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RESEARCH**

APPLICATION NUMBER:

211243Orig1s000

NON-CLINICAL REVIEW(S)

Secondary Pharmacology/Toxicology Review:

By: Ikram M. Elayan, PhD, Pharmacology/Toxicology Supervisor, Division of Psychiatry Products

NDA: 211243

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Drug: SPRAVATO® (esketamine)

Applicant: Janssen Pharmaceuticals, Inc.

Indication: Treatment-resistant depression in adults

Background: This is a 505(b)1 application for esketamine (SPRAVATO®) for the treatment of adults with treatment-resistant depression (TRD) to be administered intranasally. The proposed clinical treatment paradigm is twice weekly for four weeks as an induction phase followed by a maintenance phase of once weekly for four weeks and then once a week or every other week for continued maintenance. The maximum recommended human dose (MRHD) is 84 mg.

Esketamine is a non-competitive antagonist to the glutamate N-methyl-D-aspartate (NMDA) receptor. The sponsor conducted a comprehensive non-clinical program to address the safety of the product including: safety pharmacology studies, chronic general toxicity studies in rats and dogs, reproductive toxicity studies (including embryofetal studies in rats and rabbits that were conducted using racemic ketamine, (b) (4)), genotoxicity studies, acute/short-term neurotoxicity studies, and carcinogenicity studies (2-year study in rats and 6-month study in transgenic mice).

The non-clinical studies submitted by the sponsor were thoroughly and critically reviewed by Dr. Shiny Mathew as the primary non-clinical reviewer. Dr. Mathew found the submitted data to be adequate to support the approval of the application as indicated for adults with TRD, and I agree with her assessment. However, Dr. Mathew expressed concerns about the long-term neurotoxicity evaluation in the studies conducted by the sponsor. Her concern stems from the observation that the plasma levels at the highest dose in animal studies were at or lower than the human plasma levels and did not provide multiple-fold safety margins to the human exposures. In addition, a thorough evaluation of the brain was not conducted in the long-term studies, including lack of additional endpoints for detecting neuronal apoptosis or markers for other neurological diseases (e.g. Alzheimer's). Dr. Mathew recommended in her review that a post marketing requirement (PMR) for the assessment of higher exposures of esketamine in chronic animal studies would be needed to evaluate the long-term neurotoxic effect of esketamine on the brain (see her review in DARRTS dated 2/28/2019).

Supervisor's comments on the issues raised by the reviewer:

Even though I understand Dr. Mathew's concerns about the long-term neurotoxicity potential for esketamine based on findings in the literature for racemic ketamine, I believe that the data submitted by the sponsor for esketamine provide evidence about the safety of the doses and dosing paradigm proposed for its use under this application. Therefore, I don't agree that there is a need for a PMR to further investigate the long-term neurotoxic effects of esketamine for this application.

In addition, I acknowledge Dr. Mathew's reservations about the plasma levels obtained in animal studies conducted with this program and I agree that they were not optimal; however, the data can still be useful to establish safety for human use of esketamine as indicated in this application. The plasma levels in rats were 1.8 (C_{max}) and 0.6 (AUC) fold and in dogs 6.5 (C_{max}) and 1.3 (AUC) fold the levels obtained in humans.

These studies were conducted at the maximum feasible dose due to the insolubility of the drug in solution and the limitation of the volume that can be administered intranasally in animals. It should be pointed out that dosing was conducted on a daily basis in these animal studies while humans will be dosed intermittently (twice a week for four weeks, once a week for another four weeks, and once every other week for maintenance). As such, the plasma levels seen in animals at steady state based on chronic daily exposures might be an exaggerated effect of what is predicted in human dosing where the drug does not accumulate due to the short half-life (7-12h) and the intermittent dosing. It is worth noting that the drug is to be administered under the supervision of a medical doctor, therefore patients' misuse of the administration of the drug as described in the label is not expected.

In addition, plasma levels in animals might not reflect brain levels. It was evident from a study conducted by the sponsor (Study #FK12091) with orally administered esketamine that the brain levels were almost twice those in the plasma in rats. While the sponsor did not conduct a similar study with the intranasal (IN) route, IN administration is considered a route that could result in higher levels of administered drugs in the brain compared to the plasma due to the direct nose-to-brain route via olfactory and respiratory epithelium that might involve paracellular, transcellular, and neuronal transport (Erdo et al., 2018). Therefore, the brain of animals treated with IN route might have been exposed to even higher drug levels than those reported in plasma. The fact that no neurotoxicity was detected in rat brains using the more expansive method of brain dissection (7 sections as per Bolon et al. 2013) used in the 6-month general toxicity study might be reassuring even though only H&E staining was used. Bolon et al. (2013) indicated that CNS and PNS tissues from general toxicity studies maybe surveyed effectively using the standard H&E-stained sections. At the time when the 6-month rat study was conducted, the sponsor used the more expansive sectioning of the brain proposed by Bolon et al. (2013), even though this has been only recently recommended (Draft FDA Guidance for Industry: Developing Drugs for Treatment of Major Depressive Disorder, 2018), therefore, the sponsor did due diligence to investigate the effect on the brain. While additional staining and further investigations might be informative, these are typically needed as a second-tier approach where there is a signal from general toxicity studies that there is an observed or possible neurotoxic effect. At the doses used in this study, there does not seem to be any neuropathological findings with esketamine to warrant this second-tier approach. As I discussed earlier, even though the plasma levels in the chronic toxicity studies were not of multiple folds compared to the human plasma, the animal dosing paradigm provides assurance that it is an exaggerated effect that humans are not expected to experience based on the intermittent dosing in humans.

Dr. Mathew referred to published data in which ketamine has been reported to be associated with apoptosis in the brain of adolescent mice and monkeys (Sun et al., 2012, Yeung et al., 2010, and Li et al., 2017). However, it could be argued that such findings with ketamine might not be related to this product for two reasons:

- 1) the indication for this product currently is for adults and not for adolescents or children and an adult brain might be different from an adolescent brain that is still undergoing development, and
- 2) esketamine was negative when tested in adult brain for its neurotoxic effect by a single IN dose administration (Olney lesion study) even at very high concentrations suggesting that esketamine is different from ketamine as far as the short-term toxicity profile.

It is possible that the long-term neurotoxicity with ketamine might be stemming from its acute neurotoxicity (Olney lesion) or it could be unrelated as a different mechanism of toxicity. The studies that are described in the literature with ketamine did not address the relation between the acute and the long-term neurotoxicity. The data that the sponsor provided for their drug from acute neurotoxicity in rats and long-term studies in rats and dogs did not indicate neurotoxicity signals. I acknowledge that in the long-term studies brain histopathology evaluation was based on H&E staining only; however, this is a procedure

that is the standard for evaluations conducted in long-term general toxicity studies for any product. As mentioned earlier and based on the paper by Bolon et al., H&E is still capable of detecting neurotoxic effects if such findings are present in brain sections that are used in the general toxicity studies.

Some studies in the literature in which adult mice or rats were treated with ketamine exhibited learning and memory and/or sensorimotor gating deficits resembling those in schizophrenia (Ding et al, 2016, Sabbagh et al., 2012). Similar effects are reported in ketamine abusers and are probably expected to be seen at higher levels of esketamine. Clinical trials conducted by the sponsor assessed cognitive functions and these functions will be further assessed in an ongoing 3-year study as a PMR that the clinical team is recommending. Therefore, addressing the neurobehavioral effects of higher doses of esketamine in animals might not be needed at this time.

Finally, treatment-resistant depression is a serious and debilitating condition, and the risk-benefit ratio for this condition might be different from other less serious and debilitating conditions. Therefore, under the current circumstances and with the available data from the studies conducted with this program, it is reasonable to conclude that esketamine is adequately safe to use as indicated for this submission and for this population. If esketamine is to be used at higher doses or with more frequent administration, then future studies might be needed to provide assurance that these higher doses or more frequent dosing will not pose any safety concerns for humans. In addition, the use of esketamine in adolescents or children will require thorough evaluation at relevant doses to be used in this population to make sure that the findings reported in the literature with ketamine use are addressed and to provide ample evidence about the long-term safety of the brain in this population.

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**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

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Applicant: Janssen Research & Development
Review Division: Division of Psychiatry Products
Reviewer: Shiny V. Mathew, PhD, DABT
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Template Version: September 1, 2010

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TABLE OF CONTENTS

1	EXECUTIVE SUMMARY	7
1.1	INTRODUCTION	7
1.2	BRIEF DISCUSSION OF NONCLINICAL FINDINGS	7
1.3	RECOMMENDATIONS	10
2	DRUG INFORMATION	14
2.1	DRUG	14
2.2	RELEVANT INDs, NDAs, BLAs AND DMFs	15
2.3	DRUG FORMULATION.....	15
2.4	COMMENTS ON NOVEL EXCIPIENTS.....	15
2.5	COMMENTS ON IMPURITIES/DEGRADANTS OF CONCERN	15
2.6	PROPOSED CLINICAL POPULATION AND DOSING REGIMEN	17
2.7	REGULATORY BACKGROUND.....	17
3	STUDIES SUBMITTED	17
3.1	STUDIES REVIEWED	17
3.2	STUDIES NOT REVIEWED.....	17
3.3	PREVIOUS REVIEWS REFERENCED	18
4	PHARMACOLOGY	18
4.1	PRIMARY PHARMACOLOGY.....	18
4.2	SECONDARY PHARMACOLOGY.....	19
4.3	SAFETY PHARMACOLOGY	20
5	PHARMACOKINETICS/ADME/TOXICOKINETICS	21
5.1	PK/ADME.....	21
5.2	TOXICOKINETICS.....	24
6	GENERAL TOXICOLOGY.....	28
6.1	SINGLE-DOSE TOXICITY	28
6.2	REPEAT-DOSE TOXICITY.....	29
6.2.1	RAT REPEAT-DOSE TOXICITY USING EskETAMINE.....	29
6.2.2	DOG REPEAT-DOSE TOXICITY USING EskETAMINE.....	38
6.3	GENERAL TOXICOLOGY STUDIES USING PMI-100	42
7	GENETIC TOXICOLOGY.....	43
7.1	<i>IN VITRO</i> REVERSE MUTATION ASSAY IN BACTERIAL CELLS (AMES)	43
7.2	<i>IN VITRO</i> ASSAYS IN MAMMALIAN CELLS	44
7.3	<i>IN VIVO</i> CLASTOGENICITY ASSAY IN RODENT (MICRONUCLEUS ASSAY).....	45
7.4	OTHER GENETIC TOXICITY STUDIES	45
8	CARCINOGENICITY	46

8.1	RAT CARCINOGENICITY	46
8.2	TRANSGENIC MOUSE CARCINOGENICITY	46
9	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	47
9.1	FERTILITY AND EARLY EMBRYONIC DEVELOPMENT	47
9.2	EMBRYONIC FETAL DEVELOPMENT	50
9.2.1	RAT EMBRYONIC FETAL DEVELOPMENT	50
9.2.2	RABBIT EMBRYONIC FETAL DEVELOPMENT	53
9.3	PRENATAL AND POSTNATAL DEVELOPMENT.....	56
10	SPECIAL TOXICOLOGY STUDIES	59
11	APPENDIX/ATTACHMENTS	63

Table of Tables

Table 1: Potential impurities in drug substance and their calculated amounts.....	16
Table 2: <i>In vitro</i> binding data for esketamine, arketamine, and selected esketamine metabolites at the PCP binding site of NMDAR.....	18
Table 3: Esketamine, arketamine, and metabolites in an <i>in vitro</i> receptor binding screen	19
Table 4: PK of ketamine in mice, rats, and rabbits following single bolus IV administration.....	21
Table 5: PK of esketamine in rats administered aqueous vs. clinical formulation.....	21
Table 6: TK of esketamine in rats from the 6-month toxicity study (week 26).....	24
Table 7: TK of esketamine in dogs from the 9-month toxicity study (week 39).....	25
Table 8: TK of racemic ketamine from pregnant rats in the embryofetal toxicity study (GD17) following IN PMI-100 administration.....	25
Table 9: Bridging study conducted in pregnant rats on GD17 after IV dosing of 90 mg/kg of racemic ketamine.....	25
Table 10: TK of racemic ketamine from pregnant rabbits in the embryofetal toxicity study (GD 18) following IN PMI-100 administration.	25
Table 11: Bridging study conducted in pregnant rabbits on GD19 after IV dosing of 8 mg/kg of racemic ketamine.....	26
Table 12: TK of esketamine in rats in the 2-year carcinogenicity assay after 6 months of dosing	26
Table 13: TK of esketamine after subcutaneous administration in transgenic mice (week 25/26)	26
Table 14: TK of racemic ketamine after subcutaneous administration of PMI-100 in rats on D1 of neurotoxicity study	27
Table 15: TK of IN esketamine in female rats on D1 of neurotoxicity study	27
Table 16: TK of IN esketamine in female rats on D1 of neurotoxicity study	27
Table 17: Summary of histopathology findings in the nasal turbinates of rats from the 6-month toxicity study.....	34
Table 18 : Summary of histopathology findings in the respiratory epithelium of rats from the 6-month toxicity study.....	34
Table 19: Mean activity count in rats from the 6-month toxicity study.....	35
Table 20: Mean trial times on various test days (D1-4) in the Morris water maze test in rats from the 6-month toxicity study.	35
Table 21: Mean % of rats that failed trials (≥ 90 sec) in the Morris water maze from the 6-month toxicity study.....	36
Table 22: Reproductive toxicity endpoints examined in male rats from the 6-month toxicity study.....	36
Table 23: Reproductive toxicity endpoints examined in female rats from the 6-month toxicity study.....	37
Table 24: Summary of histopathology findings in dogs from the 9-month toxicity study.....	42
Table 25: Mating and fertility parameters in male rats in the fertility and early embryonic development study	49
Table 26: Mating and fertility parameters in female rats in the fertility and early embryonic development study	49

Table 27: Cesarean section findings from rat embryo-fetal development study with PMI-100..	52
Table 28: Offspring necropsy findings from rat embryo-fetal development study	52
Table 29: Caesarian section findings from rabbit embryo-fetal development study with PMI-100	55
Table 30: Offspring necropsy findings from rabbit embryofetal development study with PMI-100	55
Table 31: Effects of esketamine administration on parameters examined in the pre- and post-natal development study in rats.	59
Table 32: TK of noresketamine in rats from the 6-month toxicity study.	65
Table 33: TK of noresketamine in dogs from the 9-month toxicity study.	65
Table 34: TK of noresketamine in transgenic mice following 26-weeks of dosing.	65
Table 35: TK of noresketamine in rats in the 2-year carcinogenicity assay after 6 months of IN dosing.	65
Table 36: TK data from bridging studies after IV administration of racemic ketamine in non-pregnant rats.	67
Table 37: TK data from bridging studies after IV administration of racemic ketamine in dogs... ..	67
Table 38: TK parameters from F1 offsprings in the pre- and postnatal development study on PND 4 and PND12.	68

Table of Figures

Figure 1: Proposed *in vitro* metabolic pathways of esketamine in liver microsomes and S9 fractions of various species..... 63

Figure 2: Esketamine (above) and noresketamine (below) exposure in nonclinical species relative to humans. 64

Figure 3: Nasal cavity, brain and larynx dissections in the rat from the 6-month toxicity study. 66

1 Executive Summary

1.1 Introduction

This application is a 505(b)(1) NDA submitted by Janssen Research & Development (JRD) for SPRAVATO™ (esketamine). All toxicology studies reviewed under this NDA are currently owned by the applicant, regardless of who sponsored the original studies. The proposed indication is treatment-resistant depression (TRD). Esketamine is a proprietary formulation of the S-enantiomer of ketamine, which is approved only for acute use as an anesthetic agent. The maximum recommended human dose (MRHD) is 84 mg/day for an adult with a proposed dosing regimen of twice weekly during the induction phase (week 1-4), followed by a once weekly maintenance phase (weeks 5-6), and a once biweekly maintenance phase (week 9-lifetime). Esketamine has not been approved outside the U.S.

1.2 Brief Discussion of Nonclinical Findings

Esketamine, like racemic ketamine, is a noncompetitive glutamate N-methyl-D-Aspartate (NMDA) receptor antagonist. It has a higher potency at this receptor compared to the racemate, R-enantiomer, or any of its metabolites. Noresketamine (M10), the major metabolite, has a 6-fold lower affinity to the NMDA receptor compared to esketamine. *In vitro* studies demonstrated that the parent and/or noresketamine have a weak affinity (<50%) for the serotonin (5HT) transporter, opioid (mu and kappa), γ -amino butyric acid (GABA), and nicotinic acetylcholine receptors (nAChRs). Therefore, the Sponsor is suggesting that the antidepressant activity is unlikely to be mediated through these transporters/receptors.

The Intranasal (IN) route of administration was chosen for esketamine due to its low oral bioavailability in both humans and nonclinical species. It is quickly absorbed from the nasal cavity with a T_{max} of 5-30 minutes in all adult nonclinical species. Due to its high lipophilicity, esketamine distributes quickly to well-perfused tissues including the brain in mice and rats. After oral esketamine administration, the distribution of polar metabolites (M10, M4, and M9) into the brain was lower compared to the parent. Esketamine is rapidly and extensively metabolized in rat and dog liver microsomes and there are species differences in its metabolic profile. Based on human mass balance studies using radiolabeled esketamine administered orally and intravenously, the major human metabolite (i.e. >10% total circulating) is noresketamine, which was quantified in all nonclinical species. There are no unique human metabolites. In rats and humans, the major excretion pathway is through urine.

General toxicology studies with esketamine of up to 6 months in rats and 9 months in dogs were conducted to support chronic administration in humans. All pivotal toxicology studies, except the single dose acute neurotoxicity study in rats and carcinogenicity study in transgenic mice, were conducted at a maximum feasible dose (MFD) due to both the inability to achieve higher concentrations of esketamine in the test formulation and the inability to administer higher IN volumes in laboratory animals. Therefore, exposures in all toxicology studies, except

the two mentioned above, are generally at or lower than the clinical exposure. Furthermore, in chronic toxicity, reproductive toxicity, and carcinogenicity studies, where there is a large increase in body weight over the duration of dosing, the estimated dose (mg/kg/day) and exposures were substantially decreased, potentially affecting the endpoints evaluated.

The 6-month chronic toxicity study in rats was conducted using juvenile animals with dosing initiated at postnatal day (PND) 35 and with reproductive and neurobehavioral parameters evaluated as part of the study design. It should be noted that a MFD was used in this study and thus the development of tolerance to some of the clinical signs observed might be due to a decrease in dose that occurs with an increase in body weight. Minimal to slight hyperplasia of the olfactory epithelium and estrous cycle irregularities were observed in this study at an AUC exposure 0.3 times that was achieved at the MRHD of 84 mg. Reproductive function was assessed after a recovery period and showed no effect on time to mating or mating/fertility indices. In this study, learning delays were observed at all doses suggesting that there is no No Observed Adverse Effect Level (NOAEL) for this finding.

In addition to the routine parameters in a general toxicity study, the Sponsor conducted subjective gross neurological examinations in the 9-month dog study. Dose dependent increases in clinical signs such as salivation, increased activity, and incoordination were observed lasting up to 1 hour post dose and persisting throughout the study at the highest dose. Minimal to moderate olfactory epithelial atrophy and changes to the nasal cavity were observed in this study after 9 months of daily dosing, at AUC exposures that were similar to those at the MRHD of 84 mg/day. In shorter toxicity studies in dogs conducted using esketamine in aqueous vehicle, and not the clinical vehicle, clinical signs such as head shaking, vomiting, salivation, ataxia, and tremors were observed at exposures similar to or lower than the MRHD.

Based on an overall weight of evidence, esketamine was determined to be negative for genotoxicity. In a 2-year carcinogenicity study in rats (IN) and a 6-month transgenic study in mice (subcutaneous), there was no evidence of carcinogenicity observed at exposures (AUC) 0.6-times in rat and 6-times in transgenic mice compared with MRHD. Additionally, a low incidence of non-neoplastic lesions of the submucosa of the bladder was observed in males at clinical exposures after daily dosing for two years in rats. With IN administered PMI-100 [100mg/mL (10% w/v) aqueous racemic ketamine solution containing 0.002% benzalkonium chloride as an antimicrobial preservative], bladder toxicity findings had a safety margin of 1.5-times the estimated esketamine AUC exposure at MRHD after 3-months in rats while there was no safety margin for these findings in dogs after 1 month of administration.

In a fertility and early embryonic development (Segment I) study, estrous cycle irregularities were observed at an esketamine dose of 45 mg/kg/day and a delay in mating was observed ≥ 15 mg/kg/day. Due to the lack of overall changes to mating and fertility indices, the NOAEL in this study was considered 45 mg/kg/day which produce AUC exposure that was 0.6 times the AUC exposure at MRHD.

