Methylnaltrexone is an FDA approved opioid antagonist that is currently approved for the treatment of opioid-induced constipation via SC injection. The approved dose for the SC formulation is 12 mg. The applicant has developed an oral formulation and tested 150, 300, and 450 mg doses orally compared to placebo. In the current submission, the applicant is providing the results of the PK studies and efficacy and safety trial (MNTX-3201) to seek approval for the treatment of opioid-induced constipation associated with non-cancer pain.

The sponsor did not provide a PK analysis. All PK parameters were presented from individual study assessments. Primary efficacy results in the phase 3 trial 3201 indicated that there was a clear dose-response with a response rate increasing from 18% in the placebo arm to 27% for the 450 mg relistor arm. **Figure 1** and ***Table 4*** shows the results of the primary and secondary endpoints for the phase 3 trial MNTX-3201.

3. Results of Sponsor's Analysis

3.1 Exposure-Response on the Primary Endpoint

Model development was conducted with plasma PK parameters and PD endpoints combined across 6 studies. Study summaries for each of these 6 trials are provided in Table 4.

Table 4. Summary of Clinical Trials Included in the Analysis

Study	Administration Route	Dose	Patient Population	# of PK Observations Included in Analysis	# of Subjects Included in Analysis
MNTX2101	SC	Multiple dose; placebo or 12 mg QD (PK on Day 3 or 4)	OIC following orthopedic surgery	27	27
3200A3-105-US	SC and PO	Single dose; 0.15 mg/kg and 300 mg PO	Methadone maintenance	66	23
3200L2-1108-US	IV infusion	Single dose; 0.075 mg/kg, 0.128 mg/kg	Methadone maintenance	84	42
3200A3-1115-US	PO	Single dose; placebo and 150 mg	Methadone maintenance	86	26
3200A3-2201-US	PO	Multiple dose; placebo or 150 mg, 300 mg, 450 mg, 600 mg QD (PK on Day 1)	NCP	72	72
MNOC1111	SC and PO	Single dose; 12 mg SC open label followed by placebo PO or 450 mg PO	NCP	177	116
Total	-	-	-	512	306

(Source: Applicant's Summary of Clinical Pharmacology, Table 29)

A total of 512 records from 306 subjects were included in the analysis. A summary of the demographics of subjects from all studies included in the analysis are displayed in Table 5.

Table 5. Summary of Demographics for Subjects Included in the PK/PD Analysis.

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Characteristic Category or statistic	Value
Age (years)	
Mean	46.8
Median	48
Range	18 – 85
Weight (kg)	
Mean	86.3
Median	84.1
Range	30.1 - 175
Sex	
Female	181
Male	125

(Source: Applicant's Summary of Clinical Pharmacology, Table 30)

The efficacy endpoints included in this analysis were the occurrence of a bowel movement (laxation) within 4 hours after administration and the time to laxation after administration of the first dose of study drug. Blood samples for analysis of plasma MNTX concentrations were collected over different intervals across studies. Therefore, MNTX C_{max} was used to develop the PK/PD models. The effect of weight, age, sex, T_{max}, and route of administration (oral or parenteral) on the probability of laxation within 4 hours of administration and the survival function was investigated with the model development data set. Analyses were conducted with the data from MNOC1111 alone to determine if MNTX AUC_{0-4} or C_{max} was a better predictor for the probability of laxation within 4 hours after administration and to investigate the effect of the C_{max} of MNTX metabolites on the probability of laxation within 4 hours after administration of the first dose.

Logistic Regression: A logistic regression model was used to describe the effect of MNTX Cmax on the probability of experiencing laxation within 4 hours after study drug administration. The data for laxation within 4 h after dose administration was characterized as a binomial response and modeled using the following equation:

$$P(y = 1) = p = \frac{\exp(\propto + \sum_{i=1}^{n} \beta_i * Cov_i)}{1 + exp(\propto + \sum_{i=1}^{n} \beta_i * Cov_i)}$$

Time to Event Model: A TTE analysis also was conducted, assuming that the 'hazard' was the occurrence of laxation. A Weibull function was used to describe the underlying hazard rate over the 24 hour observation period. The Weibull function was described by the parameters ν and λ [dweib(ν, λ)] where ν is a constant and λ is a function of MNTX C_{max}. The effect of MNTX C_{max} on λ was described as:

$$\lambda = e^{(\beta 0 + \sum_{i=1}^{n} \beta i * Cov_i)}$$

Where β_0 is and intercept parameter and β_i and Cov_i are the coefficient and value of the i_{th} covariate, respectively, tested in the model. The Weibull function was fit to the time to laxation data in the model development data set. The parameters determined from the best fit of the model to the model development data were used to predict the survival rate of subjects in the validation data set with the following functions.

