CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

211371Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Division Diretcor
CDTL Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	211,371
Priority or Standard	Priority
Submit Date(s)	4/19/18
Received Date(s)	4/19/18
PDUFA Goal Date	12/19/18
Division/Office	DPP/ODE1/OND
Review Completion Date	9/19/18
Established Name	Brexanolone
(Proposed) Trade Name	ZULRESSO
Pharmacologic Class	Neuroactive Steroid Gamma-Aminobutyric Acid (GABA) A Receptor Positive Modulator
Code name	SAGE-547
Applicant	Sage Therapeutics
Formulation(s)	5 mg/mL solution for infusion
Dosing Regimen	60-hour infusion, target dose 90 µg/kg/h
Applicant Proposed Indication(s)/Population(s)	Treatment of post-partum depression
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	Treatment of post-partum depression

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DEPI=Division of Epidemiology; DMEPA=Division of Medication Error Prevention and Analysis; DPP=Division of Psychiatry Products; DRISK=Division of Risk Management; IO=Immediate Office; OB=Office of Biostatistics; OCP=Office of Clinical Pharmacology; ODE=Office of Drug Evaluation; OND=Office of New Drugs; OPQ=Office of Pharmaceutical Quality; OPDP=Office of Prescription Drug Promotion; OSE=Office of Surveillance and Epidemiology; OSI=Office of Scientific Investigations

Glossary

AC	Advisory committee
ADME	Absorption, distribution, metabolism, excretion
AE	Adverse event
AUC	Area under the curve
BIMF	Barkin Index of Maternal Functioning
BLA	Biologics license application
BPCA	Best Pharmaceuticals for Children Act
BRF	Benefit Risk Framework
CDER	Center for Drug Evaluation and Research
CDTL	Cross-Discipline Team Leader
CFR	Code of Federal Regulations
CSF	Cerebrospinal fluid
CGI-I	Clinical Global Impression—Improvement
CGI-S	Clinical Global Impression—Severity
CL	Clearance
C_{max}	Maximal concentration observed
CMC	Chemistry, manufacturing, and controls
CNS	Central nervous system
CRF	Case report form
CSR	Clinical study report
CSS	Controlled Substance Staff
Css	Concentration at steady state
DPP	Division of Psychiatry Products
EC_{50}	Half maximal effective concentration
ECG	Electrocardiogram
eCTD	Electronic common technical document
E_{max}	Maximal effect
EPDS	Edinburgh Postnatal Depression Scale
EPC	Established pharmacologic class
ETASU	Elements to assure safe use
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GAD-7	Generalized Anxiety Disorder 7-item Scale
GCP	Good clinical practice
GD	Gestation day
GLP	Good laboratory practice
HAM-D	Hamilton Rating Scale for Depression
HCR	Historical control range
HCRU	Health Care Resource Utilization
HD	High dose
IC_{50}	Half maximal inhibitory concentration

ICH	International Conference on Harmonization
I_{GABA}	GABA-induced chloride currents
IND	Investigational New Drug
ISE	Integrated summary of effectiveness
ISS	Integrated summary of safety
ITT	Intent to treat
IV	Intravenous
Ki	Inhibition constant
LD	Low dose
LOC	Loss of consciousness
MADRS	Montgomery-Åsberg Depression Rating Scale
MD	Mid dose
MRHD	Maximum recommended human dose
MedDRA	Medical Dictionary for Regulatory Activities
mITT	Modified intent to treat
NDA	New drug application
NME	New molecular entity
NOAEL	No observed adverse effect level
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PD	Pharmacodynamics
PHQ-9	Patient Health Questionnaire (9-item)
PK	Pharmacokinetics
PMC	Postmarketing commitment
PMR	Postmarketing requirement
PND	Postnatal day
PP	Per protocol
PPI	Patient package insert
PPD	Postpartum depression
PREA	Pediatric Research Equity Act
PRO	Patient reported outcome
REMS	Risk evaluation and mitigation strategy
SAE	Serious adverse event
SAP	Statistical analysis plan
SBECD	Sulfobutyl ether beta-cyclodextrin
SC	Saline control
SF-36	Short-form 36
$T_{1/2}$	Terminal elimination half-life
TEAE	Treatment emergent adverse event
TBPS	Tert-butylbicyclophosphorothionate
TK	Toxicokinetics
T_{max}	Time to reach maximal concentration
V_{ss}	Volume of distribution at steady-state