NMDA receptor antagonists are known to cause neuronal apoptosis in the young, developing brain in animals. These findings are well-established in literature for mice, rats, and non-human primates treated with acute anesthetic doses of racemic ketamine. Predicting that similar findings would occur with esketamine and that appropriate drug label warning will be issued for use during pregnancy and lactation, a dedicated neurotoxicity study after *in utero* drug administration was not requested from the Sponsor for esketamine. The Sponsor submitted embryofetal developmental (Segment II) studies conducted with PMI-100 in both rats and rabbits (originally sponsored by Javelin Pharmaceuticals (b) (4) reaching nominal doses through varied IN volumes for different treatment groups. In rats, there were no adverse fetal findings up to 12-fold the MRHD for the estimated esketamine AUC exposure in this study. In rabbits administered PMI-100, skeletal malformations were observed at maternally toxic doses. The estimated esketamine AUC exposure at NOAEL for these findings is 0.3 times the AUC exposure at MRHD.

In a pre- and postnatal development (Segment III) study in rats, a sensorimotor delay (i.e. Preyer response reflex) was observed at all doses of esketamine during the preweaning period without an observed effect on fetal body weight. During the postweaning period, motor activity was decreased but there was no effect on learning, habituation, sexual development, or mating and fertility in F1 offsprings. These findings were observed at exposures comparable to or lower than the MRHD.

Due to the known effect of NMDA receptor antagonists in causing neuronal vacuolation and necrosis in the sexually mature adult brain, commonly referred to as Olney lesions, several dedicated neurotoxicity studies were conducted in rats. Because of the time-sensitive nature of these findings, only those GLP studies where a 4-6 hour and 3-day sacrifice time points were utilized for the examination of neuronal vacuolation and necrosis, respectively, were considered adequate. In general, neuronal vacuolation is considered reversible while neuronal necrosis is irreversible. When a single dose of PMI-100 was administered subcutaneously to rats, neuronal vacuoles but not necrosis was observed at the highest dose. Estimating 50% of the exposure to be from esketamine, the NOAEL for neuronal vacuolation is 1.6-times and 4.5 times and the NOAEL for necrosis was 10-times and 16-times, respectively, for AUC and Cmax exposures at MRHD. In a single dose neurotoxicity study conducted with IN esketamine, exposures up to 17-fold and 23-fold the AUC and Cmax at the MRHD, respectively, did not produce neuronal necrosis.

In conclusion, even though JRD has (b) (4) generated substantial amount of nonclinical data with either IN racemic ketamine or IN esketamine, these studies have not been ideal since exposures in the nonclinical species were either less than or equal to human exposures. The AUC exposures at the highest dose in the chronic dog and rat studies were 1.3 times and 0.6 times, respectively, when compared with AUC exposure at MRHD. Through multiple dosing, it was possible to increase exposures even if the drug was IN instilled as evident by the high exposures in the pivotal single dose neurotoxicity study in rats. Therefore, the major outstanding concern for esketamine in nonclinical studies is an understanding of toxicities at higher exposures, particularly those related to the brain, after chronic-intermittent

dosing. Specifically, the Sponsor's current chronic toxicity studies only conducted routine histopathology and were not designed to address other neurotoxicity concerns such as neuronal apoptosis or protein changes that are reported after 6 months of daily administration of IV racemic ketamine in adolescent nonclinical species (Yeung et al., 2010, Sun et al., 2012 and Li et al., 2017). The estimated esketamine exposure where the positive findings occur in these studies is near the clinical exposure with IN esketamine. Having no safety margin for these potentially unmonitorable and irreversible findings is highly concerning. Considering the clinical need for this product for a life-threatening condition, the best way to characterize these toxicities is through adequate animal toxicity studies conducted at a maximum tolerated dose (MTD) in an appropriate adult species under a Post Marketing Requirement (PMR).

To reiterate, while carcinogenicity and embryofetal toxicity concerns can be adequately communicated through the drug label, we currently do not have sufficient safety information on the chronic-intermittent effect of esketamine on the brain to alleviate concerns from the published literature on racemic ketamine. Clinical studies to examine cognitive deficits after a lifetime exposure to the drug are not practical and may have multiple confounders. The current chronic nonclinical toxicity studies conducted by the Sponsor were not designed to understand if neuronal changes or cognitive deficits occur with chronic-intermittent administration. The 6-month rat toxicity study examined seven brain sections as per Bolon et al., 2013 but in the dog, sectioning did not include appropriate nuclei noted for nonrodents in the same paper. Histopathology in both species used only routine H&E staining without the use of specific markers to detect neuronal loss or changes in implicated proteins. Chronic toxicity study in rats included neurobehavioral battery which showed nonstatistically significant delays in learning but were underpowered and used juvenile rats. Chronic toxicity study in dogs included gross neurological examinations which were uninformative to understand if cognitive deficits occur. Therefore, this Reviewer believes that the Sponsor will need to commit to an adequate chronic toxicity study conducted at MTD, but following clinical frequency in an appropriate nonclinical species, chosen based on similar metabolite profile as in the human. The highest dose in this proposed study should reach several multiples of the clinical exposure and should be a dedicated neurotoxicity study to examine neuronal markers of apoptosis, necrosis, and possibly Alzheimer's disease. Additionally, this study conducted to satisfy PMR, should include a head-to-head comparison with racemic ketamine and examine functional endpoints such as locomotor activity, learning and memory, and auditory startle habituation.

1.3 Recommendations

1.3.1 Approvability

The nonclinical information submitted by the Sponsor is adequate to support approval of the product as indicated for adults with TRD, which is a life-threatening condition. However, the long-term effects on the brain will need to be explored with a designated nonclinical neurotoxicity study under a PMR.

1.3.2 Additional Nonclinical Recommendations

None

1.3.3 Labeling

Below are the recommendations for the drug label. The *Warning and Precaution* section and sections 5.10, 8.1, 8.2, 8.3 and 8.4 were written, in collaboration with the review team from the Division of Pediatric and Maternal Health, to express the neuronal apoptosis concerns in nonclinical species with racemic ketamine. Sections 12.1, 12.2, 13.1, and 13.2 were also edited/written by the Pharm/Tox team. Labeling is under negotiation with the Sponsor therefore, the following may not be the final version of the labeling.

Section 8: Use in specific populations

8.1 Pregnancy

Risk Summary

(b) (4) use in pregnant women to (b) (4) drug-associated risk of major birth defects, miscarriage, or adverse maternal or fetal outcomes. Based on findings from published animal reproduction studies with (b) (4) ketamine, SPRAVATO may cause fetal harm (*see Data*). (b) (4)

(b) (4) advise pregnant women of the potential risk to a (b) (4). There are risks to the mother associated with untreated depression in pregnancy (*see Clinical Considerations*).

Published studies in pregnant primates demonstrate that the administration of drugs that block N-methyl-D-aspartate (NMDA) receptors during the period of peak brain development increases neuronal apoptosis in the developing brain of the offspring. There are no data on pregnancy exposures in primates corresponding to periods prior to the third trimester in humans [*See Use in Specific Populations (8.2)*].

In embryofetal reproduction studies in rabbits, skeletal malformations were noted at maternally toxic doses when ketamine was IN administered with a No Observed Adverse Effect Level (NOAEL) at an estimated esketamine exposures 0.3 times the exposures at the maximum recommended human dose (MRHD) of 84 mg/day. In addition, IN administration of esketamine to pregnant rats during pregnancy and lactation at exposures that were similar to those at the MRHD resulted in a delay in sensorimotor development in pups during the preweaning period and a decrease in motor activity in the post-weaning period. The estimated background risk of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2 to 4% and 15 to 20%, respectively.

Clinical Considerations

Disease-associated maternal and/or embryo/fetal risk

A prospective, longitudinal study followed 201 pregnant women with a history of major depressive disorder who were euthymic and taking antidepressants at the beginning of pregnancy. The women who

discontinued antidepressants during pregnancy were more likely to experience a relapse of major depression than women who continued antidepressants. Consider the risk of untreated depression when discontinuing or changing treatment with antidepressant medication during pregnancy and postpartum.

Data

Animal Data

(b) (4)

When female monkeys were treated intravenously with ketamine at anesthetic dose levels in the third trimester of pregnancy, neuronal cell death was observed in the brains of their fetuses. (b) (4)

This period of brain development translates into the third trimester of human pregnancy. The clinical significance of these findings is not clear; however, studies in juvenile animals suggest neuroapoptosis correlates with long-term cognitive deficits.

Racemic ketamine was administered IN to pregnant rats during the period of organogenesis at doses of 15, 50, and 150 mg/kg/day and in pregnant rabbits at doses of 10, 30, and 100/50 mg/kg/day. The No Observed Adverse Effect level (NOAEL) for embryofetal toxicity in rats was the highest dose of 150 mg/kg/day. (b) (4)

(b) (4) the NOAEL associated with esketamine plasma exposure (AUC) is 12-times the AUC exposure at the MRHD of 84 mg/day. In rabbits, the high dose was lowered from 100 to 50 mg/kg (b) (4) due to excessive mortality in maternal does. Skeletal malformations were observed at doses ≥ 30 mg/kg/day, which were maternally toxic. The NOAEL for skeletal malformations was associated with a plasma esketamine exposure (AUC) that was 0.3 times the AUC exposure at MRHD of 84 mg/day.

Administration of esketamine HCl to pregnant rats during pregnancy and lactation at IN doses equivalent to 4.5, 15, and 45 mg/kg/day (based on a 200 gram rat) produced AUC exposures 0.07, 0.5, and 0.7 times the MRHD of 84 mg/day, respectively. Maternal toxicity was observed at doses ≥ 15 mg/kg/day. In addition, a dose response delay in the age of attainment of Preyer response reflex was observed in pups at all doses during the preweaning period (b) (4) This sensory/motor developmental measure was tested starting on postnatal day (PND) 9, and the effect normalized by PND 19 in treatment groups as compared with PND14 for the majority of the controls. There is no NOAEL for this delay in sensory/motor response observed in pups during the preweaning period. During the postweaning period, a decrease in motor activity was observed at doses ≥ 15 mg/kg which is 0.5-times the human exposure at the MRHD of 84 mg/day. The NOAEL for maternal toxicity and decreased motor activity during the postweaning period was 4.5 mg/kg/day which was associated with plasma exposure (AUC) that was 0.07-times the AUC exposure at MRHD of 84 mg/day.

8.2 Lactation

Risk Summary

Esketamine is present in human milk. There are no data on the effects of esketamine on the breastfed infant or on milk production. Published studies in juvenile animals report neurotoxicity (*see Data*). Because of the potential for neurotoxicity, advise patients that breast-feeding is not recommended during treatment with SPRAVATO™.

Data

Published juvenile animal studies demonstrate that the administration of drugs that block NMDA receptors, such as ketamine, during the period of rapid brain growth or synaptogenesis, results in widespread neuronal and oligodendrocyte cell loss in the developing brain and alterations in synaptic morphology and neurogenesis. Based on comparisons across species, the window of vulnerability to these changes is believed to correlate with exposures in the third trimester of gestation through the first several months of life, but may extend out to approximately 3 years of age in humans.

8.3 Females and Males of Reproductive Potential

Contraception

Based on published animal reproduction studies, SPRAVATO™ may cause embryofetal harm when administered to a pregnant woman [*see Warnings and Precautions (5.x) and Use in Specific Populations (8.1)*]. However, it is not clear how these animal findings relate to females of reproductive potential treated with the recommended clinical dose. Consider pregnancy planning and prevention for females of reproductive potential.

8.4 Pediatric Use

The safety and effectiveness of SPRAVATO™ in pediatric patients have not been evaluated.

Section 12: Clinical Pharmacology

12.1 Mechanism of Action

Esketamine, the S-enantiomer of racemic ketamine, is a nonselective, noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor. The mechanism by which esketamine exerts its antidepressant effect is unknown. The major circulating metabolite of esketamine (noresketamine) demonstrated activity at the same receptor with less affinity.

Section 13: Nonclinical Toxicology

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

Once-daily IN administration of esketamine at doses equivalent to 4.5, 15, and 45 mg/kg/day (based on a 200 gram rat) did not increase the incidence of tumors in a 2-year rat carcinogenicity study. At the highest dose, the AUC exposure to esketamine was lower than the human exposure (AUC) at the maximum recommended human dose (MRHD) of 84 mg. Once-daily subcutaneous administration of

esketamine up to 75/40 mg/kg/day did not increase the incidence of tumors in a 6-month study in transgenic (Tg.rasH2) mice.

Mutagenesis

Racemic ketamine was not mutagenic with or without metabolic activation in the Ames test, but was positive in an *in vitro* mouse lymphoma test in the presence of metabolic activation. Intraperitoneally-injected ketamine (b) (4) in an *in vivo* bone marrow micronucleus test in mice.

Genotoxicity with esketamine was seen in a screening *in vitro* micronucleus test in the presence of metabolic activation. However, esketamine was (b) (4) in an *in vivo* Comet assay in rat liver cells.

Impairment of Fertility

Esketamine was administered IN to both male and female rats before mating, throughout the mating period, and up to day 7 of gestation at doses equivalent to 4.5, 15, and 45 mg/kg/day (based on a 200 gram rat), which are approximately 0.05, 0.3, and 0.6-times the maximum recommended human dose (MRHD) of 84 mg/day based on mean AUC exposures, respectively. (b) (4) estrous cyclicity at the high dose of 45 mg/kg/day and increased time to mate at doses \geq 15 mg/kg/day were observed without an overall effect on mating or fertility indices. The No Observed Adverse Effect Level (NOAEL) for mating and fertility is 45 mg/kg/day which is 0.6-times the esketamine exposures at MRHD of 84 mg/day.

13.2 Animal Toxicology and/or Pharmacology

Neurotoxicity

In a single-dose neuronal toxicity study where esketamine was administered IN to adult female rats, there were no findings of neuronal vacuolation up to an estimated dose equivalent of 45 mg/kg for a 200 gram rat with an exposure difference of 1.8 and 4.5 times the clinical exposures for AUC and Cmax, respectively, to the MRHD of 84 mg/day. In a second single dose neurotoxicity study conducted with nasally administered esketamine to adult female rats, there were no findings of neuronal necrosis up to a dose equivalent of 270 mg/kg for a 200 gram rat which has an exposure difference of 18-fold and 23-fold, respectively, to AUC and Cmax exposures at the MRHD of 84 mg/day.

In a single-dose neuronal toxicity study in adult rats, subcutaneously administered racemic ketamine caused neuronal vacuolation in layer I of the retrosplenial cortex without neuronal necrosis at a dose of 60 mg/kg. The NOAEL for vacuolation in this study was 15 mg/kg. Estimating 50% of the exposure to be from esketamine, the NOAEL for neuronal necrosis is (b) (4) times and 16-times exposures and the NOAEL for neuronal vacuolation is 1 (b) (4) times and (b) (4) times, respectively, for AUC and Cmax to the clinical exposure at the MRHD of 84 mg/day. The relevance of these findings to humans is unknown.

2 Drug Information

2.1 Drug

CAS Registry Number: 33643-47-9

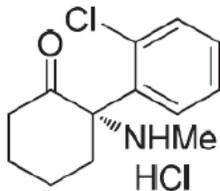
Generic Name: esketamine

Code Name: JNJ-54135419-AAC (HCl salt); JNJ-54135419-AAA (free base)

Chemical Name: (S)-2-(o-chlorophenyl)-2-(methylamino)cyclohexanone hydrochloride

Molecular Formula/Molecular Weight: C₁₃H₁₆ClNO.HCl/274.2

Structure or Biochemical Description



Pharmacologic Class: Noncompetitive NMDA receptor antagonist

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 114345 (IN esketamine): for Treatment Resistant Depression

(b) (4)

2.3 Drug Formulation

Esketamine is formulated as an aqueous nasal spray containing 161.4 mg/mL (b) (4) of esketamine HCl corresponding to a base equivalent concentration of 140 mg esketamine/mL (b) (4) containing (b) (4) ethylenediaminetetraacetic acid disodium salt (EDTA)/mL (b) (4) and (b) (4) citric acid/mL (b) (4) in water for injection, (b) (4) (b) (4) NaOH. This aqueous nasal spray is to be self-administered by the patient under the supervision of a health care professional via disposable, single-use, nasal spray device.

2.4 Comments on Novel Excipients

The drug product does not contain any novel excipients.

2.5 Comments on Impurities/Degradants of Concern

Drug substance, starting material, reagents, process intermediates, process impurities, and potential process impurities were adequately evaluated for mutagenicity potential by *in silico* evaluation using DEREK and Leadscope analyses. Of those tested, three impurities: (b) (4)

(b) (4) were

indicated to be mutagenic based on expert conclusion and found to be Ames positive. These (b) (4) mutagenic impurities are being controlled as per (b) (4). Based on process control and purging factor consideration, these impurities are not expected to occur in the final drug substance at levels above maximum allowable concentration of (b) (4)

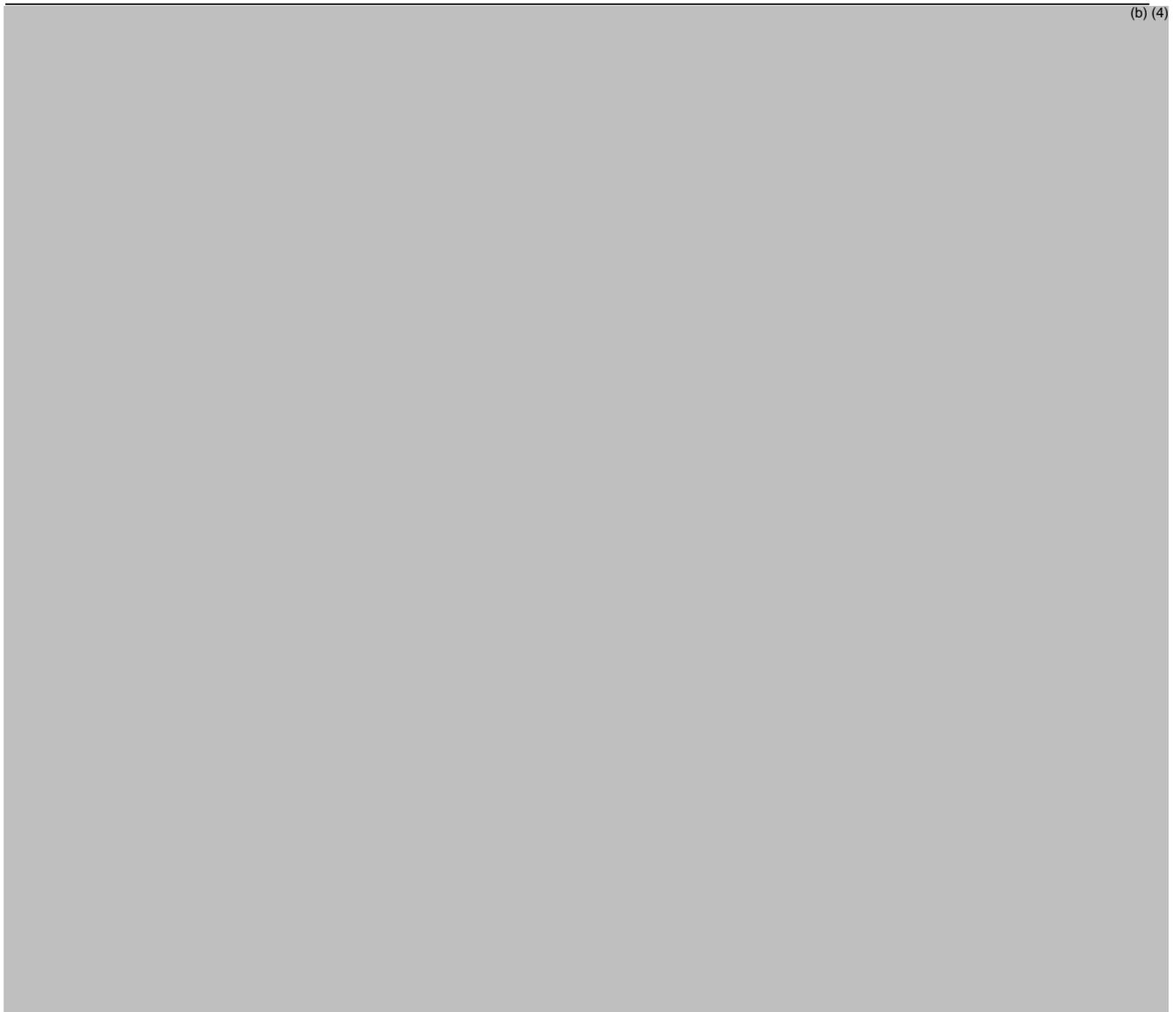
(b) (4) Therefore, the Sponsor has not specified any limits for these three mutagenic impurities and it is acceptable based on the CMC review.

The following table lists the specified organic impurities of esketamine HCl in the European Pharmacopeia. None of the impurities (A, B, C, or D) have structural alerts or concerns for mutagenicity based on FDA internal CMC assessment. Despite having no structural alerts, the Sponsor evaluated impurity (b) (4)

Based on that legislation, the Sponsor tested impurity (b) (4) in an acute oral toxicity test in rats, Ames assay, *in vitro* bovine corneal opacity and permeability (BCOP) test, *in vitro* skin corrosion and skin irritation tests, and an *in vivo* local lymph node assay in mice. All tests were negative except a positive signal in the BCOP test.