The observed proportion of subjects who experienced laxation within 4 hours after administration of placebo and after administration of MNTX versus C_{max} quintile midpoint for all routes of administration is displayed in **Figure 3**. Each C_{max} quintile range included 77 or 78 observations. Results from a total of 124 subjects were included in the calculation of the proportion of subjects who experienced laxation within 4 hours after administration of placebo. The proportion of subjects who experienced laxation within 4 hours after administration increased consistently over

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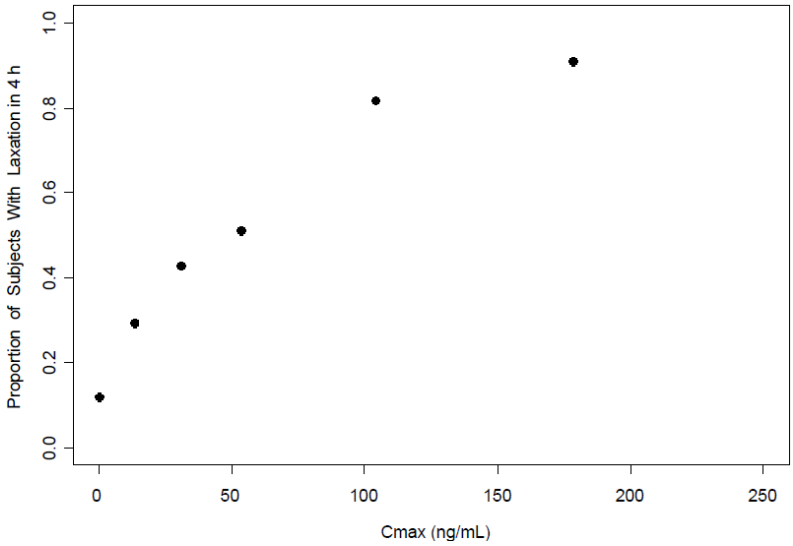
the range of observed C_{max} values and approached 1 at the greatest observed plasma MNTX C_{max} values.

Figure 3. Observed Proportion of Subjects who Experienced Laxation Within 4 Hours After Administration of MNTX or Placebo Versus The Midpoint of Each C_{max} Quintile.



(Source: Applicant's Summary of Clinical Pharmacology, Figure 5)

The observed proportion of subjects who experienced laxation within 4 hours after administration of MNTX versus C_{max} quintile midpoint for each route of administration is displayed in **Error! Reference source not found.**.

Table 6. Posterior Parameter Estimates From Logistic Regression Model Fit to the Occurrence of Laxation Within 4 Hours After Administration: Cmax and Tmax Only and Excluding Placebo Subjects**.**

Parameter	Covariate	Mean	SD	Median	95% CrI
α	-	-2.965	0.8894	-2.944	-4.776, -1.281
β_1	$\ln(C_{max}) + 1$	0.8312	0.1634	0.8272	0.52, 1.162
β_5	T_{max}	-0.4603	0.1224	-0.4587	-0.7066, -0.2294
DIC		282.4			

(Source: Applicant's Summary of Clinical Pharmacology, Table 35)

Table 7. Posterior Parameters Estimates for the Final TTE Model Fit to Results From Subjects that Received MNTX in the Model Development Data Set

Parameter	Covariate	Mean	SD	Median	95% CrI
v	-	0.6413	0.03622	0.6408	0.5716, 0.7137
β_0	-	-3.977	0.59	-3.981	-5.155, -2.803
β_1	$\ln(C_{max}) + 1$	0.5802	0.1007	0.58	0.3775, 0.7827
β_5	T_{max}	-0.1837	0.06437	-0.1819	-0.3152, -0.06333
DIC		1187			

(Source: Applicant's Summary of Clinical Pharmacology, Table 38)

Applicant's Conclusions:

- A concentration-dependent effect on laxation within 4 hours after dosing of MNTX was observed. MNTX Cmax and Tmax were significant predictors of the probability of laxation with 4 hours after the first dose.