1 Executive Summary

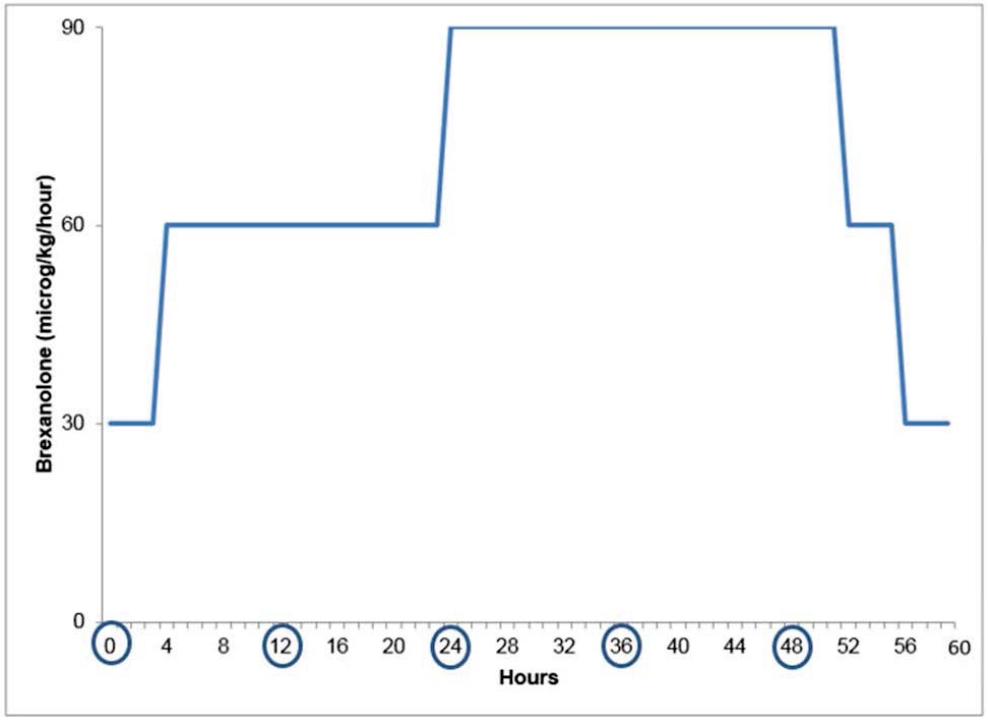
1.1. Product Introduction

Brexanolone (SAGE-547; proposed trade name Zulresso) is chemically identical to the endogenous human hormone allopregnanolone. It is a new molecular entity (NME) with the proposed indication of treatment of postpartum depression (PPD). Although its mechanism of action is unknown, it appears to be a positive allosteric modulator of gamma-aminobutyric acid type A ($GABA_A$) receptors with a binding site distinct from benzodiazepines. Brexanolone is available as a 5mg/mL solution in sulfobutyl ether beta-cyclodextrin (Captisol), which is administered as an intravenous infusion over 60 hours. Once mixed, the infusion is only stable for 12 h at room temperature and 96 h refrigerated. The dose is weight- and time-based as per the following (see also Figure 1):

- 4 hours at 30 µg/kg/hour
- 20 hours at 60 µg/kg/hour
- 28 hours at 90 µg/kg/hour
- 4 hours at 60 µg/kg/hour
- 4 hours at 30 µg/kg/hour

The product would be given once per episode of PPD.

Figure 1. Dose and Timing for Brexanolone Administration.



O=New infusion bag required.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant submitted two positive, adequate, and well-controlled trials that met the evidentiary standard for the demonstration of brexanolone's effectiveness for the treatment of postpartum depression. The studies demonstrate a clinically meaningful effect because the improvement in depressive symptoms on the Hamilton Depression Rating Scale (HAM-D) is both consistent with the effects of other, approved antidepressants and occurs much quicker than other available treatments (after 60 hours versus 4 weeks). HAM-D remission (a total score of ≥7) and response (a reduction in total score of at least 50%) also supported brexanolone's effectiveness. The Clinical Global Impression of Improvement (CGI-I) also showed statistically significant and clinically meaningful differences from placebo. Although few of the other experimental endpoints were statistically significant, they all revealed a trend of decreasing depressive symptoms with brexanolone. Notably, these included several patient-rated scales (e.g., the Barkin Index of Maternal Functioning and Edinburgh Postnatal Depression Scale).