Table 1: Potential impurities in drug substance and their calculated amounts.

(b) (4)



substance, the threshold for qualification is (b) (4)% or (b) (4)mg per day intake, whichever is lower. The level of these impurities in the carcinogenicity studies, 6-month chronic toxicity studies, and neurotoxicity studies (batches A12BD0277 and A12LD4238) are (b) (4)% and calculated to be (b) (4) in a rat based on surface area calculation. The fourth impurity (impurity D in the table above), the R-enantiomer of the drug is present at higher levels (i.e. (b) (4)%) in the clinical batch but it is also qualified in the rat (see table above). Therefore, all of the nongenotoxic organic impurities are adequately covered in toxicology studies.

The CMC reviewer, Rohit Tiwari, PhD, alerted the Pharm/Tox Reviewer to the presence of an (b) (4) not listed under ICHQ3C: (b) (4) for which risk assessment was requested from the Sponsor. The Sponsor's risk assessment notes that (b) (4) induced chromosomal aberrations in some *in vitro* and *in vivo* animal studies but it is negative in a rat and mouse carcinogenicity studies conducted by ATSDR, EPA, NTP and others. Therefore, based on a weight of evidence, it is considered to be non-genotoxic. Based on the principles outlined in (b) (4) the Sponsor calculated the permitted daily exposure (PDE) for the (b) (4) mg/day or approximately (b) (4) mg/kg/day in a 60-kg subject. The reporting threshold for (b) (4) which translates to (b) (4) ug/day for a 84 mg/day, if given daily. This (b) (4) ug/day is calculated as (b) (4) ug/kg/day in a 60-kg weighing patient, which is (b) (4)-fold below the tentative PDE value of (b) (4) mg/kg/day. Therefore, the Sponsor's conclusion that the risk of adverse impact in patients is low is reasonable.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed indication is TRD. The proposed starting dose is 56 mg/day in adults with a proposed dosing regimen of twice weekly during the induction phase (week 1-4), followed by a once weekly maintenance phase (weeks 5-6), and a once biweekly maintenance phase (week 9-lifetime). Subsequent doses after Day 1 of dosing may be either 56 or 84 mg/day.

2.7 Regulatory Background

JRD is the original developer of IN esketamine. (b) (4)

3 Studies Submitted

3.1 Studies Reviewed

All submitted pivotal studies, in various species were reviewed in detail, except the neurotoxicity studies in juvenile animals.

3.2 Studies Not Reviewed

Preliminary dose range finding studies and non-pivotal studies for deciding nonclinical safety of esketamine and ketamine were not reviewed in detail. In addition, because the approval of TRD will be based on clinical trials in adults, juvenile animal toxicity studies were not reviewed.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

No primary pharmacology studies were conducted for esketamine by the Sponsor. However, based on published studies conducted with racemic ketamine, esketamine is assumed to be a noncompetitive antagonist channel blocker at the N-methyl-D-aspartate receptors (NMDARs). NMDARs are one of three ionotropic receptors in the CNS that bind to the endogenous excitatory neurotransmitter, glutamate. The other two receptor subtypes in this class are α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainic acid (Review by Newcomer, et al., 2000). The blockade of NMDARs is thought to be responsible for clinical effects such as perceptual and dissociative alteration, analgesia, amnesia, hypnosis, sedation, and anesthesia. However, whether the antidepressant action is facilitated by the same receptors is yet to be established. More recent literature suggests that the antidepressant activity of ketamine may be mediated by AMPA receptors (Zanos, et al., 2016). Interestingly, literature reports also suggest that arketamine may be more efficacious than esketamine based on animal models (Zhang, et al., 2014; Yang et al., 2015).

Metabolites of esketamine

The phase 1 human metabolites of esketamine are noresketamine (M10), (2S, 6S) hydroxynoresketamine (M4), and ketoreduced (2S, 6S) hydroxynoresketamine (M19). Thirteen *in vitro* binding studies were conducted to determine the pharmacological profile of esketamine, arketamine, and esketamine metabolites.

Multiple ligand binding sites exist on the NMDARs such as the competitive glutamate site, coagonist glycine site, allosteric binding sites and binding sites within the pore (e.g. Phencyclidine (PCP)) (Van Dongen, 2009). The results of the *in vitro* binding studies showed that esketamine exhibits antagonism at the PCP binding site within the pore of the NMDAR with an IC_{50} value 0.45 μ M. Arketamine and noresketamine also bind at the PCP binding site of the NMDAR but with less potency (see table below for details on binding affinities).

Table 2: *In vitro* binding data for esketamine, arketamine, and selected esketamine metabolites at the PCP binding site of NMDAR.

Compound	IC_{50}	K_i
Esketamine	0.45 μ M	0.26 μ M
Arketamine	1.8 μ M	1.0 μ M
Noresketamine (M10)	2.6 μ M	1.5 μ M
2S,6S-HNK (M4)	>10 μ M	>10 μ M

Studies (DD17104, DD18012, DD18013) were conducted to better understand the subunit selectivity of racemic ketamine, arketamine, esketamine, and esketamine metabolites using CHO cells expressing

human recombinant NMDARs: GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, and GluN1/GluN2D. The results revealed that ketamine, esketamine, and arketamine act as nonselective antagonists of the various subtypes of NMDARs, with arketamine having a lower potency than the former two compounds. Of the selected metabolites of esketamine tested, noresketamine antagonized activity at all NMDAR subtypes with potency approaching that of arketamine.

4.2 Secondary Pharmacology

To establish secondary pharmacology, esketamine and various esketamine metabolites [M10, M4, M19, 2S, 5S-5-hydroxynorketamine (M5), and 2S-5, 6-dehydronoresketamine (2S-5, 6-DHNK) (M9)] were screened *in vitro* for binding on a series of receptors, ion channels, and transporters (see Table 3 below). Besides the NMDAR binding site, esketamine displaced binding at the 5HT transporter (31%), kappa opioid (KOR) (38.3%), and μ -opioid (MOR) (49.9%) receptors. Ketamine, and arketamine also displaced binding at the latter two receptors. It is estimated that ketamine and esketamine, respectively, displace MOR binding with K_i of 41.7 and 11-28.8 μ M and KOR binding with a K_i of 28.2 and 23.4 μ M. Considering the 22-fold difference between K_i values of esketamine on MOR (11 μ M) versus NMDAR (0.5 μ M), the Sponsor rationalizes that the effect at MOR is not likely to mediate antidepressant action at subanesthetic dose.

Table 3: Esketamine, arketamine, and metabolites in an *in vitro* receptor binding screen

Receptor	% inhibition for Esketamine (10 μ M)	% inhibition for ketamine (10 μ M)	% inhibition for arketamine (10 μ M)	% inhibition for M4 (10 μ M)	% inhibition for M9 (10 μ M)	% inhibition for M10 (10 μ M)	% inhibition for M19 (10 μ M)
μ -opioid receptor	49.9%	29.2%	24.9%	NS	NS	NS	NS
κ -opioid receptor	38.3%	NS	NS	NS	NS	NS	NS
5HT transporter	31.0%	NS	NS	NS	NS	NS	NS
BZD	NS	NS	NS	62.9%	NS	NS	NS
GABAA	NS	NS	NS	36%	NS	NS	NS

NS=No significant effects

At 10 μ M concentration, esketamine-derived metabolite 2S,6S-HNK (M4) inhibited the binding of reference agonists to central benzodiazepine receptors and GABAA-gated chloride channels by 63% and 36%, respectively (study No. 100030088_FK1207). However, this effect was not confirmed in a subsequent study in the rat cerebral cortex (concentration-dependent *in vitro* CEREP binding assays) where both esketamine and arketamine metabolites 2S,6S-HNK and 2R,6R-HNK showed \leq 25% binding to central BZD site or Cl^- Channel (GABA-gated) even at the highest tested concentration of 100 μ M.

Published data report that ketamine, esketamine, and arketamine block the nicotine-induced current at a low micromolar concentration range in transfected rat nicotinic acetylcholine receptors (nAChRs) (Moaddel et al, 2013). In a study (DD17053) conducted by the Sponsor using cells transfected with human nAChR $\alpha 7$, (R,S) DHNK failed to inhibit these receptors whereas Moaddel, et al., 2013 reported that the same metabolite blocked rat nAChR $\alpha 7$ current with an IC_{50} of 50 nM. The Sponsor hypothesizes that the discrepancy between published data and in-house data may be attributed to species-specific sensitivity. However, a head-to-head comparison of the human and rat nAChRs would be needed to resolve this discrepancy. In study No. DD17063 examining functional activity of racemic ketamine, esketamine, arketamine, and esketamine metabolites at human nAChR $\alpha 7$, none of the compounds exhibited significant antagonist activity, except for 2S-5,6 DHNK (M9) which reduced activity by 36% when tested at 100uM.

Overall, the pharmacology studies conducted by JRD conclude that esketamine is a nonselective and a noncompetitive antagonist of NMDARs. Of the various phase 1 metabolites generated in humans, only noresketamine (M10), but not others (i.e. M4, M5, M9, and M19), is confirmed to have activity at NMDARs.

Established Pharmacologic Class

The current list of established pharmacological class (EPC) has two listings for N-methyl-D-Aspartate (NMDA) receptor antagonists: 1. Dextromethorphan; which is described as an “uncompetitive NMDAR antagonist” and 2. Memantine; which is described as “NMDA receptor antagonist”. The KETALAR® label refers to the racemate as nonbarbiturate general anesthetic with the statement “mechanism of action is primarily due to antagonism of NMDA receptors in the central nervous system.” In the current proposed label, the Sponsor is referring to the drug as “glutamate receptor modulator” in the highlights and in the mechanism of action section 12.1 as “a non-competitive, subtype non-selective, activity-dependent glutamate receptor modulator”. However, to keep simplicity of EPC class, it was determined that the EPC of esketamine should be referred to as a “noncompetitive NMDA receptor antagonist.”

4.3 Safety Pharmacology

CNS safety pharmacology was evaluated as part of the 6-month toxicology study in rats using Functional Observational Battery (FOB), Morris Water Maze, and locomotor activity (refer to the 6-month study under section 6.2.1). Cardiovascular pharmacology was evaluated as part of the 9-month dog study using electrocardiography (refer to the 9-month dog study under section 6.2.2). Additionally, the following cardiovascular and respiratory studies were conducted under Safety Pharmacology.

Study/Study No.	Finding
hERG Assay/ JRD, TOX11340 (GLP)	$IC_{50} = 214 \mu M$ (58.7 $\mu g/mL$). IC_{50} based safety margin is approximately 337-fold the plasma concentration of esketamine at MRHD (174 ng/mL).
Cardiovascular and Respiratory (Conscious non-telemetered Beagle Dog)/TOX11501 (GLP)	Single escalating doses of 0.3, 1 or 3 mg /kg of esketamine was IV administered to male beagle dogs resulting in increases in heart rate, blood pressure (systolic, diastolic, and mean), and heart related shortening of QT-interval in a dose-dependent manner. The effects on heart rate, blood pressure, and QT-interval were noted in all dose levels, whereas heart rate related shortening of the PQ-interval and an increase in respiration rate was only observed at 3 mg eq/kg. No drug related arrhythmias were noted throughout the study.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Type of Study	Major Findings																															
Absorption (Study Nos. FK1034, FK 10368, and FK10710)	<p>Oral bioavailability was low for dogs (1.3%) as compared to >54% for IN bioavailability. C_{max} was reached 5-30 min after IN administration in all adult nonclinical species for the parent and 30 min-2h for noresketamine (M10) metabolite.</p> <p>Table 4: PK of ketamine in mice, rats, and rabbits following single bolus IV administration</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>Mice^a</th> <th>Rats^a</th> <th>Rabbits^b</th> </tr> </thead> <tbody> <tr> <td>AUC_{last} (ng.h/mL)</td> <td>1700</td> <td>1540</td> <td>400</td> </tr> <tr> <td>CL (L/kg/h)</td> <td>0.37[†]</td> <td>3.3[†]</td> <td>12.6</td> </tr> <tr> <td>V_d (L/kg)</td> <td>0.10[†]</td> <td>2.9[†]</td> <td>2.3</td> </tr> </tbody> </table> <p>^adose = 20 mg/kg in isotonic NaCl solution at pH 4.8; ^bdose = 5 mg/kg isotonic NaCl solution at pH 4.8; AUC_{last}: area under the curve from zero to the time of the last quantifiable concentration; CL: clearance; V_{ss}: volume of distribution at steady-state; [†]For mice and rats the CL and V_d are expressed as L/h and L, respectively.</p> <p>In comparison with IN formulation of esketamine in an aqueous solution, the formulation containing EDTA and citric acid (i.e. clinical formulation) produced half as much C_{max} but bioavailability was 88% (interindividual variability was moderate in this study) (Table 5). Toxicology studies conducted early in drug development were done with the less bioavailable formulation containing water.</p> <p>Table 5: PK of esketamine in rats administered aqueous vs. clinical formulation</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>Solution A: water^a</th> <th>Solution B: EDTA citric acid^a</th> </tr> </thead> <tbody> <tr> <td>C_{max} (ng/mL)</td> <td>730</td> <td>356</td> </tr> <tr> <td>T_{max} (h)</td> <td>0.083-0.25</td> <td>0.083-0.5</td> </tr> <tr> <td>AUC_{0-inf} (h.ng/mL)</td> <td>573¹</td> <td>506²</td> </tr> <tr> <td>F rel (B vs. A)%</td> <td>–</td> <td>88.3</td> </tr> </tbody> </table> <p>^aDose= 3mg/rat; ¹N=4; ²N=2</p>	Parameter	Mice ^a	Rats ^a	Rabbits ^b	AUC _{last} (ng.h/mL)	1700	1540	400	CL (L/kg/h)	0.37 [†]	3.3 [†]	12.6	V _d (L/kg)	0.10 [†]	2.9 [†]	2.3	Parameter	Solution A: water ^a	Solution B: EDTA citric acid ^a	C _{max} (ng/mL)	730	356	T _{max} (h)	0.083-0.25	0.083-0.5	AUC _{0-inf} (h.ng/mL)	573 ¹	506 ²	F rel (B vs. A)%	–	88.3
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Type of Study	Major Findings
<p>Distribution Study No. FK12091</p>	<p>The Sponsor did not conduct radiolabeled tissue distribution or quantitative whole body autoradiography studies in animal species using esketamine. After oral dosing of esketamine in rats, brain:plasma concentration ratios were 3-4 for M19, 1.8 for esketamine, 1.2 for M4, and 0.3-0.4 for both M10 and M9.</p> <p>From literature, after 5 min IV dose of ketamine, the highest esketamine concentration was observed in kidney, lung, fat, and heart at 3 h post infusion (Edwards and Mather, 2001). Esketamine and ketamine are known to cross the placental barrier and it is rapidly distributed to fetal tissues including the brain (Chang et al., 1974) .</p>
<p>Metabolism In vitro study: Study No. FK10473</p>	<p>Esketamine was rapidly and extensively metabolized in rat and dog liver microsomes and S9 fractions and in human liver microsomes. Much slower and less extensive metabolism was observed in mouse microsomes. In rat induced liver S9 fraction, high turnover was observed. A total of 12 metabolites were observed <i>in vitro</i>, 7 of which are present in humans.</p> <p>The major metabolic pathway in all species was N-demethylation at the secondary amine (M10; noresketamine). Other pathways involved are oxidation on the cyclohexanone moiety (M2, M4, and M5), oxidative deamination (M11), and keto reduction (M12) of M10. Additionally, further oxidation of the cyclohexanone moiety of esketamine (M6), was observed. In mice and human microsomes, the major metabolite was M10 and in rat and dog microsomes, the major metabolite was M2. In dog and rat microsomes, additional oxidation of M10 to M1 was observed. (See Figure 1 in the Appendix for <i>in vitro</i> metabolic pathways of esketamine).</p> <p>In humans, noresketamine (N-demethylation) was the major circulating metabolite in plasma (i.e. 12-14% of total circulating drug material). This was followed by M19 (keto-reduction of M4), M4 (N-demethylation and hydroxylation at the 6-position), M5 (2S, 6S-HNK), and M17 (O-glucuronidation of M4), which were present at >25% of parent AUC. Noresketamine is accepted to be generally qualified in nonclinical species as its exposure levels in rat general toxicity, Segment I, Segment III, and carcinogenicity study are near the MRHD (0.5 fold) in humans (see Figure 2 in the Appendix for Sponsor's safety margin estimation). Segment II studies were not conducted with esketamine, but rather with PMI-100. Norketamine, of which 50% is noresketamine, is formed when ketamine is administered; however, the exposure of level of neither norketamine nor noresketamine was directly quantified in those studies. Nevertheless, the label for this drug indicates that the drug is not recommended for pregnant and lactating women.</p> <p>The Sponsor did not conduct <i>in vivo</i> metabolism studies in nonclinical species with unlabeled esketamine.</p> <p><i>In vitro</i>, CYP2B6 and CYP3A4 play major roles in esketamine metabolism whereas CYP2A6 and CYP2B6 play a major role in noresketamine metabolism.</p> <p><i>In vitro</i>, esketamine was not a major CYP inhibitor; M10 weakly inhibits CYP3A4 (IC₅₀=1.9 uM with testosterone as substrate).</p>

Type of Study	Major Findings
<p>Excretion Study No. FK10757</p>	<p>After a single IV dose of 5 mg/kg ³H-esketamine to male rats, 30-41% radioactivity was excreted in the urine. No biliary excretion study for esketamine was performed in animals.</p> <p>There is literature evidence that esketamine is excreted in human breast milk therefore animal studies were not conducted.</p>
<p>Other PK studies Study No.: FK10602 , FK10995, FK12057, FK12092, FK 12099, FK12111, FK12229</p>	<p>The Sponsor conducted additional studies to characterize other metabolites which represented >25% of esketamine AUC, such as M4, M5, M9, M10, and M19. Of these, M19 and M4 are present at 3-4 fold higher levels in humans when esketamine was administered IN than PO or IV. In nonclinical species (rats and dogs) M4, but not M19, had adequate coverage after IN administration. Similarly, M19 was not adequately covered in the Sponsor's transgenic mice carcinogenicity study. However, it was shown that M19 forms under experimental conditions in the presence of S9 in an Ames assay and mouse lymphoma assay when incubated with both ketamine and esketamine. Therefore, it can be concluded that M19 has been qualified in routine genotoxic studies.</p> <p>Published data suggest that tertiary amines can react with nitrous acid to form N-nitroso compounds which are potentially genotoxic (Brambilla and Martelli, 2007; Mensinga, et al., 2003). However, there was no measurable concentration of N-nitrosoesketamine formed in simulated gastric fluid under fasted or fed conditions.</p>

5.2 Toxicokinetics

<p>TK data from general toxicology studies</p> <p><i>Rat: 26-week IN study with reproductive phase (Study No. TOX10768)</i></p> <ul style="list-style-type: none"> • Samples collected at 5 min, 15min, 30 min, 2 hrs, 6hrs, and 24 hrs on D1 and week 26 of dosing. • TK parameters were estimated using Analyst 1.6.2 computer application program. • NOAEL is 3 mg/rat (15 mg/kg/day) 	<p>Sponsor calculated exposures for both esketamine and noresektamine in all pivotal toxicity studies. After clinical administration of 84 mg of IN esketamine, the C_{max} and AUC exposures for esketamine are 174 ng/mL and 530 ng.h/mL and for noresektamine 250 ng/mL and 1784 ng.h/mL, respectively. Due to the IN toxicity studies being conducted at MFD, there was little or no exposure margin for both esketamine and noresektamine compared to MRHD. Based on the 2-year carcinogenicity study in rats, C_{max} and AUC exposure margins for esketamine are 1.8 times and 0.6 times for esketamine, respectively, and 2.3 and 0.9 fold, respectively, for noresektamine to the MRHD. In dogs, the C_{max} and AUC based exposure ratios for esketamine compared to the MRHD were 6.5 and 1.3-fold, respectively, and approximately 1.3 and 0.5 fold, respectively, for noresektamine. In transgenic mice, the C_{max} and AUC based exposure ratios for esketamine compared to the MRHD were 27 and 6 -fold, respectively, and approximately 19 and 6- fold for noresektamine. Noresektamine exposure data are provided in the Appendix (see Table 32, Table 33, Table 34, and Table 35).</p> <p><u>Rat*</u></p> <p>T_{max}: 5-15 minutes post dose</p> <p>Accumulation: exposures are decreased on week 26 compared with D1 and are likely due to having no weight correction for dose administration.</p> <p>Dose proportionality: not dose proportional.</p> <p>Sex differences: females less exposure than males at doses ≤3.0 mg/rat and more than males at 9 mg/rat.</p> <p>*Esketamine exposure data from the 6-month toxicity study is unreliable due to <i>ex vivo</i> contamination of samples. <u>For all safety margin calculations, exposure data from 2-year carcinogenicity study were used.</u></p> <p>Table 6: TK of esketamine in rats from the 6-month toxicity study (week 26).</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>Sex</th> <th>0.9 mg/rat</th> <th>3.0 mg/rat</th> <th>9.0 mg/rat</th> </tr> </thead> <tbody> <tr> <td rowspan="2">AUC_{0-t} (ng.h/mL)</td> <td>M</td> <td>1610</td> <td>1900</td> <td>3560</td> </tr> <tr> <td>F</td> <td>304</td> <td>1790</td> <td>4100</td> </tr> <tr> <td rowspan="2">C_{max} (ng/mL)</td> <td>M</td> <td>3520</td> <td>11300</td> <td>13400</td> </tr> <tr> <td>F</td> <td>1110</td> <td>9480</td> <td>20300</td> </tr> </tbody> </table>	Parameter	Sex	0.9 mg/rat	3.0 mg/rat	9.0 mg/rat	AUC _{0-t} (ng.h/mL)	M	1610	1900	3560	F	304	1790	4100	C _{max} (ng/mL)	M	3520	11300	13400	F	1110	9480	20300
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Dog: 9-month IN toxicity study (Study No. TOX10701)

- Samples were collected at 5 min, 15 min*, 0.5hr, 1 hr, 4hr, and 7 hr on D7 and week 39.
- TK parameters were estimated using WinNonlin PK software.
- NOAEL is 48 mg/dog (6 mg/kg/day)

TK data from reprotox studies

Rat: embryo-fetal development (Study No. 3542.11)

- Samples collected 0, 0.25, 0.5, 1, 4, and 8 hours after IN administration using varying volumes on GD 6 and 17 for the main study. For bridging study, samples were collected 15 min prior to administration and 5 min, 15 min, 30 min, and 2 h after dosing.
- TK parameters were estimated using WinNonlin PK software.
- NOAEL for maternal toxicity is 50 mg/kg/day; NOAEL for embryofetal toxicity is 150 mg/kg/day).