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- The effect of MNTX Cmax on the probability of laxation within 4 hours after administration and the time to laxation are independent of route of administration.
- MNTX metabolites do not have a significant effect on the clinical endpoints investigated relative to systemic exposure to the parent compound.
- MNTX Cmax rather than AUC0-4 is a better predictor of the probability of laxation within 4 hours after administration of the first dose.
- The model demonstrated that a meaningful clinical effect can be observed at the plasma MNTX concentrations observed after an oral dose of 450 mg.

Reviewer's Comments:
The applicant's exposure response relationship (developed using the phase 1 and phase 2 studies listed in Table 4) appears to overestimate the phase 3 responses for each dose group when considering the Cmax for each respective dose. Additionally, as the review team is not considering the primary endpoint the key endpoint supporting approval, the agency requested the applicant evaluate exposure response for the key secondary endpoint, the endpoint that the review division is focusing on for supporting an efficacy assessment.

3.2 Exposure-Response Analysis on the Key Secondary Endpoint

The FDA submitted an information request to the applicant on 12/8/2015. Below are the applicant's responses to this request. This request was made to ascertain the nature of the exposure-response relationships for the key secondary endpoint. The request was also to address whether Cmax or AUC is the relevant exposure metric for this secondary endpoint and to not that these analyses should be performed in a patient population relevant to in the indication (non-cancer related pain).

Agency Request:

1. Reconduct your exposure-response analysis (for laxation with 4 hrs) using data from only the relevant/indicated population (opioid-induced constipation [OIC] from non-cancer pain).

Applicant's Response:

Results:
A data set including only studies MNTX2101 and MOA7282201 was formatted. As in the original analysis, for non-placebo patients, Cmax was adjusted by the transformation:

$$adjCmax = \ln(Cmax) + 1$$

Placebo patients were assigned an adjCmax value and Tmax of 0 as in the original analysis.
Included in this data set were adjCmax, Tmax, route of administration, and whether laxation occurred within 4 hours after dosing for non-cancer pain patients only. Logistic regression was performed in R (version 3.1.3) using the GLM package. Results are given in Table 2.

Table 8. Logistic Regression Results from Non-Cancer Pain Population, Laxation within 4 hours.

Parameter	Estimate	Standard Error of the Estimate	P value
Intercept	-1.90280	0.57397	0.000916
Beta(adjCmax)	0.26797	0.11273	0.017445
Beta(Tmax)	-0.01268	0.12579	0.919713
Beta(route, 1 = oral)	0.11144	0.56057	0.842416

(Source: Applicant's Response to Clinical Pharmacology Information Request, Table 2)

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Discussion:
The logistic regression for studies MNTX2101 and MOA7282201 showed a statistically significant effect of Cmax on probability of laxation within 4 hours of dosing, with a higher Cmax predicting a higher probability of laxation (Table 8). The estimate of the coefficient for the Tmax effect was not different from 0.

Agency Request:
2. Conduct a similar exposure-response analysis for your key secondary endpoint compared to that done for your primary efficacy endpoint:

- ***"Responder endpoint: Proportion of subjects who responded to study drug during Weeks 1 to 4, where a responder is defined as ≥3 RFBM/week, with an increase of ≥1 RFBM/week over baseline, for ≥3 out of the first 4 weeks of the treatment period.***
- ***The analysis should test both Cmax and AUC as metrics of methylnaltrexone exposure. This analysis should also be performed in only the relevant/indicated population (OIC from non-cancer pain)."***

Applicant Response:

Results:
As pharmacokinetic data were not available in study MNTX3201, Cmax and AUC were simulated using the established pharmacokinetic model from studies MNTX2101 and MOA7282201 for non-cancer pain patients only. Cmax and AUC for patients from study MNTX3201 were estimated using the patients' weight and the non-cancer pain pharmacokinetic model. Logistic regression was again done in R using the GLM package. Route of administration was not included in this regression, as there was only one value (oral). As in the analysis for studies MNTX2101 and MOA7282201, the Cmax was adjusted using the relationship below:

$$adjCmax = \ln(Cmax) + 1$$

Where AdjCmax is the adjusted Cmax value and ln is the natural logarithm. AUC was similarly adjusted. Placebo patients were assigned an adjCmax, Tmax, and adjAUC value of 0.
Results of the logistic regression are given in **Table 9** (Cmax) and **Table 10** (AUC).

Table 9. Logistic Regression Results for Study MNTX3201, Cmax Model.