1.3. Benefit-Risk Assessment

Benefit-Risk Integrated Assessment

Brexanolone (to be marketed as Zulresso) is chemically identical to the endogenous human hormone allopregnanolone. It is a new molecular entity not currently marketed anywhere in the world for any indication. Brexanolone's proposed indication is treatment of postpartum depression (PPD). PPD is a major depressive episode with onset during pregnancy or within 4 weeks of delivery. As with other forms of depression, it is characterized by sadness and/or anhedonia and may present with symptoms such as cognitive impairment, feelings of worthlessness or guilt, or suicidal ideation. Because of the risk of suicide, PPD is considered a life-threatening condition. It also can have profound negative effects on the maternal-infant bond and later infant development. Although there are many approved antidepressant medications, none are specifically approved for PPD. The initial hypothesis behind brexanolone's mechanism of action led the Division to believe that it might be uniquely effective for PPD. Therefore, we did not require studies in non-PPD major depressive disorder.

The endogenous hormone allopregnanolone increases during pregnancy and reaches a peak during the third trimester. After delivery, allopregnanolone levels abruptly fall. Initially, the Applicant believed brexanolone might be effective for PPD as an allopregnanolone replacement. However, researchers have found that allopregnanolone levels do not predict PPD. Brexanolone's mechanism of action is, therefore, unknown. It appears to be a positive allosteric modulator of $GABA_A$ receptors with a binding site distinct from benzodiazepines.

Brexanolone is administered as a 60-hour infusion—including a titration and taper—with a target dose of 90 µg/kg/h. One submitted study included a target dose of 60 µg/kg/h for the 60-hour infusion. Evidence of efficacy was assessed in three controlled studies: 547-PPD-202A, 202B, and 202C. The primary efficacy endpoint in these studies was change from baseline on the Hamilton Depression Scale at 60 hours after start of the brexanolone infusion. All three studies showed a reduction in depressive symptoms with brexanolone infusion.

The Applicant submitted sufficient information to adequately assess brexanolone's safety profile. The Division did not require the exposure numbers suggested for chronic conditions (based on the International Council of Harmonisation guidance) because the drug is administered as a one-time infusion. The Agency's major safety concern is the possibility of sudden loss of consciousness (LOC) during the infusion (6 of 140 women exposed to brexanolone). After examining dose, timing of dose, blood level, concurrent medications, available medical history, and patient characteristics (e.g., age, body mass index) we found no relationships between these factors and the LOC events. Because LOC can be abrupt, and there is no way to predict the event, the Agency did not feel the risk could be mitigated solely through labeling. Therefore, this product will be approved with a risk evaluation and mitigation strategy (REMS). Aside from the risk of LOC, brexanolone appeared reasonably well-tolerated.