Rabbit: embryo-fetal development (Study No. 3542.13)

- Samples collected 0, 0.25, 0.5, 1, 4, and 8 hours after IN administration using varying volumes on GD 6 and 18. For bridging study samples were collected at 2 min, 5 min, 10 min, 15 min, 30 min, 1 h, and 2 h post dosing on GD19.
- TK parameters were estimated using WinNonlin
- Maternal toxicity and embryofetal NOAEL is 10 mg/kg/day.

Dog

Accumulation: exposures increased over time.

Dose proportionality: dose proportional

Sex difference: In general AUC exposure was greater in males and C_{max} exposure was greater in females.

Table 7: TK of esketamine in dogs from the 9-month toxicity study (week 39).

Parameter	Sex	24 mg/day	48 mg/day	72 mg/day
AUC _{0-t} (ng.h/mL)	M	361	523	771
	F	159	630	584
C _{max} (ng/mL)	M	489	944	1100
	F	328	1260	1160

Rat

Table 8: TK of racemic ketamine from pregnant rats in the embryofetal toxicity study (GD17) following IN PMI-100 administration.

Parameter	15 mg/kg	50mg/kg	150 mg/kg
AUC _{0-∞} (ng.h/mL)	920	7770	12950
C _{max} (ng/mL)	1950	14130	21730
T _{max} (hr)	0.25	0.25	0.25
T _{1/2} (hr)	0.3	1.12	1.67

Table 9: Bridging study conducted in pregnant rats on GD17 after IV dosing of 90 mg/kg of racemic ketamine.

Parameter	ketamine	esketamine	norketamine	Ratio esk/ket
AUC _{0-∞} (ng.h/mL)	32807	16095	NC	0.491

NC: not calculated.

Rabbit

Table 10: TK of racemic ketamine from pregnant rabbits in the embryofetal toxicity study (GD 18) following IN PMI-100 administration.

Parameter	10 mg/kg	30 mg/kg	50 mg/kg
AUC _{0-∞} (ng.h/mL)	NA	930	1530
C _{max} (ng/mL)	50	1900	2310
T _{1/2} (hr)	NA	0.39	NA

NA: Not able to determine;

<p>TK data from Carcinogenicity studies</p> <p>Rat: 2-year carci study after daily IN dosing (Study No. TOX10702)</p> <ul style="list-style-type: none"> • Samples collected at 5 min, 15 min, 30 min, 2 hr, 6 hr, and 24 hr on D1, during month 3 and month 6. • TK parameters were estimated using Phoenix (WinNonlin) software. • NOAEL is 9 mg/day. <p>Transgenic mice: 6 month carcinogenicity assay after daily IN dosing (Study No. TOX11233)</p> <ul style="list-style-type: none"> • Samples collected at 0.25, 0.5, 1, 4, 6 and 8 hours post dosing on D1 and D177. • TK parameters were estimated using Phoenix software. • NOAEL is 75/40 mg/kg/day. 	<p>Table 11: Bridging study conducted in pregnant rabbits on GD19 after IV dosing of 8 mg/kg of racemic ketamine.</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>ketamine</th> <th>esketamine</th> <th>norketamine</th> <th>Ratio esk/ket</th> </tr> </thead> <tbody> <tr> <td>AUC_{0-∞} (ng.h/mL)</td> <td>386</td> <td>119</td> <td>915</td> <td>0.308</td> </tr> </tbody> </table>	Parameter	ketamine	esketamine	norketamine	Ratio esk/ket	AUC _{0-∞} (ng.h/mL)	386	119	915	0.308													
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	<p>Table 12: TK of esketamine in rats in the 2-year carcinogenicity assay after 6 months of dosing</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>Sex</th> <th>0.9 mg/day</th> <th>3 mg/day</th> <th>9 mg/day</th> </tr> </thead> <tbody> <tr> <td rowspan="2">AUC_{0-t} (ng.h/mL)</td> <td>M</td> <td>19.6</td> <td>60.7</td> <td>253</td> </tr> <tr> <td>F</td> <td>36.1</td> <td>256</td> <td>366</td> </tr> <tr> <td rowspan="2">C_{max} (ng/mL)</td> <td>M</td> <td>59.5</td> <td>81.8</td> <td>201</td> </tr> <tr> <td>F</td> <td>76.3</td> <td>253</td> <td>428</td> </tr> </tbody> </table>	Parameter	Sex	0.9 mg/day	3 mg/day	9 mg/day	AUC _{0-t} (ng.h/mL)	M	19.6	60.7	253	F	36.1	256	366	C _{max} (ng/mL)	M	59.5	81.8	201	F	76.3	253	428
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<p>Table 13: TK of esketamine after subcutaneous administration in transgenic mice (week 25/26)</p> <table border="1"> <thead> <tr> <th>Parameter</th> <th>Sex</th> <th>10 mg/kg</th> <th>25 mg/kg</th> <th>75/40 mg/kg</th> </tr> </thead> <tbody> <tr> <td rowspan="2">AUC_{0-t} (ng.h/mL)</td> <td>M</td> <td>980</td> <td>2460</td> <td>3980</td> </tr> <tr> <td>F</td> <td>409</td> <td>901</td> <td>2210</td> </tr> <tr> <td rowspan="2">C_{max} (ng/mL)</td> <td>M</td> <td>1570</td> <td>3780</td> <td>5990</td> </tr> <tr> <td>F</td> <td>786</td> <td>1490</td> <td>3540</td> </tr> </tbody> </table>	Parameter	Sex	10 mg/kg	25 mg/kg	75/40 mg/kg	AUC _{0-t} (ng.h/mL)	M	980	2460	3980	F	409	901	2210	C _{max} (ng/mL)	M	1570	3780	5990	F	786	1490	3540	
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TK data from neurotoxicity studies

Study No. 3542.9B: 28-day neurotoxicity study after SC administration of PMI-100

- Samples collected at 0.25, 0.5, 1, 3, 6, and 24 hrs on D1 and D28
- TK parameters were estimated using WinNonlin software.
- NOAEL for neuronal vacuolation is 15 mg/kg.
- NOAEL for neuronal necrosis is 60 mg/kg

Study No. TOX10415

- Samples collected at 5 min, 15 min, 30 min, 60 min, 120 min, 480 min, and 24 hrs.
- TK parameters were estimated using WinNonlin software.
- NOAEL is 9 mg/rat for neuronal vacuolation

Study No. TOX11374

- Samples collected at 3 min, 20 min, 30 min, 45 min, 2 h 15 min, 6hr 15 min, and 24 h after first instillation for vehicle and HD groups, at 3 min, 10 min, 20 min, 35 min, 2 hr 05 min, 6 h 05min, and 24 h after first instillation of the LD and at 3 min, 15 min, 25 mi, 40 min, 2h 10 min, 6 h 10min, and 24 h after first IN instillation for the MD groups.
- TK parameters were estimated using Analyst Software.
- NOAEL is 72mg/rat for neuronal necrosis.

Table 14: TK of racemic ketamine after subcutaneous administration of PMI-100 in rats on D1 of neurotoxicity study

Parameter	Sex	4 mg/kg	15 mg/kg	60 mg/kg
AUC _{0-last} (ng.h/mL)	M	176	987	5879
	F	162	1638	10714
C _{max} (ng/mL)	M	360	2037	6067
	F	397	1577	5523

Table 15: TK of IN esketamine in female rats on D1 of neurotoxicity study

Parameter	Sex	0.9 mg/rat	3 mg/rat	9 mg/rat
AUC _{0-t} (ng.h/mL)	F	57.8	261	936
C _{max} (ng/mL)	F	92.5	157	791

Table 16: TK of IN esketamine in female rats on D1 of neurotoxicity study

Parameter	Sex	36 mg/rat	54 mg/rat	72 mg/rat
AUC _{0-t} (ng.h/mL)	F	5400	9430	45800 ¹
C _{max} (ng/mL)	F	2380	4070	10245 ¹

¹ N=2; highly variable data since existing 2 animal data points are >3 fold apart.

6 General Toxicology

6.1 Single-Dose Toxicity

Rat

Three non-GLP studies were conducted to determine esketamine exposure when ketamine was administered. In study No. TOX 10457, all rats (3/sex) died within 15 minutes during an IV infusion of 30 minutes with 150 mg/kg/day racemic ketamine (aqueous solution, pH 4.5, NaCl until isotonic) after exhibiting signs such as severe sedation and decreased activity. At 120 mg/kg/day, 2/3 males died shortly at the end of infusion but the females survived. A dose of 30 mg/kg by a 30 min IV infusion was well-tolerated with abnormal respiration and ataxia lasting up to 2 hours postdose. See the Table 36 in the Appendix for exposure data in rats from these bridging studies.

A single dose oral study at doses ≤ 160 mg/kg/day followed by a 14-day repeated dose toxicity study at doses ≤ 160 mg/kg/day (non-GLP; Study No. TOX 12233) were conducted with esketamine HCl in S-D rats (8-9 weeks of age). In the single dose phase, the dose was lowered to ≤ 120 mg/kg/day and in the repeated dose, the dose was lowered to ≤ 40 mg/kg/day in females due to severity of the clinical signs. Ataxia was observed immediately after dosing followed by decreased general activity ≥ 80 mg/kg/day and ≥ 40 mg/kg/day in females. Catalepsy was observed ≥ 160 mg/kg/day in males and ≥ 40 mg/kg/day in females. After these CNS signs resolved, the animals showed excitation. With repeated dosing, severity and duration of CNS signs decreased. There was a minimal increase in calcium and inorganic phosphate in males at 160 mg/kg/day. Minimal centrilobular hypertrophy was observed at 160 mg/kg/day correlating with increased liver weights. Dose-related accumulation of hyaline droplets in the corticotubular epithelium in the kidney was observed in males ≥ 40 mg/kg/day. The NOAEL in the 14-day study was 40 mg/kg/day for males (C_{max} 314 ng/mL and AUC_{0-24h} was 425 ng.hr/mL) and 10 mg/kg/day for females (C_{max} 203 ng/mL and AUC_{0-24h} was 262 ng.hr/mL).

Dog

A non-GLP study (TOX 13114) was conducted in the beagle dog to explore various formulations of esketamine (IV, IN, oral, sublingual). Clinical signs such as decubitus, moderate to severe decreased activity, tremors, excitation and/or hemorrhagic vomit were observed ≥ 28 mg/dog, depending on the route of administration. Best bioavailability was observed via the oral dose using an oral thin film. A bridging study (TOX10458) was conducted in the dog to determine esketamine exposures after a single IV ketamine (aqueous solution, pH 4.5, NaCl until isotonic) administration to correlate findings from shorter studies conducted with PMI-100 (see Table 37 in the Appendix showing the PK bridging data in dogs). In this study, ataxia, decubitus, decreased general activity, salivation, and licking were observed after a single IV dose ≥ 0.3 mg/kg, lasting up to 2 hrs.

6.2 Repeat-Dose Toxicity

6.2.1 Rat Repeat-Dose Toxicity using Esketamine

Summary of studies shorter than 6 months in duration in rat:

A 3-month repeat dose IN toxicity study in SPF SD rats followed by a 1-month recovery period (6-7 weeks 10/sex/group for main study; 5/sex/group for recovery) was conducted (GLP; TOX 10517). Doses of esketamine HCl (batch no. A12BD0277) were IN instilled (0.5 ml/rat) at doses of 0.9, 3, or 9 mg equivalent/rat once daily or 9 mg eq/rat once a day for three days per week for 3 months. This study had two controls: one to complement daily dosing and the second to complement intermittent dosing. The vehicle in this study was not the clinical vehicle but rather demineralized water adjusted to pH 4.5 ± 0.1 with NaOH or HCl. Slight salivation and increased general activity were observed 30 minutes post dosing throughout the duration of the study ≥ 3 mg eq/rat. After daily dosing, ataxia was seen ≥ 3 mg eq/rat until day 4 and occasionally afterwards. With intermittent dosing, similar CNS signs were observed except that ataxia was seen until 4th treatment (D7). There were no clinical observations on treatment free days and severity and frequency decreased over time. Histopathology showed a minimal decrease in mucus content of goblet cells in the respiratory epithelium, noted particularly in the epithelium lining the nasal septum at level 1 at 9 mg eq/rat. Morphologically, this change appeared as hypercellularity in this localized area due to the disappearance of large quantities of cytoplasm (mucus vacuoles). In the daily dose group, the frequency was increased as compared with intermittent dosing (i.e. all or nearly all rats affected in the daily dosing group compared with 2M and 4F affected in the intermittent group). There were no histopathology findings in the recovery group. The NOAEL in this study is the mid dose of 3mg/eq (C_{max} is 172 ng/mL and AUC_{0-24h} is 176 ng.hr/mL).

Study title: Esketamine hydrochloride (JNJ-54135419-AAC): Toxicity study by IN administration to Crl:CD (SD) rats for 26 weeks with a reproductive phase

Study no.:	TOX 10768
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 4, 2013
GLP compliance:	Yes (b) (4)
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-54135419-AAC, 2 batches used: A12BD0277 (from weeks 1-10; purity 99.7%); A12LD4238 (from weeks 11 to termination, purity: 100.3%)

Key Study Findings

- This rat toxicity study was conducted as a combination of chronic and juvenile toxicity studies with dosing initiated on postnatal day (PND) 35 and included evaluation of neurobehavioral and reproductive toxicity endpoints. In general, CNS clinical signs reflected the anesthetic properties of the drug with apparent tolerance developing over time. Unexpected to the predicted anesthetic properties, there was a dose response increase in activity at ≥ 15 mg/kg/day in the open field test which often reached statistical significance. The NOAEL for this finding was 4.5 mg/kg/day.
- A drug effect was observed on spatial learning in Morris Water maze. An increase in latency to reach platform and higher failure rate were observed at week 13/14 of dosing (but not at week 5/6) with statistical significance observed on D2 of that week in males at 45 mg/kg/day. A similar trend was seen without reaching statistical significance at other time points. After repeated drug administration of nearly 6 months, the learning delay was observed at all doses.
- A drug effect on estrous cycle was not observed at weeks 6-7 of dosing but rather after 1 week of recovery at ≥ 45 mg/kg/day. At that time point, 25% of HDF compared to 15% of controls had irregular estrous cycles. Reproductive capacity was assessed only after 3 weeks of recovery but not in the presence of drug. No mating delay or effect on mating/fertility indices was observed in this study in the absence of drug.
- Minimal to slight hyperplasia of the olfactory epithelium was observed in both males and females ≥ 45 mg/kg/day.
- The appearance of tolerance to clinical signs and the lack of consistent findings for other endpoints in this study may be due to the lack of body weight correction for dosing over the course of the study (see the dose section below).
- NOAEL in this study is the mid dose of 15 mg/kg/day for nasal findings and estrous cycle delays but there is no NOAEL for the learning deficits observed in this study. The rat NOAEL is 0.3* - times for AUC and 0.8-times for Cmax as compared to the esketamine exposures at the MRHD of 84 mg/day (530 ng.h/mL for AUC and 174 ng/mL for Cmax for esketamine).

*Esketamine exposures used here to calculate safety margins were from the carcinogenicity study in rats because of contamination of PK samples in this study.

Methods

Doses: 0, 0.9, 3, 9 mg eq/rat/day (estimated to be 4.5, 15, and 45 mg/kg/day for a 200 gram rat; To note: at the end of 26 weeks, male rats weighed as much as 600 gram which means that the highest dose received was 15 mg/kg/day)

Frequency of dosing: Once daily for 26 weeks.

Route of administration: IN instillation

Dose volume: All treatment groups received the same volume (50uL/day; 25ul/nare) with varying drug concentration in vehicle.

Formulation/Vehicle: Pyrogenic free water/water for injection with (b) (4) citric acid and (b) (4) EDTA, pH4.5

Species/Strain: Rat/Crl:CD (SD)

Number/Sex/Group: 20/sex/dose for main study group

Age: PND 35 at the start of dosing

Weight: 108-199g (M); 93-157g (F)

Satellite groups: TK group: 6/sex/dose for all doses except vehicle control which had 3/sex/dose; reproductive phase group: 20/sex/dose

Unique study design: The Sponsor integrated endpoints similar to a juvenile toxicity study into this chronic toxicity study using PND 35 rats at the start of dosing. After 13 weeks of dosing, the reproductive phase group rats were given a 6-week dose free period during which mating and fertility parameters were assessed. Neurobehavioral endpoints were assessed on week 5 (first 10 main study rats), week 13 (reproductive phase animals), and week 21 (last 10 main study rats). Dosed females were checked for estrous cycle phases using vaginal smears for 15 days during treatment (weeks 6 and 7 of treatment) and for 15 days prior to pairing (starting after at least one week of recovery). Animals were paired at approximately 20 weeks of age for up to 2 weeks. Following pairing, mating was confirmed using pipette lavage. Reproductive phase females were killed on D14 after mating, or on D10 after last day of pairing for the females which failed to mate. Reproductive phase males were killed after the majority of the reproductive phase females, which was after six weeks of recovery. In the reproductive phase females, each ovary/uterine horn, number of corpora lutea, implantation sites, resorption sites

and embryos were assessed. (See appendix Figure 3 for brain and nasal histopathology sectioning)

Deviation from study protocol: None affecting the interpretation of study results

Observations and Results: Changes from Control

Parameters	Major findings
Mortality	No test article related mortality. Three deaths/early termination in main/reproductive phase groups [1CM (repro), 1CM (main), 1MDF (main)]. Two TK deaths due to failure to revive from anesthesia.
Clinical Signs	Dose-dependent increase in the incidence of unsteady gait (all animals affected at the highest dose), overactive and underactive behavior in all dosed males and females. The clinical signs started within 5-10 minutes of drug administration and lasted for 20-30 minutes post dose. These findings decreased in frequency and magnitude with repeat dosing with no signs observed after week 16 of dosing in main study animals.
Body Weights	unremarkable
Ophthalmoscopy	unremarkable
Hematology	unremarkable
Clinical Chemistry	unremarkable
Urinalysis	unremarkable
Gross Pathology	unremarkable
Organ Weights	A decrease in absolute and relative ovarian weight was observed in females at the end of 26 weeks. This finding was slight (i.e. <20%) but statistically significant at all doses. The relative weights were 0.093g, 0.078g, 0.076g, and 0.083g for all C, LD, MD, and HD respectively. This decrease in ovarian weight occurred in the absence of correlating histopathology findings.
Histopathology Adequate battery: Yes; Brain sectioning conducted as per Bolon et al., 2013	<i>Nasal turbinates</i> Minimal to slight hyperplasia of the olfactory epithelium was observed in 12/20 HDM and 10/20 HDF, and in a single MDF (see Table 17 below). <i>Respiratory epithelium</i> There was an increase in goblet cells in the respiratory epithelium of the septum in all animals, including vehicle controls. While a dose-dependent relationship is not clear in males, mid and high dose females have twice as many incidences of this finding compared with controls (see Table 18 below for the Sponsor's summary). This may be the result of IN route of administration but a test article relationship cannot be excluded due to increased frequency in females.
Special Evaluation: Locomotor activity	FOB was conducted at the time of anticipated peak plasma concentration (5-15 minutes after dosing). In FOB, total mean activity count in an open field showed a dose related increase (18-90%) which reached statistical significance at the mid and high doses on weeks 5 and 21 (see Table 19). There were no dose related effects for total locomotor activity (home cage) which was performed 1 hr prior to dose administration (i.e. 20-22 hrs post dose). Considering the drug's short half-life, it may have been completely excreted at the time when this endpoint was measured.