Parameter	Estimate	Standard Error of the Estimate	P value
Intercept	-0.47733	0.14326	0.000862
Beta(adjCmax)	0.04045	0.02410	0.093270
Beta(Tmax)	0.03686	0.08033	0.646342

(Source: Applicant's Response to Clinical Pharmacology Information Request, Table 3)

Table 10. Logistic Regression Results for Study MNTX3201, AUC Model.

Parameter	Estimate	Standard Error of the Estimate	P value
Intercept	-0.48180	0.14063	0.000612
Beta(adjCmax)	0.11010	0.06101	0.071128
Beta(Tmax)	0.01950	0.08416	0.816731

(Source: Applicant's Response to Clinical Pharmacology Information Request, Table 4)

Discussion:
The responder analysis was based on predicted pharmacokinetic quantities (Cmax and AUC) from the typical values of a pharmacokinetic model based on two other studies in the same population. As such, the robustness of this analysis is unclear. This analysis failed to reject the

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null hypothesis of no effect of Cmax (**Table 9**) and AUC (**Table 10**) on responder status, although the trend is consistent with the previous analysis in which the independent variable was measured, rather than predicted.

Agency Request:

3. For both of the above analyses use the established exposure-response relationship to simulate the dose-response for each study used in the analysis and for the phase three trial MNTX3201.

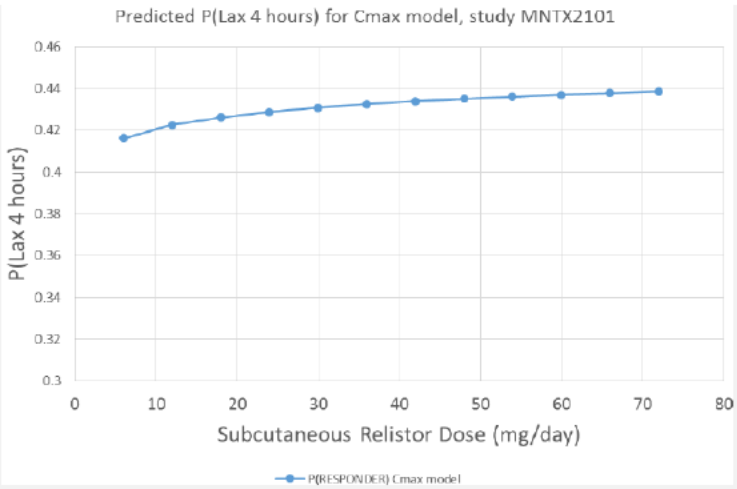
Applicant Response:

Results:
The dose-response relationships for all three studies (MNTX2101, MOA7282201, and MNTX3201) were estimated by using the expected values of parameters from the pharmacokinetic analysis of studies MNTX2101 and MOA7282201. This pharmacokinetic model was used to predict the typical value for Cmax and AUC for oral doses from 50 mg to 600 mg for study MNTX320, Cmax for oral doses from 50 mg to 600 mg for study MOA7282201, and Cmax for subcutaneous doses of 6 to 72 mg for study MOA7282201. The logistic regression models for the specific studies (MNTX3201 and combined MNTX2101 and MOA7282201) described above were then used to predict the probability of responder = 1 (MNTX3201) or laxation within 4 hours after the dose (MNTX2101 and MOA7282201). The dose-response relationships are shown in Figure 1, Figure 2, and Figure 3.

Discussion:
Figure 4, Figure 5, and Figure 6 show the predicted dose-response relationship for Relistor from studies MNTX2101, MOA7282201, and MNTX3201, respectively. These figures suggest that there is a consistent dose-response relationship across all studies; however, the relationship is relatively shallow. The between subject variability in the pharmacokinetic model was very high. This is not uncommon with low bioavailability drugs. As a result, the predicted values for Cmax and AUC for patients in study MNTX3201, calculated from the parameter values from studies MNTX2101 and MOA728220, likely had a poor correlation with the (unmeasured) actual Cmax and AUC. This would result in a similar poor correlation between the simulated values for Cmax and AUC and responder status for MNTX3201. As such, it is not unexpected to have limited power to demonstrate the pharmacokinetic/ pharmacodynamic relationship between both Cmax and AUC and the responder endpoint.