<p>Special Evaluation: Continued Functional Observational battery</p>	<p>Other FOB findings were consistent with that for an anesthetic. These included the following: dose related incidences of salivation and cold paws in all dosed animals; a dose dependent increase in the incidence of abnormal gait (unsteady/uncoordinated/elevated/flattened/unstable) during the 2-minute observation assessment in weeks 5, 13, and 21. Slow or poorly coordinated righting reflex, increased distance in landing footsplay and reduced forelimb/hindlimb grip strength (~30%) was observed at all three time points at the HD compared with controls. For most of the anesthetic related findings, there was a reduction in magnitude and/or incidence over time.</p>
<p>Special Evaluation: Continued; Morris Water maze</p>	<p><u>There was a drug effect on spacial learning (i.e. mean trial times) observed starting on week 13/14 (see Table 20).</u> Due to the high variability in data and small sample size (only 10/sex/group), statistics cannot be fully relied on in this study (i.e. 11.3 sec, 12.9 sec, 17.2 sec and 27.3 sec in CM, LDM, MDM and HDM, respectively) but there seems to be a trend effect. On week 13/14, day 2, there was a clear dose response delay in reaching the platform in all drug treated males, which was statistically significant at the HD. HDM had a delay of 2.4 fold compared with controls at this time point (p<0.01). On the later test dates in week 21/22, this initial delay is still observed but at a smaller magnitude and not reaching statistical significance.</p> <p><u>Additionally, a drug effect was observed on the % of animals that failed the test on D1 (i.e. those animals that had ≥ 90 sec to reach the platform) (see Table 21).</u> For this parameter, a dose response is clear in both sexes by week 21/22 reaching a 20-30% increase in failure rate at the HD compared with controls. Based on the fewer number of rats failing by D4, it is clear that they are able to learn after repeated trials (i.e. no apparent effect of drug on memory retention in this study).</p>
<p>Special Evaluation: Continued; Reproductive Toxicity</p>	<p>During the treatment period, there were statistically significant variations in body weight in reproductive phase males. However, overall, the body weight gain in dosed animals was similar to controls. In recovery, MDM and HDM had a 17% and 25% increase in body weight gain that reached statistical significance at high dose. See Table 22 for reproductive toxicity endpoints tabulated from Sponsor's data.</p> <p>During the gestational period, body weights were not different over days 0-6, but between days 6-14, all dosed females had a 16% decrease in body weight gain, which reached statistical significance at MD and HD. During the 13-weeks of drug dosing, there were no significant differences in absolute body weights or body weight gains between treated rats and controls (see Table 23).</p> <p>Vaginal smears were done for two weeks on weeks 6-7 and on week 14 (1 week after recovery) (see methods for details). Whereas there was no drug effect on estrous cycling in weeks 6-7, an effect on estrous cycles was observed at the second time point (i.e. recovery). At this time point, <u>slightly less number of HDF (75%) had normal 4 day cycles as compared with 85% in Controls.</u> Unlike the observed mating delays in the Segment I study, <u>this study did not show a delay in mating because mating was conducted after 3 weeks of recovery and not in the presence of drug.</u> Overall, similar to Segment I study, there was no effect on mating and fertility indices. <u>It is likely that effect on estrous cycle in this study may be age-related.</u></p> <p><u>The post implantation loss was 62% and 38% higher in LDF and HDF compared with controls but not following a clear dose response. There were no other salient findings in other litter data parameters (Table 23).</u> HCD was not provided for reproductive and neurobehavioral parameters from 6-month chronic toxicity studies.</p>

LD: low dose; MD: mid dose; HD: high dose; HCD: historical control data.

Reviewer's Comments: The dosing scheme (mg/rat) in this study is skewing the data, particularly those related to learning and behavior. Since there is no body weight correction for dosing, the animals are receiving more dose of the drug earlier in the study than towards the end of six months when they gain weight (i.e. 45 mg/kg/day when 200 gram vs. 15 mg/kg/day when 600 gram).

Table 17: Summary of histopathology findings in the nasal turbinates of rats from the 6-month toxicity study.

The table below is excerpted from TOX 10678, P.67. Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat).

Group/sex Dose (mg eq./rat/day)	1M 0	2M 0.9	3M 3	4M 9	1F 0	2F 0.9	3F 3	4F 9
Olfactory epithelium – Hyperplasia								
Minimal	0	0	0	8	0	0	1	7
Slight	0	0	0	4	0	0	0	3
Total	0	0	0	12	0	0	1	10
Number of tissues examined	19	20	20	20	20	20	19	20

Table 18 : Summary of histopathology findings in the respiratory epithelium of rats from the 6-month toxicity study.

The table below is excerpted from TOX 10678, P.67. Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat).

Group/sex Dose (mg eq./rat/day)	1M 0	2M 0.9	3M 3	4M 9	1F 0	2F 0.9	3F 3	4F 9
Respiratory epithelium – Increased goblet cells - Septum								
Minimal	4	8	10	4	4	3	7	6
Slight	3	9	1	1	1	2	3	5
Total	7	17	11	5	5	5	10	11
Number of tissues examined	19	20	20	20	20	20	19	20

Table 19: Mean activity count in rats from the 6-month toxicity study.

The table below is created from data from TOX 10678. Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat); N=10/sex/dose.

Mean Activity count in open arena						
Doses; estimated	Males			Females		
	Week 5	Week 13	Week 21	Week 5	Week 13	Week 21
0	21.5	22	17.3	27.7	26.5	24.2
4.5 mg/kg/day	24.1	20.9	20.4	28.7	28.1	27.2
15 mg/kg/day	28.9**	20.7	24.2*	36.7*	32	26.6
45 mg/kg/day	37.9**	28.0	40.4**	51.6**	31.6	43.9**

Table 20: Mean trial times on various test days (D1-4) in the Morris water maze test in rats from the 6-month toxicity study.

Tables are created from data taken from the Sponsor's submission (Study No. TOX10768, P. 167-172). Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat); N=10/sex/dose.

Mean Trial times (Seconds) on Week 5/6								
Doses; estimated	Males				Females			
	D1	D2	D3	D4	D1	D2	D3	D4
0	52.1	21.8	11	12.6	40	17.3	15.6	12.4
4.5 mg/kg/day	44.1	22	19	15.8	56.9	19.8	17.7	12
15 mg/kg/day	42.3	22.5	13.7	8.3	48.9	14.6	15.1	9.2
45 mg/kg/day	41.4	28.1	15	16.5	38.9	16.8	9.4	9.1

Mean Trial times (Seconds) on Week 13/14								
Doses; estimated	Males				Females			
	D1	D2	D3	D4	D1	D2	D3	D4
0	42.6	11.3	8.2	6.9	48	18.9	11.5	11.4
4.5 mg/kg/day	40	12.9	8.2	9.4	60.3	22	13.3	11.6
15 mg/kg/day	45.5	17.2	13.0	10.8	49.2	22.7	15.3	8.6
45 mg/kg/day	56.5	27.3**	14.6	7.4	56.9	17	11.5	9.5

Mean Trial times (Seconds) on Week 21/22								
Doses; estimated	Males				Females			
	D1	D2	D3	D4	D1	D2	D3	D4
0	53.8	13.3	10	8.5	47.6	31.3	14.2	9
4.5 mg/kg/day	54.5	24.7	6.6	10.2	49.3	30.4	15	13.6
15 mg/kg/day	51.3	24	10.2	7.7	57.9	30	15.9	11.4
45 mg/kg/day	62	21.6	9.5	5.9	55.7	22	19.6	10.6

Table 21: Mean % of rats that failed trials (≥ 90 sec) in the Morris water maze from the 6-month toxicity study.

Tables are created from data taken from the Sponsor's submission (Study No. TOX10768, P. 167-172). Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat); N=10/sex/dose.

Day 1 of testing on respective weeks						
Doses; estimated	% of Males with at least 1 failed trial (90 sec)			% of Females with at least 1 failed trial (90 sec)		
	Week 5/6	Week 13/14	Week 21/22	Week 5/6	Week 13/14	Week 21/22
0	50	70	55.6	50	50	50
4.5 mg/kg/day	70	60	70	60	70	60
15 mg/kg/day	40	60	60	50	50	70
45 mg/kg/day	70	70	80	40	80	70

Day 4 of testing on respective weeks						
Doses; estimated	% of Males with at least 1 failed trial (90 sec)			% of Females with at least 1 failed trial (90sec)		
	Week 5/6	Week 13/14	Week 21/22	Week 5/6	Week 13/14	Week 21/22
0	0	0	0	0	0	0
4.5 mg/kg/day	0	0	0	0	0	0
15 mg/kg/day	0	10	0	0	0	0
45 mg/kg/day	20	0	0	0	10	0

Table 22: Reproductive toxicity endpoints examined in male rats from the 6-month toxicity study.

Table is created from data taken from the Sponsor's submission (Study No. TOX10768). Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat); N=20/dose.

Parameter	Estimated Dose, mg/kg/day			
	0	4.5	9	45
Body weight gain Week 0-13 (g)	347	375	364	363
Body weight gain Recovery 0-6 (g)	52	55	61	65*
No. males paired	19	20	20	20
No. males mated	18	20	20	20
Fertility index (%)	89	95	100	90
Relative testes weight (g)	3.86	3.888	3.963	3.788
Relative Epididymis weight (g)	1.503	1.503	1.521	1.457
Epididymal sperm concentration	Not examined			
% motile sperm	Not examined			
% abnormal sperm	Not examined			

Table 23: Reproductive toxicity endpoints examined in female rats from the 6-month toxicity study.

Table is created from data taken from the Sponsor's submission (Study No. TOX10768). Esketamine doses were 0, 0.9, 3 and 9 mg/rat (0, 4.5, 9 and 45 mg/kg/day for a 200 gram rat); N=20/dose.

Parameter	Estimated Dose, mg/kg/day			
	0	4.5	15	45
Absolute Body weight	300	298	296	304
Week 0-13 (dosing period)	173	174	171	181
Recovery period 0-3 weeks (g)	-1	-5	-3	-4
Absolute body weight on GD 14	355	350	349	357
Body weight gain GD 0-6 (g)	19	23	22	23
Body weight gain GD 6-14 (g)	37	31	31*	31*
% Regular estrous cycle of 4 days (wks 6-7)	95	100	100	95
% regular estrous cycle during recovery	85	90	100	75
Mating index for F (%)	95	100	100	100
Fertility index for F (%)	90	95	100	90
Corpora lutea (mean no. per animal)	16.6	15.7	15.8	16.7
Implantations (mean no. per animal)	15.8	14.2	14.8	15.9
Early Resorptions (mean no. per animal)	1.3	1.8	1.4	1.8
Live embryo	14.6	12.4	13.4	14.1
Preimplantation loss (%)	5.4	10.5	6.9	5.4
Postimplantation loss (%)	8.6	14.0	8.7	11.9

6.2.2 Dog Repeat-Dose Toxicity using Esketamine

Summary of studies shorter than 9 months in duration in dog:

A 3-month repeated dose IN toxicity study of JNJ-54135419-AAC in the beagle dog with a 1-month recovery period/Study No. 10524 (GLP). Male and female beagle dogs (4/sex/group) were administered esketamine HCl (Batch no. A12BD0277) doses of 0, 24, 48 or 72 mg equivalent/dog/day twice daily (7 days a week) for 3 months or at 72 mg equivalent/dog/day for 3 days each week for three months. The volume administered in this study was 0.4 mL/dog/day and given twice daily with 15 minutes in between dosing. This study had two controls: one to complement daily dosing and a second to complement intermittent dosing. The vehicle in this study was not the clinical vehicle but rather demineralized water adjusted to pH 4.5 ± 0.1 with NaOH or HCl. A 1-month recovery period was conducted in this study where 2/sex/group served as recovery animals for the two control and two high dose groups. Clinical signs were as expected for an anesthetic and included head shaking, transient vomiting, salivation, ataxia (up to 1 hr post dosing), slight tremors (on a few occasions for approximately the first 3 weeks of dosing), and/or increased/decreased activity following a dose response. Increased heart rate ($\uparrow 20$ bpm), without changes to other EKG parameters, was observed at all doses (including intermittent dosing) starting at 1 month of dosing into three months of dosing and the effect did not fully recover. Decreased (6%) potassium levels were observed starting at the MD after 1 month of dosing. There were no histopathological changes (signed path report is present). The NOAEL in this study is the high dose of 72 mg eq/dog/day ($C_{max}=791$ ng/mL, AUC 550 ng.hr/mL).

Reviewer's Comments: *This study is not conducted at MTD but rather at MFD. The high dose tested in this study has no exposure difference to the clinical dose and the vehicle tested here is not the clinical vehicle. Brain sectioning for nonrodents was not done as per Bolon et al., 2013 since this study was conducted prior to the publication of the position paper.*

Study title: JNJ-54135419-AAC: A 9-month toxicity study by the IN route in dogs

Study no.: TOX 10701
Study report location: (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: Oct 23, 2013
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: JNJ-54135419-AAC, Batch No. A12LD4238, 100.3%

Key Study Findings

- Clinical signs were transient and included salivation, increased activity, uncoordination characterized by unsteady gait, head drop and collision with the pen furniture, in a dose responsive manner within 15 minutes of drug administration for up to 1 hr post dose; lasting up to 2 hours at the high dose of 12 mg/kg/day. Increased activity and uncoordination persisted throughout the study at 12 mg/kg/day.
- Minimal to moderate olfactory epithelial atrophy in males ≥ 8 mg/kg/day but not in females. Focal minimal intra-epithelial accumulation of pale eosinophilic material in the dorsal meatus at level III of the nasal cavity was observed at all doses in males and at ≥ 8 mg/kg/day in females.
- The NOAEL in this study is 8 mg/kg/day based on clinical signs and nasal histopathology changes. At this dose the mean C_{max} and AUC at NOAEL are 1102 ng/mL and 576 ng.hr/mL for C_{max} and AUC, respectively. This is 6.3 times and 1.08 times the esketamine exposure for C_{max} and AUC at the MRHD of 84 mg/day (174 ng/mL for C_{max} and 530 ng.h/mL for AUC for esketamine).

Methods

- Doses: 0, 24, 48, and 72 mg eq/dog/day (estimated to be 4 mg/kg/day, 8 mg/kg/day, and 12 mg/kg/day for a male dog that is about 6 kg; to note, at the end of 9 months, male dogs weighed as much as 10 kg which means that the highest dose received was 7.2 mg/kg/day)
- Frequency of dosing: Twice daily dosing for 39 weeks
- Route of administration: IN instillation
- Dose volume: Each animal received 2 instillations in each nostril (100 ul/nostril) on each treatment day. Both daily instillations were given at 15 ±5 min interval (total dosing volume 200 uL per nostril or 400 uL per animal) on each day. Doses were given using a pipette syringe with attached approximately sized plastic tip.
- Formulation/Vehicle: Water for irrigation, citric acid, EDTA, pH 4.5 via NaOH and/or HCl
- Species/Strain: Beagle Dog
- Number/Sex/Group: 4/sex/dose
- Age: 5-6 months at initiation
- Weight: At the initiation of dosing, 5.3-7.6 kg for M and 5.5-7.2 kg for F.
- Satellite groups: TK was conducted in main study animals
- Unique study design: Neurological examination was conducted on all animals once during pretrial period then prior to the first dose on D1, and during weeks 14, 27, and 38. Neurological exam included a subjective observation of animal in its home pen of the functions controlled by the cranial nerves comprising: vision, eye movements, mastication, swallowing, facial expression, breathing, tongue, neck and head movement. Animals were removed from home pens to examine head movement/symmetry, head muscle tone and eye reaction (menace reflex). Also, proprioception and postural reaction tests such as proprioceptive position, hopping and visual and tactile placing reactions.
- Deviation from study protocol: None affecting the overall interpretation of results.

Observations and Results: Changes from Control

Parameters	Major findings
Mortality	No premature mortalities
Clinical Signs	Drug related clinical signs included salivation, increased activity, incoordination (unsteady gait, head drop, collision with pen furniture and/or tentative placement of limbs) occurring in a dose responsive manner within 15 minutes postdose lasting up to 1 hr post dose. At higher doses, the effects were seen up to 2 hours post dose. Increased activity and incoordination was observed for the first 6 weeks, 15/16 weeks and up to 34 weeks respectively at the LD, MD, and HD.
Body Weights	Unremarkable
Ophthalmoscopy	Unremarkable
ECG	Unremarkable
Hematology	<i>No drug related findings</i>
Clinical Chemistry	Changes were noted but none were clearly drug related or of large magnitude.
Urinalysis	Unremarkable
Gross Pathology	Unremarkable
Organ Weights	Unremarkable
Histopathology Adequate battery: Yes, for most tissues but not for the brain since sectioning <u>was not conducted as per Bolon et al., 2013.</u>	<p>Minimal to moderate olfactory epithelial atrophy was observed in 1/4 males at MD and 3/4 males at the HD. Additionally, there was a dose response finding of focal minimal intra-epithelial accumulation of pale eosinophilic material in the dorsal meatus at level III of the nasal cavity at all doses in males and at MDF (see Table 24).</p> <p>The pathology summary and the Sponsor's summary notes vacuolation in the sub-ependymal neuropil around the third ventricle in 1 MDM. Since this finding was not present in the HDM, the pathologist concludes that this is unrelated to drug administration. Peer review, by the Sponsor's pathologist, notes general agreement with overall interpretation and conclusion of the study. Peer review mentions "brain sections from all dogs were evaluated." But does not discuss any brain finding concerns.</p> <p>Additionally, cellular debris consisting of multinuclear cells and swollen spermatogenic cells present in the tubular lumen were seen in the testes and epididymides of 2 LDM and 2 HDM. The pathologist considers this finding to be part of normal background in young adult male dogs. This conclusion sounds reasonable. Individual animal observations showed minimal to mild congestion/inflammation of the urinary bladder in 1 HDM, 1MDF, and 1HDF. However, these were not noted in the pathology report suggesting that the pathologist considered these findings within background.</p>
Special Evaluation: Neuro exam	No drug related abnormalities were noted.

LD: low dose; MD: mid dose; HD: high dose.

Table 24: Summary of histopathology findings in dogs from the 9-month toxicity study

Table is excerpted from Study No. TOX10768, P. 904.

Group	Males				Females			
	1	2	3	4	1	2	3	4
Dose (mg eq./day)	0	24	48	72	0	24	48	72
No. animals examined	4	4	4	4	4	4	4	4
Brain (No. Examined)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Vacuolation, mild, subependymal, III ventricle	0	0	1	0	0	0	0	0
Epididymis (No. Examined)	(4)	(4)	(4)	(4)	-	-	-	-
Cellular debris, minimal	0	0	0	2	-	-	-	-
Nasal cavity (No. Examined)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(3)
Olfactory epithelial atrophy, dorsal meatus, level III	0	0	1	3	0	0	0	0
Minimal	0	0	0	1	0	0	0	0
Mild	0	0	0	1	0	0	0	0
Moderate	0	0	1	1	0	0	0	0
Eosinophilic accumulation, focal, olfactory epithelium, dorsal meatus, level III, minimal	0	1	2	2	0	0	2	2
Testis (No. Examined)	(4)	(4)	(4)	(4)	-	-	-	-
Cellular debris, minimal	0	2	0	2	-	-	-	-

Reviewer's Comments: This study is not conducted at MTD but rather MFD. Brain sectioning was not conducted as per nonrodent sampling mentioned in Bolon et al., 2013 therefore, fewer brain sections than noted in the current draft Major Depressive Disorder guidance. The finding of neuropil vacuolation around the third ventricle in 1 male at the mid dose is a concern due to the nature of its location which may have been missed at higher doses due to the lack of adequate brain sections. At the same time, the pathologist's evaluation of this case as incidental since it was not seen at HD, may be reasonable. The neurological exams conducted in this study were routine subjective observations and not a dedicated specialized exam.

6.3 General Toxicology Studies Using PMI-100

The general toxicity studies with PMI-100 have been reviewed by Dr. Baishali Kanjilal.

Toxicology studies of up to 3 months were conducted by the previous owner, Javelin Pharmaceuticals (b) (4) in which PMI-100 (100mg/mL (10% w/v) aqueous racemic ketamine solution containing 0.002% benzalkonium chloride as an antimicrobial preservative) was administered via IN route. These studies were conducted with varying volumes of the drug to reach nominal doses. JRD performed several tolerability/TK studies with IV racemic ketamine (aqueous solution, pH 4.5, NaCl until isotonic; without benzalkonium chloride) in nonpregnant and pregnant rats, pregnant rabbits, and dogs to be used as bridging studies to estimate exposure to esketamine from studies conducted with PMI-100. When IV racemic ketamine was administered, circulating esketamine exposures were approximately 40%, 50%, and 30% in nonpregnant rats, dogs, and pregnant rats, respectively (see Appendix Table 36, Table 37 and section 5.2). It is important to note that different vehicles and different routes of administration were pursued in the bridging studies as compared with Javelin's toxicology studies.

IN administration of ≥ 75 mg/kg/day and SC administration of ≥ 40 mg/kg/day in rats and IN administration of ≥ 75 mg/kg/day of PMI-100 in CD-1 mice produced transient clinical signs that were consistent with anesthetic doses of the drug such as wobbly gait, decreased activity, impaired mobility, circling, prostration, labored breathing, and ataxia. MTD was reached at an IN dose of 250 mg/kg/day and >80 mg/kg/day when administered subcutaneously in rats and at >250 mg/kg/day IN for mice. Three months of IN administration of PMI-100 in mice produced minimal to mild squamous metaplasia and acute inflammation of the nasal cavity at 90 mg/kg/day with NOAEL at 60 mg/kg/day (AUC=2,646 ng.hr/mL for racemic ketamine). A 3-month study in SD rats showed test article related minimal squamous metaplasia and hyperplasia of the nasal cavity and bladder/kidney calculi and/or minimal inflammation/mucosal hyperplasia of the urinary bladder at ≥ 60 mg/kg/day (AUC at LOAEL=9,489 ng.hr/mL and AUC at NOAEL=1,711 ng.hr/mL for racemic ketamine). After 28-day dosing in dogs, gross necropsy showed the following findings in the bladder: multiple red foci and reddened mucosa, minimal chronic inflammation, hyperplasia of the transitional epithelium, and hemorrhage at ≥ 20 mg/kg/day. The NOAEL for the findings in the bladder in this study is 4 mg/kg/day (AUC at NOAEL=64 ng.hr/mL for racemic ketamine). These findings did not fully reverse after 2 weeks of recovery.

Estimating 50% esketamine exposure at NOAEL, nasal cavity findings in mice and rats have a 1.5-2.5-fold safety margin to the clinical exposure at MRHD of 84mg/day. For bladder findings, there is a 1.5-fold safety margin in rats, however, in dogs there is no safety margin to the clinical AUC exposure at the MRHD.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title/Number: Bacterial Reverse mutation assay using racemic ketamine (PMI-100)*/Study No. AA34UN.503.BTL

Key Study Findings:

- PMI-100 (racemic ketamine; batch No. 46855) was negative for mutagenicity in bacterial cells in a valid Ames test.

GLP compliance: Yes

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 uvrA (pKM101); doses ≤ 5000 μ g/plate in water with Benzalkonium chloride; +/- S9.

Study is valid: Yes

***Reviewer's Comments:** Esketamine was never tested in an Ames assay but by definition, 50% of the racemate should be the S-enantiomer. Genotoxic studies were accepted as valid and negative during the IND phase of this drug.

Study title/Number: Bacterial Reverse Mutation Assay of (b) (4) (Impurity A; (b) (4)) / Study No. TOX 12465

Key Study Findings:

- JNJ (b) (4) (batch No. I16KB4361) was negative for mutagenicity in bacterial cells in a valid Ames test.

GLP compliance: Yes

Test system: *Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 uvrA (pKM101); doses ≤ 5000 µg/plate in DMSO; +/- S9.

Study is valid: Yes

Reviewer's Comments: (b) (4) is an impurity in the (b) (4) of esketamine. Based on the Sponsor's CMC section (2.3.S.3, table 4), there is no structural alert for this substance in silico and since it is negative in an Ames assay, its levels are controlled as a nonmutagenic impurity.

7.2 In Vitro Assays in Mammalian Cells

Study title/Number: In vitro mammalian cell gene mutation test (L5178Y/TK=-/ Mouse Lymphoma assay) / Study No. AA34UN.704.BTL

Key Study Findings:

- PMI-100 (racemic ketamine; batch no. 46855) was negative for clastogenicity in mouse lymphoma cells without activation in both a 4-hr and 24-hr exposure and positive with S9 with a 4-hour exposure in a valid in vitro mammalian cell gene mutation test.

GLP compliance: Yes

Test system: L5178Y mouse lymphoma cells; doses ≤ 125 µg/mL in water +S9 and ≤ 75 µg/mL in water.

Study is valid: Yes

Study title/Number: JNJ-54135419: In vitro micronucleus test in human TK6 cells/TOX10413

Key Study Findings:

- Esketamine HCl (batch no. 0142911P) was negative for clastogenicity in human lymphocytes in the absence of S9 activation in both a 4-hr and 24hr exposure and positive with S9 with a 4-hour exposure in a valid in vitro micronucleus assay.

GLP compliance: No

Test system: Human TK5 lymphoblastoid cell line; doses ≤ 54.6 µg/mL in DMSO +S9 and ≤ 500ug/mL µg/mL in DMSO -S9

Study is valid: Yes

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title/Number: PMI-100: Mammalian Erythrocyte Micronucleus test/AA34UN.123.BTL

Key Study Findings:

- PMI-100 (batch No. 46855) was not clastogenic in a valid *in vivo* micronucleus assay after IP injections of ≤ 150 mg/kg plasma concentrations 34-times clinical exposure.
- Mortality occurred at 400 mg/kg. Convulsions observed at 150 mg/kg.

GLP compliance: Yes

Test system: rat, bone marrow micronuclei; single intraperitoneal injections of 37.5, 75 or 150 mg/kg; bone marrow was collected at 24 and 48 hrs after treatment.

Study is valid: Yes

7.4 Other Genetic Toxicity Studies

In Vivo Comet Assay in Rodent (DNA damage)

Study title/ number: In vivo Comet assay in liver cells following intravenous infusion of JNJ-54135419-AAC in rats/Study No. TOX10530

Key Study Findings:

- Esketamine HCl (batch no. A12BD0277) was not genotoxic in a valid *in vivo* Comet assay after IV infusions of ≤ 50 mg/kg which is 11 times the AUC exposure at MRHD of 84 mg.

GLP compliance: Yes

Test system: rat (7-13 weeks old) liver samples; 30 minute IV infusions of 10, 30 or 50 mg/kg for 3 consecutive days in water, pH 4.5.

Study is valid: Yes

Reviewer's Comments: This study did not test the clinical vehicle containing citric acid and EDTA. The TK data were determined in a separate study (TOX 12321 (non-GLP) using batch no A12LD4238 of esketamine HCl). AUC for the esketamine was 5860 ng.hr/mL but Cmax was not determined. For the metabolite (JNJ64115922) AUC_{0-t} was 548 ng.hr/mL and Cmax 74.5 ng/mL.

8 Carcinogenicity

8.1 Rat Carcinogenicity

Study title: JNJ-54135419-AAC: A 104-week carcinogenicity study by the IN route in rats/ Study No.673195

Male and female S-D rats (65/sex/group) were IN administered (via instillation; 50ul/rat) mg/rat dose of esketamine HCl [in water for irrigation with (b) (4) citric acid and (b) (4) EDTA (pH 4.5)] estimated at 0 (saline), 0 (vehicle), 4.5, 15, and 45 mg/kg/day for a 200 gram rat. Males were terminated at 104 weeks and females were terminated at 105 weeks after a drug holiday from 102-105 weeks. Numerical incidence for Leydig cell adenoma was 1, 0, 1, 1, and 3, respectively, for saline, vehicle, LD, MD, and HD. However, the p value (p=0.04) did not reach Executive Carcinogenicity Assessment Committee's (E-CAC) criteria for statistical significance for common tumors. Even in the absence of statistical significance, this Reviewer had considered this tumor finding to be relevant considering the distribution of NMDARs in the male reproductive system and delays in mating observed in the Segment I study. The administration of drug in this study (mg/rat) where dosing was done without body weight correction and the observed body weight decreases at the HD may have impacted tumor formation. There were no other treatment related increases in tumor incidence in either sex according to E-CAC criteria for statistical significance. Two HDM in this study had non-neoplastic lesions of the submucosa of the bladder which could be drug-related considering ketamine's known effect on this organ in rats and dogs from Javelin's toxicity studies and in humans (Skeldon and Goldenberg, 2014). The mean AUC exposure at the highest dose of 45 mg/kg/day is 309 ng/mL which is 0.58-times the clinical exposure at the MRHD of 84 mg.

8.2 Transgenic Mouse Carcinogenicity

Study title: JNJ-54135419: 26 week repeated dose subcutaneous carcinogenicity study in Tg.rasH2 mice/Study No. AD64VG.7S8R.BTL

Male and female Tg. ras H2 mice (25/sex/dose) were subcutaneously injected doses of esketamine HCl [in water with (b) (4) citric acid and (b) (4) EDTA (pH 4.5)] at 0 (saline), 0 (vehicle), 10, 25, and 75 mg/kg/day for 26-weeks. Due to high mortality, ECAC advised the Sponsor to provide a drug holiday of 1-week at the high dose and lower all doses to 5, 10, and 30 mg/kg/day during the interim phase of the study. The Sponsor implemented the drug holiday and lowered the high dose to 40 mg/kg/day but did not lower doses in the other two dose groups. FDA statistical analysis showed a statistically significant increase in mortality at the mid and high doses in male mice. The overall number of male mice surviving until the end of the study was adequate. There were no treatment related increases in tumor incidence in either sex per the E-CAC criteria for statistical significance. The mean AUC exposure at 40 mg/kg/day is 3,095 ng/mL which is

5.8-times the clinical exposure at the MRHD of 84 mg/day.

Reviewer's Comments: The full review of the carcinogenicity studies and E-CAC meeting minutes can be found under the IND 114345.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: JNJ-54135419-AAC: IN Fertility study in the rat

Study no.:	TOX 10529
Study report location:	Archives of JRD
Conducting laboratory and location:	 (b) (4)
Date of study initiation:	January 23, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-54135419-AAC, A12BD0277, 99.7%

Key Study Findings

- A dose dependent decrease in body weight gain (15%) was seen in males at doses ≥ 15 mg/kg/day while a dose dependent increase (10-16 %) was seen in females prior to mating at doses ≥ 4.5 mg/kg/day. This increase reversed during the gestational period (GD1-7).
- Estrous cycle irregularities were observed at the high dose of 45 mg/kg/day and an increase in time to mate was observed at ≥ 15 mg/kg/day. This delay did not affect overall mating and fertility indices.
- The overall NOAEL in this study (based on no change in mating and fertility indices) is 45 mg/kg/day which is 0.6* times the esketamine exposure for clinical AUC at the MRHD of 84 mg/day (530 ng.h/mL for AUC for esketamine).
*Exposure data is the average of male and female rats based on week 26 of the 6-month chronic study.

Methods

Doses:	0, 0.9, 3, and 9 mg eq/rat/day (estimated to be 0, 4.5, 15, and 45 mg/kg/day of esketamine for a 200 gram rat)
Frequency of dosing:	Once daily
Dose volume:	All treatment groups received the same volume (50uL/day; 25ul/nare) with varying drug concentration in vehicle.
Route of administration:	IN instillation
Formulation/Vehicle:	Demineralized water adjusted to pH 4.5 ± 0.1 with NaOH or HCl
Species/Strain:	Rat/SPF Sprague Dawley (CrI: CD)
Number/Sex/Group:	22/sex/dose; M rats were 53-70 days old at dosing; F rats were 49-63 days old at dosing
Satellite groups:	None
Study design:	Males were dosed for 4 weeks prior to pairing, during pairing and up to termination. Females were dosed for 14 days prior to pairing, during pairing and up to Day 7 of pregnancy.
Deviation from study protocol:	None affecting interpretation of study results

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	Transient ataxia observed in 2/22 MDM&F and 11/22 HDM and 21/22 HDF in the early part of the dosing period. Salivation was also observed in most MD and HD rats. No clinical signs at the LD.
Body Weights	<p>Premating period: A significant decrease in body weight gain of 15% was observed in males at the MD and HD during the dosing period prior to mating (see Table 25). A statistically significant increase of >70% in body weight gain was observed in all dosed females weeks 2-4. Body weight changes were independent of food consumption.</p> <p>Mating/Gestation: A decrease in body weight gain of 18% was observed in males at MD and HD during the mating period. A dose dependent decrease in body weight gain (10-16%) was observed in all treated females from GD 1-7, reaching statistical significance in MD and HD. This finding reversed on GD 8-13 with an observed increase in body weight gain (>10%) in treated females. These changes were independent of food consumption (see Table 26).</p>
Necropsy Findings	No drug related effects.

<i>Mating and Fertility and Pregnancy parameters</i>	<p>Estrous cycle: A drug effect on estrous cycles was observed at all doses in this study. While all the CF had cycles of ≤ 4 days, 2/22, 3/22, and 6/22 females at LD, MD, and HD, respectively, were either acyclic or had irregular estrous cycles (i.e. some longer and some shorter than 4-5 days) (see Table 26). Percent of females with regular cycles were 100%, 90%, 86%, and 73% in controls, LD, MD, and HD respectively. Of those that had irregular cycles, 7%, 6%, and 15% had cycles of greater than 5 days at LD, MD, and HD, respectively (see Table 26).</p> <p>Mating and Fertility: Compared with concurrent (2.23) and HCD (2.58), MD and HD had an increase in mean days to mate of 2.82 and 2.82, respectively. Specifically, a total of 5 pairs (3/22 in MD and 2/22 in HD) failed to mate in this study within the first 5 days. Three of these pairs (2 at the MD and 1 at the HD) mated within a 10-day period and were pregnant at necropsy. In the other two cases (one from each dose), the pairs were separated and the females were paired with a male from the same group which had previously mated, finally resulting in successful mating on day 13 and day 17. There was no drug effect on overall copulation or fertility rate.</p> <p>Pregnancy: Pre-implantation loss was higher in all treatment groups compared to concurrent controls (2.8%, 12.35%, 6.02%, 8.95% in Control, LD, MD, HD, respectively). The values in LD and HD are higher than mean HCD values (6.56%). However, examining individual data, it appears that this difference is driven by a few dams. The postimplantation loss and resorptions at the HD was higher than HCD but not from concurrent control. There were no other notable changes to fertility or litter parameters.</p>
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C: Control; LD: low dose; MD: mid dose; HD: high dose; HCD: historical control data

Table 25: Mating and fertility parameters in male rats in the fertility and early embryonic development study

Parameter	Estimated doses (mg/kg/day)				HCD
	0	4.5	15	45	
Body weight gain (Week 1-4) (g)	78.5	75.4	65.9*	67*	
Body weight gain (Week 4-8) (g)	127.7	124.0	107.5*	109.2*	
No. of males that were paired	22	22	22	22	
No. of males mated	22	22	21	21	
Copulation index (%)	100	100	95.5	95.5	100
Mean number of days prior to mating	2.23	1.95	2.82	2.82	2.58
No. of fertile males	22	22	21	21	
Fertility index	100	100	95.5	95.5	96.3

HCD: historical control data, *P<0.05

Table 26: Mating and fertility parameters in female rats in the fertility and early embryonic development study

	Estimated doses (mg/kg/day)				HCD
	0	4.5	15	45	
Body weight gain (Week 2-4) (g)	8.9	15.2*	16.6*	15.1*	
Body weight gain (GD 1-7) (g)	37.3	33.7	31.2*	32.2*	
Body weight gain (GD8-13) (g)	29.4	32.4	34.2*	32.4	
# of rats (regular estrous cycles)	22	20	19	16	

# of rats (Irregular estrous cycles i.e. other than 4-5 day cycles)	0	0	0	3	
# of rats (Acyclic)	0	2	3	3	
% of F with < 4 days	0	0	0	1	
% of F with 4 days [§]	100	93	94	84	
% of F with 5 days	0	4	2	6	
% of F with 6-10 days	0	0	0	6	
% F with >10 days	0	3	4	3	
Embryo-fetal parameters					
Corpora lutea (mean no. per animal)	15.7	16.2	16.5	17.6*	16.81
Implantations (mean no. per animal)	15.6	14.7	16	16.2	15.6
Resorptions (mean no. per animal)	1.27	0.50*	0.95	1.14	0.83
Preimplantation loss (%)	2.8	12.35	6.02	8.95	6.56
Postimplantation loss (%)	8.04	3.44	5.93	7.12	5.64

HCD: historical control data

[§]This % of rats does not include those that were acyclic.

* P<0.05

Reviewer’s comments: *Effect of vehicle on fertility was not tested in this study. The Sponsor attributes increase in time to mate in this study to female estrous cycle irregularities. However, due to mating of treated rats (i.e. both males and females were treated), this mating delay could be mediated by an effect of drug on one sex or both sexes. However, overall mating and fertility indices (i.e. total number of rats mated and found pregnant) are not affected. The Sponsor notes that estrous cycle irregularities observed in this study is consistent with published literature on ketamine’s known effect on suppression of circulating progesterone, testosterone, and 17β estradiol.*

9.2 Embryonic Fetal Development

9.2.1 Rat Embryonic Fetal Development

Study title.: A developmental toxicity (Segment II) study in Sprague-Dawley rats with PMI-100

Study no.:	3542.11
Study report location:	 (b) (4)
Conducting laboratory and location:	
Date of study initiation:	October 11, 2001
GLP compliance:	Yes; except for mating conducted in a non-GLP lab
QA statement:	Yes
Drug, lot #, and % purity:	Ketamine HCl, Lot No. 42727, 100.6%

Key Study Findings

- Maternal toxicity NOAEL is the 50 mg/kg/day based on the severity of clinical signs related to anesthetic properties of the drug at 150 mg/kg/day. A drug related embryofetal toxicity is not evident in this study.
- Deviation in concurrent control data as compared to HCD is attributed to large and varying volumes of instillation used in this study.
- Assuming that 50% of circulating drug species is esketamine when PMI-100 is administered, there is no evidence of teratogenicity in rats at a dose of up to 12-fold the MRHD (Cmax=174 ng/mL and AUC=530 ng.h/mL for esketamine in the clinic) of 84 mg/day.

Methods

Doses:	0, 15, 50, and 150 mg/kg/day
Frequency of dosing:	Once daily
Dose volume:	Varying dosage volumes of 1500, 150, 500, and 1500 uL/kg/day, respectively for doses of 0, 15, 50 and 150 mg/kg/day.
Route of administration:	IN instillation
Formulation/Vehicle:	Water for injection
Species/Strain:	Crl:CD (SD)IGS BR rats
Number/Sex/Group:	25F/group
Satellite groups:	6F/dose were assigned for TK
Study design:	Time mated female rats were dosed from GD6 through GD17. Fetuses were delivered by C-section on GD20 and examined.
Deviation from study protocol:	None affecting the interpretation of results

Observations and Results

Parameters	Major findings
Mortality	No drug related effect.
Clinical Signs	Drug related anesthetic effects such as salivation, wobbly gait and impaired mobility, and/or decreased activity were observed at the MD and HD. In addition, ocular discharge, dark material around nose, rough coat, and nystagmus were also observed in MD and HD.
Body Weights	Absolute body weight (uncorrected) was not significantly different between control and treated rats (i.e. not >10%). Body weight gain (↓12%) at the HD between GD 6-17 and reached statistical significance. Corrected maternal body weight change was ↓14% and ↓9.6% for MD and HD, respectively.
Necropsy findings Cesarean Section Data	Corpora lutea, implantation sites, live fetuses, # of male and female pups, and postimplantation loss were lower in this study compared with HCD (See Table 27). There was a drug effect following a dose response in the mean number of male and female pups (53% M in C vs. 48.5% in HD; 46.7% F in C vs. 51.5% in HD). These numbers were out of the range for the HCD of the lab. Fetal body weight on the other hand was higher in concurrent control than HCD.

Necropsy findings Offspring	A tabular summary of malformations and variations that appear to change with treatment provided in Table 28 below. For many of the endpoints, concurrent control appears to have an increased incidence compared with treatment and HCD.
Toxicokinetic	See tabular summary of TK data in section 5.2. Tmax was reached within 15 minutes. Drug accumulation appeared to have occurred based on the doubling of AUC exposure by GD 17.