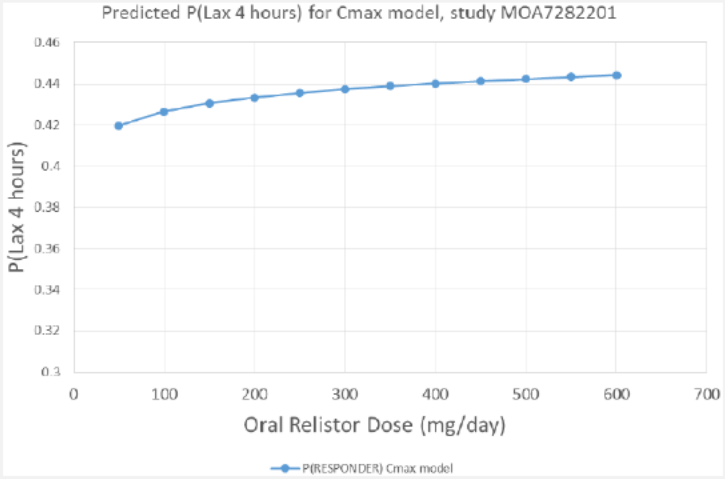
Figure 4. Dose-response relationship between subcutaneous Relistor dose and the probability of laxation within 4 hrs based on Cmax logistic model for study MNTX2101.

Reference ID: 3942818



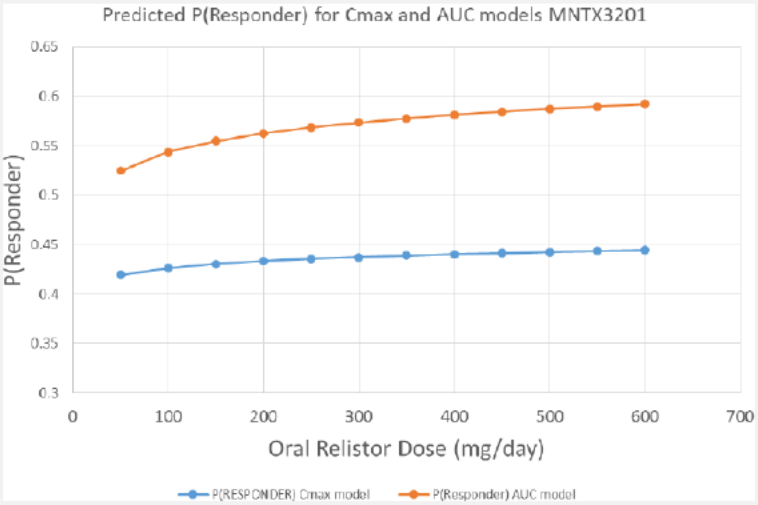
(Source: Applicant's Response to Clinical Pharmacology Information Request, Figure 1)

Figure 5. Dose-response relationship between oral Relistor dose and the probability of laxation within 4 hrs based on Cmax logistic model for study MOA7282201.



(Source: Applicant's Response to Clinical Pharmacology Information Request, Figure 2)

Figure 6. Dose-response relationship between oral Relistor dose and the responder endpoint based on Cmax (blue) and AUC (orange) logistic model for study MNTX3201



(Source: Applicant's Response to Clinical Pharmacology Information Request, Figure 2)

Reviewer's Comments:
The ability to evaluate the exposure-response for the key secondary endpoint is limited by the fact that PK data were not collected in that phase 3 trial, the only trial where the secondary endpoint was evaluated. Regardless, the applicant, simulated PK concentration for each individual in this trial and were still able to establish a logistic regression relationship with Cmax. The relationship was not established with AUC since AUC was similar between the SC and IV formulations despite the large difference in dose and while the AUC was similar between the different doses and routes of administration, there appeared to be better efficacy with the SC dose than the oral dose. The trend in efficacy was consistent with the trend in Cmax, higher efficacy with the higher Cmax from the SC dose. Thus Cmax was associated with the exposure response relationship. Despite a trend for increasing efficacy with increasing Cmax, no dose increase was recommended as this would push the AUC higher than previously studied and the exposure-response curve appeared to offer little to no additional benefit above the 450 mg oral dose.

4 Listing of Analyses Codes and Output Files

File Name	Description	Location in \\cdsnas\pharmacometrics\
PKPD_2ndEndpoint_NCPain.R	Reviewer's exposure response data assessment	\Reviews\PM Review Archive\2016\Relistor_NDA208271_JCE\ER Analyses

Reference ID: 3942818

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

DILARA JAPPAR
06/07/2016

JUSTIN C EARP
06/07/2016

NITIN MEHROTRA
06/07/2016

SUE CHIH H LEE
06/08/2016