LD: low dose; MD: mid dose; HD: high dose; HCD: historical control data

Table 27: Cesarean section findings from rat embryo-fetal development study with PMI-100

Segment 2 study parameters (PMI-100) (varying volumes)					
Dose (mg/kg/day)	0	15	50	150	HCD range
Body weight gain in g (GD 6-17) F0	67	72	61	59*	
Corrected body weight change in g	73	74	63*	66	
Corpora lutea (per animal)	13	14.2	14.3	13.7	16.3-19.3
Implantation sites (# per animal)	11.6	13	13.2	12.3	15.1-18.2
Pre implantation loss (# per animal)	1.4	1.2	1.1	1.5	0.7-2.3
Live fetuses (per animal)	11.2	12.5	12.5	11.7	13.9-17.3
Males (mean %)	53	51	46.7	48.5	44-49
Females (mean %)	46.7	48.9	53.3	51.5	49-51
Postimplantation loss(# per animal)	0.4	0.5	0.7	0.5	0.6-1.6
Fetal body weight (g)	4	3.8	3.8	3.7	3.5-3.8

Table 28: Offspring necropsy findings from rat embryo-fetal development study with PMI-100

Segment 2 study parameters (PMI-100) malformations (varying volumes)					
Dose (mg/kg/day)	0	15	50	150	HCD range
% with visceral malformations fetal (litter)	10 (26)	4 (20)	3 (12)	3 (8)	0-0.6 (0-4.5)
Total % with malformations fetal (litter)	10 (30)	4 (20)	3 (16)	4 (12)	0-1 (0-8)
Seg 2 study parameters (PMI-100) variations					
% with Sternebrae malaligned slight/moderate fetal (litter)	16 (48)	15 (56)	17 (56)	22 (54)	11-44 (42-96)
% with Reduced ossification of the 13 th rib fetal (litter)	0.7 (4)	0 (0)	0.6 (4)	1.4 (8)	0-8 (0-4)

Reviewer's comments: This study did not test the effect of the commercial drug substance or vehicle on embryofetal parameters. The methodology used in this study (i.e. the large amounts and varying instillation volumes) adds additional stress on the maternal dams and makes the interpretation of the results difficult.

9.2.2 Rabbit Embryonic Fetal Development

Study title.: A developmental toxicity (Segment II) study in New Zealand white rabbits with PMI-100

Study no.:	3542.13
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 14, 2001
GLP compliance:	Yes; except for mating conducted in a non-GLP lab
QA statement:	Yes
Drug, lot #, and % purity:	Ketamine HCl, Lot No 42727, 100.6%

Key Study Findings

- Maternal toxicity characterized by decreased body weight gain and feed consumption occurring at ≥ 30 mg/kg/day.
- Developmental toxicity characterized by skeletal malformations and observed at ≥ 30 mg/kg/day.
- The NOAEL for maternal and embryofetal toxicities is 10 mg/kg/day which is estimated to be 0.3* times the AUC exposure at MRHD.

*Ketamine exposures could not be determined at IN 10 mg/kg/day dose in the Segment II study in rabbits. The esketamine estimate here is derived from PK bridging data with 8mg/kg/day IV ketamine assuming linearity of exposure (at 10 mg/kg/day of ketamine administration, AUC exposure for esketamine is 148 ng.hr/mL). Therefore, the esketamine exposure here may be overestimated.

Methods

Doses:	0, 10, 30 and 100/50 mg/kg/day
Frequency of dosing:	Daily
Dose volume:	varying IN volumes of 1000/500, 100, 300 and 1000/500 uL/kg/day respectively for 0, 10, 30 and 100/50 mg/kg/day
Route of administration:	IN instillation
Formulation/Vehicle:	Sterile water for injection
Species/Strain:	New Zealand white rabbits
Number/Sex/Group:	20F/group
Satellite groups:	TK: 3F/group
Study design:	Pregnant rabbits were dosed from GD6-GD18. The fetuses were delivered by C-section on GD29 and

examined.

Deviation from study protocol: Yes. Due to unexpected mortality, the high dose was lowered to 50 mg/kg/day by lowering volume to 500ul/kg/day (in both HD and control groups) after the first 5 days of dosing. Therefore, the does received the lower dose starting on GD 11 until the remainder of the dosing period.

Observations and Results

Parameters	Major findings
Mortality	Five HDF were found dead on GD 7/8. Two HDF from the TK group was added to the main study to meet the minimum requirement for the study.
Clinical Signs	One MDF aborted on GD25 and 1 HDF delivered early on GD 28. Dose dependent clinical signs as expected for an anesthetic drug such as wobbly gait and decreased activity were observed at both MD and HD. In addition, wetness around nares, salivation, dilated pupils, and ocular discharge were noted in these F. Lateral recumbency, shallow breathing, eyes dark in color were all noted in HDF. One LDF had wetness around nares. In premature decedents or those that aborted early, the clinical signs included decreased activity, decreased food consumption, lateral recumbency, wobbly gait, shallow breathing, eyes dark in color, reddish nasal discharge, and/or soft/mucoid stools.
Body Weights	At the end of the dosing period, HD dams had a 4% decrease in absolute body weight which reached a statistical significance. A↓ in mean body weight gain of 12%, 50%, and 57% was observed in LD, MD, and HD; respectively, compared to control, during the dosing period (GD 6-18). This difference reached statistical significance at both the MD and HD. When dosing was stopped (GD 18-29) this effect reversed for LD and MD but not for the HD dams (see Table 27). Gravid uterine weights were not significantly different from one another. When corrected maternal body weight (maternal body weight change-gravid uterine weight) was evaluated, a significant effect of drug on maternal body weight was also observed for the MD group (i.e. ↓33%) and all the weight gain at the HD was from gravid uterine weight (see Table 27) .
Necropsy findings Cesarean Section Data	Mean number of post implantation loss was >2x in all dose groups compared with control but not strictly dose-dependent (i.e. 0.2, 0.4, 1, and 0.7 for C, LD, MD, and HD, respectively) and within HCD (see Table 29).
Necropsy findings Offspring	External malformations such as anal atresia and/or short tail were present in a total of 3 fetuses in 2 litters at the HD. Fused kidney, a visceral malformation was present in 1 HDF. The litter incidences for skeletal malformations at the high dose were as follows: intraparietal bipartite (1/15), jugals misshapen (1/15), cervical vertebral anomaly (2/15), vertebral anomaly with/without rib anomaly (3/15), thoracic centrum anomaly (1/15), fused ribs (1/15), and costal cartilage anomaly (1/15). Total skeletal malformations, were increased based on fetus and litter number at the MD and HD from concurrent controls (see Table 30). Total number of malformations was increased at all doses and above the HCD for the lab.
Toxicokinetic	See tabular summary of TK data in section 5.2. Tmax was reached within 15 minutes. There was no drug accumulation in this study.

LD: low dose; MD: mid dose; HD: high dose

Table 29: Caesarian section findings from rabbit embryo-fetal development study with PMI-100

Segment 2 study parameters (PMI-100) (varying volumes) rabbit					
Dose (mg/kg/day)	0	10	30	100/50	HCD range
Body weight gain (D 6-D18) (g)	233	204	116*	100*	---
Body weight gain (D18-29)	241	237	299	209	---
Gravid uterine weight (g)	507	502	526	515	---
Corrected maternal body weight change (g)	124	129	82	-1	---
Pregnancy rate (%)	95	100	85	75	70-100%
Corpora lutea (mean#/animal)	9.5	9.7	10.4	10	8.6-10.4
Implantation site (mean #/animal)	8.3	8.8	9.8	9.3	5.7-10
Preimplantation loss (mean number per animal)	1.2	0.9	0.6	0.7	0.4-2.9
Postimplantation loss (mean number per animal)	0.2	0.4	1.0	0.7	0.2-1.1
Early resorption mean # per animal)	0.1	0.2	0.6	0.3	0.2-0.9
Late resorptions mean# per animal)	0.1	0.3	0.4	0.3	0-0.4

*= $p < 0.05$ **Table 30: Offspring necropsy findings from rabbit embryofetal development study with PMI-100**

Segment 2 study parameters (PMI-100) (varying volumes) rabbit					
Dose (mg/kg/day)	0	10	30	100/50	HCD range
Short tail (fetal) (%)	0	0	0	2%	---
Short tail (litter) (%)	0	0	0	13%	---
Cervical vertebral anomaly (fetal) (%)	0	0	0	3%	0-1.91%
Cervical vertebral anomaly (litter) %	0	0	0	13%	0-11.76%
Vertebral anomaly with/without rib anomaly (fetal) (%)	1.2%	0.6%	2%	3%	0-2.33%
Vertebral anomaly with/without rib anomaly (litter) (%)	5%	5%	12%	20%	0-15%
Fetus with skeletal malformations (%)	3%	3%	8%	8%	0-6%
Litters with skeletal malformations (%)	16%	25%	47%	40%	0-31%
Total malformations (fetal)	4.5%	4.2%	11%	9%	0-6.38%
Total malformation (litter)	26%	35%	58%	46%	0-31%

9.3 Prenatal and Postnatal Development

Study title: Esketamine hydrochloride (JNJ-54135419): study for effects on pre- and postnatal development in the Crl: CD (SD) rat by IN administration

Study no.:	TOX10766
Study report location:	(b) (4)
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 23, 2013
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	JNJ-54135419-AAC, A12LD4238, 100.3%

Key Study Findings

- Maternal toxicity (F0 dams) occurred at ≥ 15 mg/kg/day based on a transient decrease in body weight gain and decrease in food consumption from GD 6-10 but reaching control levels at the end of the gestation period. Adverse clinical signs were observed in maternal dams throughout gestation and lactation at ≥ 45 mg/kg/day.
- A dose response delay in Preyer response reflex was observed independent of a drug effect on body weight in this study. On D14, only 69.4%, 66.1%, and 52.8% at LD, MD, and HD reached this development milestone compared with 84.3% in controls. This effect normalized by PND19.
- In the postweaning period, motor activity was decreased at ≥ 15 mg/kg/day without an effect on learning, habituation, sexual development, mating, and fertility.
- The NOAEL for maternal toxicity is 4.5 mg/kg/day, no NOAEL for pup physical development during the pre-weaning period and 4.5 mg/kg/day for post weaning development which is 0.07-times[†], <0.09-times[‡], and 0.09-times[‡] times; respectively, the human exposure ($C_{max}=174$ ng/mL and $AUC=530$ ng.h/mL for esketamine) at the MRHD of 84 mg/day.

[†] There were no TK data from maternal dams available in this study. Exposure data were taken from female animals in the 2-year carcinogenicity study in rats.

[‡] PND 4 AUC values are used for exposure margin calculation in F1 offspring (see table 38 in the Appendix).

Methods

Doses: 0, 0.9, 3, and 9 mg eq/rat/day (estimated to be 0, 4.5, 15 and 45 mg/kg/day of esketamine for a 200 gram rat).

Frequency of dosing: daily

Dose volume: All treatment groups received the same volume (50uL/day; 25ul/nare) with varying drug concentration in vehicle.

Route of administration: IN instillation

Formulation/Vehicle: Pyrogen free water/water for injection with (b) (4) citric acid and (b) (4) EDTA (pH 4.5)

Species/Strain: Crl: CD (SD) rat

Number/Sex/Group: 22/sex/dose

Satellite groups: No satellite group; TK was conducted in F1 offspring on PND4 and PND12

Study design: Pregnant rats (F0 dams) were dosed from gestational day (GD) 6 to day 20 of lactation, inclusive. Blood was collected from 4 F1 pup culls on PND 4 and 12 but not from F0 dams. In addition to routine parameters, Preyer response reflex was assessed daily from D9 of age until achieved or D19 of age in the F1 offsprings. Brain weights were taken for F0 dams and F1 offsprings. Brain histopathology, targeting cingulate gyrus/retrosplenial cortices, was done on 5 randomly selected F0 dams, pups on PND4, PND12, and PND21, and adults from F1. Preyer response testing was done as follows: individual pups were gently restrained in the hand of the observer. With background noise kept to a minimum, a dog clicker was used to generate a sound of (75-85dB) approximately 8cm behind the pup and assessed for response. Reactions such as ear or head twitching or jumping were deemed a positive response and the pup was considered to have passed the test.

Deviation from study protocol: None affecting interpretation of study results

Observations and Results

Generation	Major Findings
F0 Dams	<p><u>Mortality</u>: Two deaths: 1CF found dead after parturition; 1LDF euthanized humanely on D23 due to difficulties in parturition.</p> <p><u>Clinical signs</u>: At the HD, unsteady gait, salivation, and decreased activity were observed from first day of dosing (GD6) until D18 of lactation in nearly all animals; one female had circling behavior</p>

	<p>on D4 of lactation. These signs were observed 5-15 min after dosing and were not observed at the end of the working day, except for salivation. These signs decreased in incidence and severity at lower doses.</p> <p><u>Body weight</u>: There were no changes to absolute body weights in this study but there were statistically significant decreases in body weight gain at MD and HD (35% and 41% lower than control, respectively) initially from GD 6-10. This decrease was accompanied by a decrease in food consumption at the MD and HD during the same time period. The decrease in body weight returned to control levels by the end of gestation. Absolute body weight and body weight gain were less than 10% from D1 to D 21 of lactation.</p> <p><u>Uterine content</u>: A slight increase in gestational length is observed at the HD (i.e. 64% of control animals delivered by GD22 in comparison to only 50% at the HD). However, all F had delivered by GD 23 (No HCD provided for gestational length). There were also no significant differences in pup sex ratios, pup birth weight, or pup body weight gain.</p> <p><u>Necropsy/Histopathology</u>: H&E staining in brain sections at three levels targeting the cingulate gyrus/cores of the retrosplenial cortices showed no salient changes.</p>
F1 Generation	<p><u>Survival</u>: No drug effect</p> <p><u>Clinical signs</u>: No drug effect</p> <p><u>Body weight</u>: No drug effect</p> <p><u>Physical development</u>: There was a dose-dependent delay in age of attainment of Preyer response reflex where it was tested from PND9-19. By D14 (M&F combined), 84.3% of control vs. 69.4, 66.1, and 52.8% had attained this reflex at LD, MD, and HD (no HCD provided for this parameter) (Table 31). The mean age for this reflex reached a statistical significance at the HD (14.5 days vs. 13.8 days in control). This delay was not correlated with a concurrent decrease in body weight at the same age. All pups attained Preyer reflex by D19. There was no drug effect on other preweaning examinations like pupil reflex, auditory startle response or air righting day.</p> <p><u>Neurological assessment</u>: After weaning, pups (N=20/sex/dose) were tested on motor activity (PND 22), prepulse inhibition of auditory startle response (PND24/25), and Morris Water (MW) maze (PND 31). High beam breaks (rearing activity) was nonstatistically decreased in F1 offspring by 22% in both MDM and HDM and low beam break (ambulatory activity) was decreased by 12% at HDM. Effect is not clear in F. In MW maze, dose dependent decreased number of sector entries (30%) and decreased trial time (30%) in HDF, reaching statistical significance for the former on D2. No HCD provided for postweaning neurological assessment.</p> <p><u>Reproduction</u>: There was no esketamine effect on balanopreputial separation or vaginal opening. No effect on precoital interval, mating performance and fertility in F1 rats.</p> <p><u>Organ weight</u>: Brain weights were measured in F1 pups. There were decreases in absolute brain weight but not when corrected for body weight.</p> <p><u>Necropsy/Histopathology</u>: No brain histopathological changes were noted in 5 randomly selected pups on PND 4, 12, 21, and adults from F1 generation with H&E stain.</p> <p><u>Toxicokinetics</u>: See Table 41 in the Appendix for exposure data from the F1 pups on PND 4 and PND 12.</p>
F2 Generation	<p><u>Litter data</u>: No effect on drug treatment on F0 generation observed in F2 litter parameters such as mean corpora lutea, implantations, resorptions, live embryos, and pre/post implantation losses.</p>

Table 31: Effects of esketamine administration on parameters examined in the pre- and post-natal development study in rats.

PPND study parameters					
Dose (mg/kg/day; estimated)	0	4.5	15	45	HCD (mean)
Body weight gain (GD 6-10)F0	17	14	11**	10**	—
Body weight gain (GD 6-20) F0	114	118	113	112	—
% passed Preyer reflex on D14 F1	84.3	69.4	66.1	52.8	—
Total Locomotor activity (high beam) (males)F1	182.9	172.5	142.8	141.8	—
Total Locomotor activity (low beam) (males)F1	626.6	611	614	545.7	—
Total Locomotor activity (high beam) (females) F1	177.7	153.8	141.5	171.2	—
Total Locomotor activity (low beam)(females) F1	617	550	517.6	599.4	—
Preimplantation loss F2 (%)	5	6.7	4.8	6.2	7.2
Postimplantation loss F2 (%)	8.3	7	4.5	6.3	6.2

Reviewer's comments: The drug administration as conducted in this study (mg/rat) would suggest that maternal dams received less exposure to esketamine as their body weight increased. Even in the presence of less than optimal dosing paradigm, there was a dose dependent delay in Preyer response reflex (sound-induced movement of the external part of the ear) suggesting that the drug causes an initial delay in sensorimotor development which normalizes over time. Preyer reflex is the elicitation of startle response to auditory stimuli and has been widely used for hearing in rodent. This test is effective for identifying profound sensorineural hearing loss and effect on brainstem response in experimental animals. It is well known that NMDA receptor antagonists like ketamine and MK-801 impair EEG/ERG readout and therefore mimic the EEG deficits observed in patients with schizophrenia. The finding of normalization of the delay by PND 19 and lack of drug effect on PPI when tested on PND24/25 would suggest that the drug does not have a long-lasting consequence on sensory gating in F1 offspring.

10 Special Toxicology Studies

Other Toxicology Studies

The Sponsor of this NDA has submitted a total of 10 neurotoxicity studies including dose range finding studies, those for PMI-100, those for IN esketamine, and those examining neuronal apoptosis in juvenile animals. These studies are: TOX 10820: tolerability study for dose selection for the pivotal 2-week neurotox study, TOX 10950: 2-week repeat dose IN neurotoxicity study using esketamine, TOX12438: range finding study in juvenile rats, TOX13050: 2-week intermittent dosing neurotoxicity study using esketamine in juvenile rats, TOX11374: single dose neurotoxicity study in female rat using esketamine, TOX 11334: single dose TK study using esketamine, Study No. 3542.9A: acute neurotoxicity study in rats using PMI-100, Study No. 3542.9B: 28-day neurotoxicity study using PMI-100, TOX JIR00009: PMI-100 vehicle effect on neurotoxicity, and TOX10415: single dose neurotoxicity study using IN

esketamine in rats. The neurotoxicity studies in juvenile rats will not be reviewed under this NDA since the indication here is not relevant for pediatric population. For Olney lesion toxicity evaluation, the appropriate sacrifice time points are 2-4 hours after the Tmax and 3-days after a single dose for neuronal vacuolation and necrosis, respectively, for most drugs. Of the studies conducted in adult rats, only valid studies are summarized below.

Esketamine HCl (JNJ 54135419-AAC): Single dose neurotoxicity study by IN administration to female rats/ Tox 10415 (GLP; signed pathology report)

Adult female Crl:CD (SD) rats (12-14 weeks of age; 10F/dose/timepoint) were administered esketamine hydrochloride (Batch No. 0142911P) doses of 0 (pyrogen free water pH 4.5), 0.9, 3, and 9 mg/rat (0, 4.5, 15 and 45 mg/kg/day of esketamine for a 200 gram rat) via IN instillation (50 ul/rat). The sacrifice time points in this study were 4 hours and 7 days post dose. The MK-801 positive controls showed minimal to moderate neuronal vacuolation at the 4-hr time point but were euthanized prematurely due to humane reasons on D2. Therefore, the 4-hr time point is valid and negative for neuronal vacuolation but not the 7-day time point for necrosis. The AUC_{0-∞} exposure is 936 ng.h/mL and Cmax is 791ng.h/mL which is 1.8 and 4.5 times clinical exposures (AUC=530 ng.h/mL and Cmax=174 ng/mL for esketamine) at the MRHD of 84 mg/day.

JNJ54135419: A single dose IN neurotoxicity study in the female rat/Tox11374: (GLP; signed path report).

Sprague-Dawley (Crl:CD) rats (12-14 weeks; 12-15F/group/time point) were IN administered esketamine HCl (batch No. 0142911P) at doses of 0 (citric acid (b) (4), EDTA (b) (4) and NaOH to PH 4.5), 36, 54 or 72 mg eq/rat (100ul/rat/instillation) via twice, thrice or 4-times in one day. These doses are estimated to be 180, 270 and 360 mg/kg for a 200 gram rat. Sacrifice time points were 48 and 96 hours post dose. There were a total of 4 deaths at the highest dose (2 main and 2 TK). Premature decedents showed ataxia, bradypnea, severely decreased activity, decubitus, catalepsy, salivation, and/or narrowed palpebral fissures. At necropsy, 2 main study animals showed discoloration in lungs and/or hemmorrhage in the pleural cavity. Rats that survived also showed decreased activity, ataxia, audible respiration with the first two occurring for 1.5 hrs, <3 hr, and >3 hrs postdose at LD, MD, and HD. This study did not examine the appropriate sacrifice time point for neuronal vacuolation. However, this is a valid and negative study for the observation of necrosis at a dose of 72 mg/eq (lower doses were not histopathologically analyzed). Exposure at 72 mg eq had a Cmax of 10,245 ng/mL and AUC of 45,800 (only N=2 TK). Due to the high variability of TK between these two animals, comparison to the next lower dose of 54 mg eq/rat (270 mg/kg) where Cmax is 4070 ng/mL and AUC is 9430 ng.hr/mL (N=4) may be appropriate. A dose of 54 mg eq/day has a safety margin of 23-and 17-fold to clinical exposures to Cmax and AUC, respectively (Cmax=174 ng/mL and AUC=530 ng.h/mL for esketamine) to the MRHD of 84 mg/day.

An acute subcutaneous neurotoxicity study in rats with PMI-100/Study No. 3542.9A (GLP; signed pathology report)

Sprague-Dawley (CrI:CD(SD)IGS BR rats (11-12 weeks old; 4/sex/group/sacrifice timepoint) were subcutaneously administered a single dose of PMI-100 (batch No. 46855) at 0, 4, 15, and 60 mg/kg. This study used sacrifice time points of 6 hrs, 24 hrs, 72 hrs, and 2 weeks post dosing and stains such as H&E, Fluoro-Jade/DAPI, and amino cupric silver (de Olmos method). Functional Observation Battery (FOB) and Biel water maze learning/memory testing was done in this study. The Biel water maze study design was: day 5 (swimming trial), 6-9 (learning trial), 10-11 (rest days), and day 12 (memory trial). Clinical signs were as expected for anesthetic drug lasting 1 hr postdose at ≥ 15 mg/kg. Minimal neuronal vacuolation was observed in layer 1 of the retrosplenial cortex of females at 60 mg/kg at the 6 hr sacrifice time point with H&E staining. Minimal neuronal degeneration observed in 2/4 F at 60 mg/kg with amino cupric silver staining was noted to be within background considering the increased sensitivity of the stain. A subcutaneous dose of 0.5 mg/kg of MK-801 produced mild to moderate vacuolation in layers 2 and 3 of the retrosplenial cortex of females and minimal to mild degree of neuronal necrosis with H&E again only in females. The ketamine exposures in female rats at NOAEL for vacuolation in this study are 1,577 ng/mL for C_{max} and 1,686 ng.hr/ml for AUC_{0-∞}. NOAEL for neuronal degeneration is 60 mg/kg which provides exposures in female rats of 5,523 ng/mL for C_{max} and 10,781 ng.hr/ml for AUC_{0-∞}. Presuming that 50% of the circulating species is esketamine when ketamine is administered, the exposure in females for esketamine at the NOAEL for vacuolation is 788 ng/mL for C_{max} and 843 ng.hr/mL for AUC. Similarly, the presumed esketamine exposure in females at the NOAEL for neuronal degeneration (e.g. the HD used in this study) is 2,761 ng/mL for C_{max} and 5,390 ng.hr/mL for AUC. Therefore, the NOAEL for vacuolation provides a safety margin of 4.5-fold for C_{max} and 1.6-fold for AUC to clinical exposures at the MRHD of 84 mg/day. The NOAEL for degeneration provides a safety margin of 16-fold for C_{max} and 10-fold for AUC to clinical exposures at the MRHD of 84 mg/day.

There was also a 28-day repeat-dose neurotoxicity study (Study No. 3542-9b) with sacrifice time points at 6 hrs, 24hrs, 48 hrs, and 14 days after the last dose. Doses of PMI-100 were exactly the same as that used in the single dose study. The results of a Biel water memory test showed a non-statistically significant but a dose dependent increase in trial time on D3&4 (learning delay) and on memory recall trial (memory delay) (nonstatistically significant) in males but no effect was observed in females. In this study, MK-801 positive control rats did not show vacuolation nor neuronal necrosis with H&E staining. With amino cupric silver stain, minimal to moderate late stage neuronal and axonal degeneration was observed in 2/4 females but not males. Occasional minimal necrosis in a HDM treated with 60 mg/kg PMI-100 was noted as background by the pathologist.

Reviewer's Comments: *The single dose study (study No. 3542.9A) using PMI-100 was not conducted at MTD based on the transient clinical signs observed. The sacrifice time point for neuronal vacuolation was appropriate and valid, however, the sample size was small. The sacrifice time point in the 28-day study (Study No. 3542.9B) was not appropriate to detect vacuolation/degeneration as seen by the lack of findings in positive control females and the sample size was too small to be considered an adequate Olney study. The single dose acute toxicity study with*

PMI-100 is a well conducted study based on histopathology, time points of sacrifice, and tissue sectioning although only a small sample size was used; therefore, the results from the single dose study are accepted.

11 Appendix/Attachments

Figure 1: Proposed *in vitro* metabolic pathways of esketamine in liver microsomes and S9 fractions of various species.

(Excerpted from the Sponsor's Nonclinical overview, P.33)

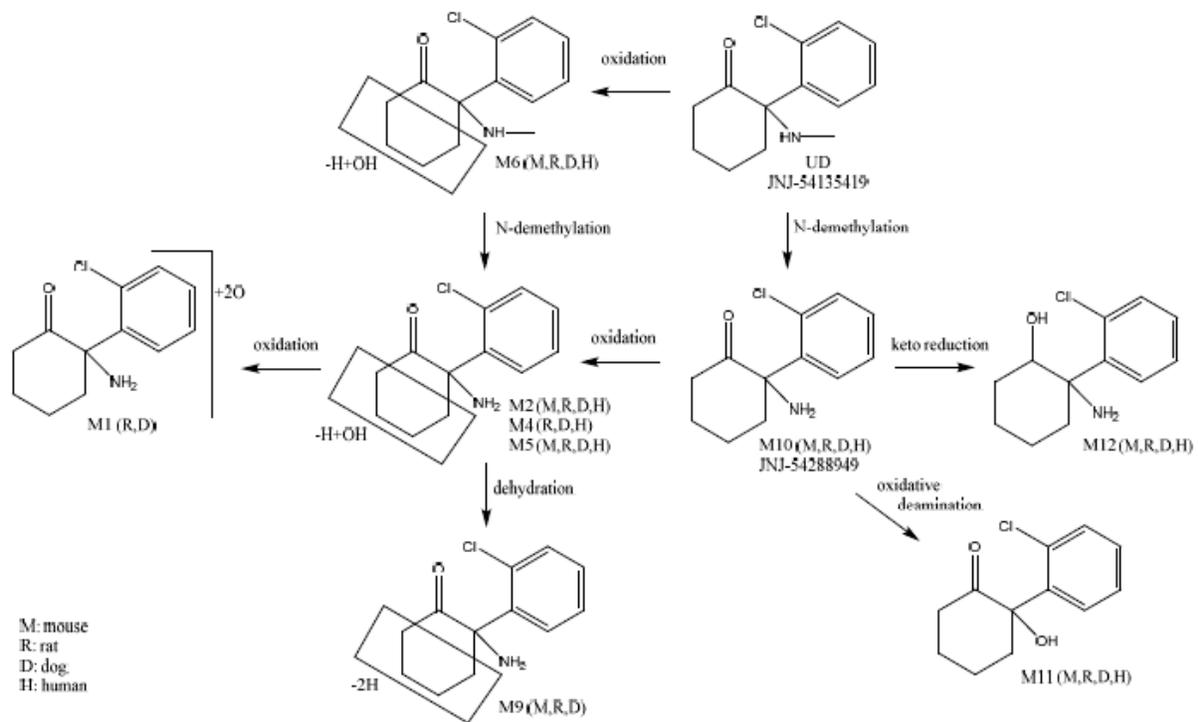


Figure 2: Esketamine (above) and noresketamine (below) exposure in nonclinical species relative to humans.

(Excerpted from the Sponsor’s nonclinical overview, P. 45-46).

Study	Esketamine Dose (route)	Mean C _{max} (ng/mL)	Mean AUC (ng.h/mL)	Animal-to-Human Exposure Ratio		Study Number
				C _{max}	AUC	
ESKETAMINE						
Human	84 mg (intranasal)	174 (M, F) ^a	530 (M, F) ^b	-	-	
Transgenic mouse 6-M carcinogenicity (SC)	75/40 mg/kg ^c	5,990 (M); 3,540 (F)	3,980 (M); 2,210 (F) ^d	34.4 (M); 20.3 (F)	7.5 (M); 4.2 (F)	Mod4.2.3.4.2/TOX11233 and Section 4.6
Rat 3-month toxicology NOAEL	9 mg/day (intranasal)	225 (M); 439 (F)	382 (M); 585 (F) ^e	1.3 (M); 2.5 (F)	0.7 (M); 1.1 (F)	Mod4.2.3.2/TOX10517
Rat 6-month toxicology NOAEL	9 mg/day (intranasal)	ND ^f	ND ^f	ND ^f	ND ^f	Mod4.2.3.2/TOX10768 and Section 4.4
Rat liver Comet	50 mg/kg/day (intravenous)	6,790 (M); 9,350 (F) ^g	No value	39.0 (M); 53.7 (F)	ND	Mod4.2.3.3.2/TOX10530
Rat 2-year carcinogenicity	9 mg/day (intranasal)	201 (M); 428 (F)	253 (M); 366 (F) ^h	1.2 (M); 2.5 (F)	0.5 (M); 0.7 (F)	Mod4.2.3.4.1/TOX10702 and Section 4.6
Rat single-dose neurotoxicity	9 mg (intranasal)	791 (F)	936 (F) ⁱ	4.5 (F)	1.8 (F)	Mod4.2.3.7.7/TOX10415
Rat single-dose neurotoxicity	72 mg (intranasal)	10,245 (F)	45,800 (F) ^j	58.9 (F)	86.4 (F)	Mod4.2.3.7.7/TOX11374
Rat 2-week neurotoxicity	54 mg/day (intranasal)	2,950 (F)	6,030 (F) ^e	17.0 (F)	11.4 (F)	Mod4.2.3.7.7/TOX10950
Dog 3-month toxicology NOAEL	72 mg/day (intranasal)	817 (M); 765 (F)	452 (M); 649 (F) ^e	4.7 (M); 4.4 (F)	0.9 (M); 1.2 (F)	Mod4.2.3.2/TOX10524
Dog 9-month toxicology NOAEL	72 mg/day (intranasal)	1,100 (M); 1,160 (F)	771 (M); 584 (F) ^h	6.3 (M); 6.7 (F)	1.5 (M); 1.1 (F)	Mod4.2.3.2/TOX10701 and Section 4.4

(Continued)

Study	Esketamine Dose (route)	Mean C _{max} (ng/mL)	Mean AUC (ng.h/mL)	Animal-to-Human Exposure Ratio		Study Number
				C _{max}	AUC	
NORESKETAMINE (M10)						
Human	84 mg (intranasal)	250 (M, F) ^b	1,784 (M, F) ^b	-	-	
Transgenic mouse 6-M carcinogenicity (SC)	75/40 mg/kg ^c	5,500 (M); 4,100 (F)	12,500 (M); 7,400 (F) ⁱ	22.0 (M); 16.4 (F)	7.0 (M); 4.1 (F)	Mod4.2.3.4.2/TOX11233 and Section 4.6
Rat 3-month toxicology NOAEL	9 mg/day (intranasal)	300 (M); 877 (F)	722 (M); 2,666 (F) ^e	1.2 (M); 3.5 (F)	0.4 (M); 1.5 (F)	Mod4.2.3.2/TOX10517
Rat 6-month toxicology NOAEL	9 mg/day (intranasal)	278 (M); 762 (F)	1,360 (M); 1,490 (F) ^d	1.1 (M); 3.0 (F)	0.8 (M); 0.8 (F)	Mod4.2.3.2/TOX10768 and Section 4.4
Rat liver Comet	50 mg/kg/day (intravenous)	5,590 (M); 8,820 (F) ^g	No value	22.4 (M); 35.3 (F)	ND	Mod4.2.3.3.2/TOX10530
Rat 2-year carcinogenicity	9 mg/day (intranasal)	406 (M); 763 (F)	968 (M); 2,140 (F) ^h	1.6 (M); 3.1 (F)	0.5 (M); 1.2 (F)	Mod4.2.3.4.1/TOX10702 and Section 4.6
Rat single-dose neurotoxicity	9 mg (intranasal)	1,220 (F)	5,960 (F) ⁱ	4.9 (F)	3.3 (F)	Mod4.2.3.7.7/TOX10415
Rat single-dose neurotoxicity	72 mg (intranasal)	7,140 (F)	40,150 (F) ^j	28.6 (F)	22.5 (F)	Mod4.2.3.7.7/TOX11374
Rat 2-week neurotoxicity	54 mg/day (intranasal)	2,870 (F)	9,890 (F) ^e	11.5 (F)	5.5 (F)	Mod4.2.3.7.7/TOX10950
Dog 3-month toxicology NOAEL	72 mg/day (intranasal)	178 (M); 240 (F)	125 ^k (M); 400 (F) ^e	0.7 (M); 1.0 (F)	0.07 (M); 0.2 (F)	Mod4.2.3.2/TOX10524
Dog 9-month toxicology NOAEL	72 mg/day (intranasal)	296 (M); 338 (F)	446 (M); 371 (F) ^h	1.2 (M); 1.4 (F)	0.3 (M); 0.2 (F)	Mod4.2.3.2/TOX10701 and Section 4.4

^a Highest mean exposure at 84 mg intranasal (ESKETINTRD1001, healthy subjects); ^b Highest mean exposure at 84 mg intranasal (ESKETINTRD1012, healthy elderly subjects; ≥75 years); ^c 75 mg/kg/day from Day 1 (both sexes) to Day 116 (male) or Day 121 (female); 40 mg/kg/day from Day 124 (male) and Day 122 (female) to Day 177; ^d AUC_{0-24h}; ^e AUC_{0-24h}; ^f Esketamine exposure levels are considered unreliable due to possible contamination as a result of blood sampling from the sublingual vein following intranasal dosing; ^g Mean concentration at 25 min after start of the 30-min infusion for esketamine, and for noresketamine at 30 min after the end of the infusion. No AUCs were determined; ^h AUC_{0-1h}; ⁱ AUC_{0-8h}; ^j AUC_{0-8h}; ^k AUC_{0-1h} instead of AUC_{0-24h}

biw: twice weekly; F: female; M: male; ND: no data; SC: subcutaneous

Table 32: TK of noresketamine in rats from the 6-month toxicity study.

Parameter	Sex	0.9 mg/rat	3.0 mg/rat	9.0 mg/rat
AUC _{0-t} (ng.h/mL)	M	246	554	1360
	F	408	891	1490
C _{max} (ng/mL)	M	119	260	278
	F	252	435	762

Table 33: TK of noresketamine in dogs from the 9-month toxicity study.

Parameter	Sex	24 mg/dog	48 mg/dog	72 mg/dog
AUC _{0-t} (ng.h/mL)	M	105	295	446
	F	103	276	371
C _{max} (ng/mL)	M	147	235	296
	F	145	244	338

Table 34: TK of noresketamine in transgenic mice following 26-weeks of dosing.

Parameter	Sex	10	25	75/40
AUC _{0-t} (ng.h/mL)	M	3380	8050	12500
	F	2020	3960	7400
C _{max} (ng/mL)	M	1870	3510	5500
	F	1520	2490	4100

Table 35: TK of noresketamine in rats in the 2-year carcinogenicity assay after 6 months of IN dosing.

Parameter	Sex	0.9 mg/day	3 mg/day	9 mg/day
AUC _{0-t} (ng.h/mL)	M	215	468	968
	F	392	979	2140
C _{max} (ng/mL)	M	143	227	406
	F	233	510	763

Figure 3: Nasal cavity, brain and larynx dissections in the rat from the 6-month toxicity study.

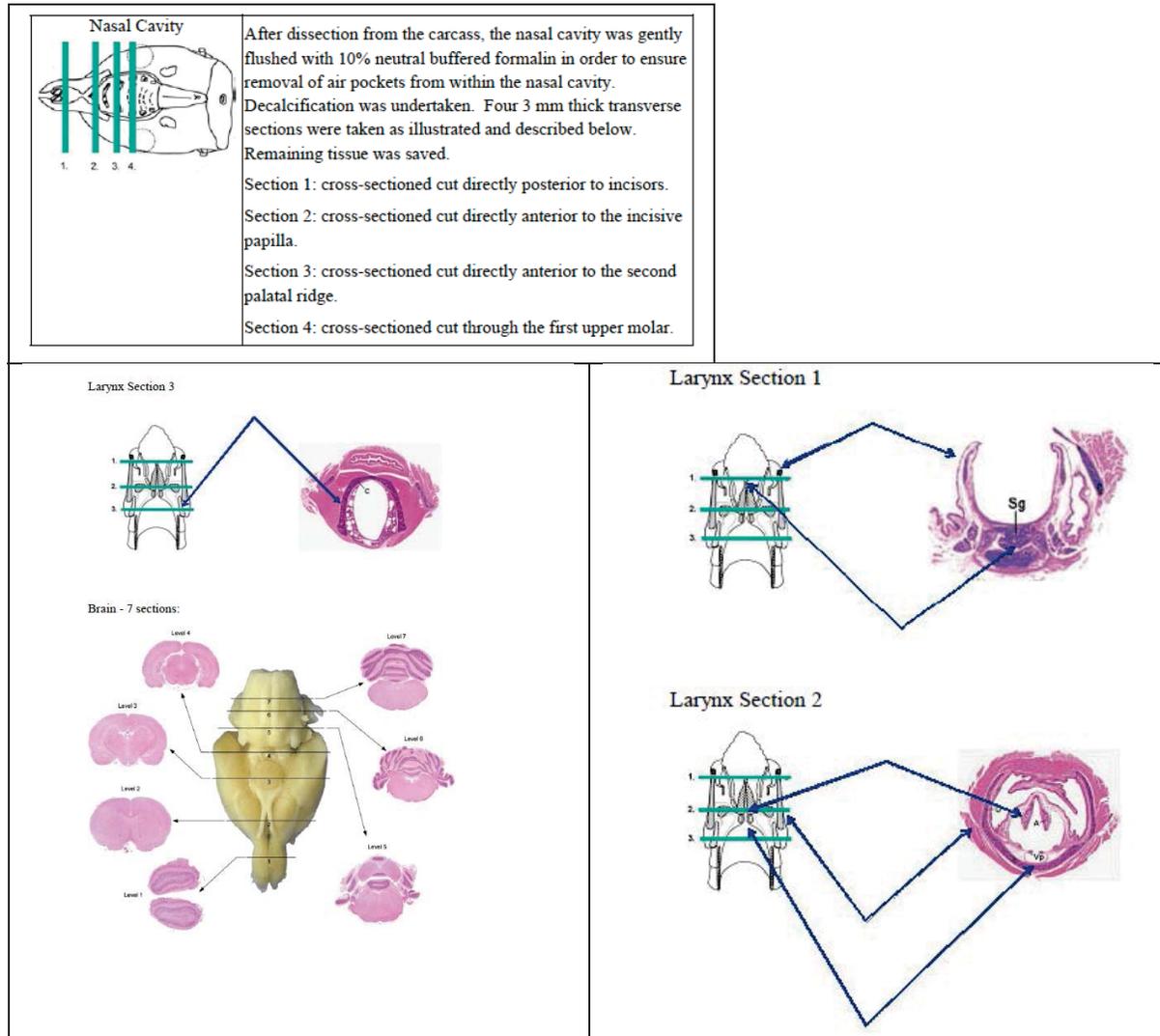


Table 36: TK data from bridging studies after IV administration of racemic ketamine in non-pregnant rats.

Table is excerpted from TOX 10457, P.2

JNJ-644059 (Ketamine)				
	Male		Female	
Dose (mg eq./kg)	30	120	30	120
C _{max} (ng/ml)	4053	9950	7057	27433
T _{max} (h)	0.50	0.42	0.50	0.64
AUC _{0-inf} (ng.h/ml)	5117 ¹	8861 ^{2,3}	8005 ¹	42224 ³

¹ n=2; ² n=1; ³ AUC_{0-24h}**Table 37: TK data from bridging studies after IV administration of racemic ketamine in dogs.**

Table is excerpted from TOX 10458, P.8

Sex	AUC _{0-inf} (ng/h/ml)					
	Male			Female		
Dose (mg eq./kg)	0.3	1	6	0.3	1	6
Ketamine	24.5	92.9	785	21.2	77.4	621
Esketamine	10.5 ¹	48.2	406	11.4	38.6	315
Norketamine	12.3 ^{1,2}	46.0	233	10.4 ²	52.3 ¹	289 ¹
Ratio Esketamine /Ketamine	0.441	0.519	0.517	0.538	0.499	0.507

¹ n=1; ² AUC_{0-24h}

Table 38: TK parameters from F1 offsprings in the pre- and postnatal development study on PND 4 and PND12.

Table is excerpted from TOX 10766, P.57

Dose level (mg eq./rat/day)	C_{max} (ng/mL)			
	Day 4		Day 12	
	Males	Females	Males	Females
0.9	29.9	25.4	BQL	BQL
3	63.8	65.7	BQL	BQL
9	1120	215	30.3	38.9

Dose level (mg eq./rat/day)	AUC_{0-t} (ng.h/mL)			
	Day 4		Day 12	
	Males	Females	Males	Females
0.9	65.6	40.6	- ^a	- ^a
3	200	210	- ^a	- ^a
9	1830	791	74.8	43.8

^a Mean plasma concentration could not be calculated at any sampling time

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/s/

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02/28/2019 08:57:04 AM

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02/28/2019 10:20:26 AM
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