Considering the seriousness of PPD, the lack of identified effective treatments, and the risks and benefits of brexanolone, the review team recommends approval. We do not believe additional studies are needed to further characterize the LOC risk. However, we recommend additional efficacy studies to determine whether the infusion can be given in an interrupted manner (e.g., only during the daytime) or shortened—potentially broadening available administration settings. We will require a nonclinical postmarketing study to quantify the risk to neurons in the third trimester. This nonclinical study will determine if brexanolone is similar to other GABA-acting drugs in this regard.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• PPD is a major depressive episode with onset during pregnancy or within 4 weeks of delivery. Although diagnosis uses the same criteria, the timing of onset may indicate a different etiology than non-postpartum depression. • Approximately 12% of U.S. women experience PPD. • Women with PPD are at risk for suicide and have impairments in daily function (including maternal-infant bonding).	PPD is potentially debilitating and life-threatening. It affects a substantial number of U.S. women. PPD is defined as major depressive disorder "with post- (or peri-) partum onset." However, the timing of this condition suggests a somewhat different etiology compared to non-postpartum major depression (involving hormone level fluctuations).
Current Treatment Options	• There are no drugs approved specifically for PPD. • Drugs approved for treatment of major depressive disorder can be used to treat PPD. • There is little direct evidence that available antidepressants adequately treat PPD. Almost all studies are small and include confounding treatments (such as concurrent psychotherapy). • All available antidepressant treatments (including psychotherapy and electroconvulsive therapy) require several weeks to demonstrate an antidepressant effect.	There is little evidence from controlled trials that available antidepressant treatments are effective for PPD. In addition, all available antidepressant treatments require weeks before their effect is seen.
Benefit	• The evidence of brexanolone's effectiveness comes from three studies: 547-PPD-202A, 202B, and 202C. The primary efficacy endpoint in all three studies was change from baseline in the Hamilton Depression Rating Scale (HAM-D) at Hour 60. All three studies were randomized, double-blind, and placebo-controlled. • 202A was a Phase 2 proof-of-concept study comparing brexanolone 90	The data submitted with this NDA meets the evidentiary standard for approval. A majority of women with PPD who receive the brexanolone infusion are expected to see benefit in their depressive symptoms. These benefits are expected to occur more quickly

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	µg/kg/h (n=10) to placebo (n=11) in patients with severe PPD (HAM-D > 26). Brexanolone was significantly superior to placebo with a least square mean difference of -12.2. • 202B was a Phase 3 study comparing brexanolone 90 µg/kg/h (n=45) and brexanolone 60 µg/kg/h (n=47) to placebo (n=46) in patients with severe PPD (HAM-D > 26). Brexanolone 60 µg/kg/h was significantly superior to placebo with a least square mean difference of -5.5. Brexanolone 90 µg/kg/h was significantly superior to placebo with a least mean square difference of -3.7. • 202C was a Phase 3 study comparing brexanolone 90 µg/kg/h (n=54) to placebo (n=54) in patients with moderate PPD (HAM-D 20 to 25). Brexanolone was significantly superior to placebo with a least square mean difference of -2.5. • Separation from placebo was present between 24 and 48 hours after starting the infusion in all three studies. • Pooled dosing arms at Day 30 showed that patients treated with brexanolone 90 µg/kg/h and 60 µg/kg/h were still superior to placebo-treated patients with least square mean differences of -2.5 and -4.7, respectively. • The nature of the infusion ensured that investigators knew the patient's adherence to treatment. • The enrolled population underrepresented the non-white/non-black population of the United States (e.g., Asian). However, there is no reason to suspect efficacy would different in these underrepresented groups.	than with currently available antidepressant treatments and remain relatively stable for 30 days. There were insufficient numbers of subjects to conclude that certain subgroups would experience differential benefits. Although the Applicant studied both 90 and 60 µg/kg/h doses, they did not examine variability in duration of infusion. Consequently, we know that a 60-hour continuous infusion is effective, but we do not know if a shorter duration or interrupted infusion (i.e., only given for a few hours per day) would be as effective. Therefore, we recommend post-marketing commitments to study the efficacy of an interrupted infusion and a short-duration infusion (i.e., 24 hours).
Risk and Risk Management	• The safety database included 140 patients with PPD exposed to brexanolone. The median age was 27 years. Approximately 20% of brexanolone-exposed and placebo patients were taking a concomitant oral antidepressant. • The most concerning adverse reaction was loss of consciousness	The LOC events have the potential to put both the patient and her infant at risk (e.g., from falls, drops, smothering). Additionally, although there were no vital sign abnormalities associated with brexanolone in the 202 studies,

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	(LOC) in 6 patients exposed to brexanolone (4%). We observed no relationship between LOC and dose, timing of dose, blood level, concurrent medications, available medical history, and patient characteristics (e.g., age, body mass index). LOC was abrupt in some cases. All resolved within 60 min after immediately stopping the infusion; no other intervention was required. • Minor adverse reactions occurring in >2% of patients exposed to any brexanolone, and twice the rate of placebo, were sedation/ somnolence (15%), dizziness/vertigo/light-headedness (12%), dry mouth/thirst (5%), and flushing (3%). • There was no association between brexanolone and suicidal ideation or behavior. • There was no effect of brexanolone on the QT interval. • There was no effect of brexanolone on laboratory values. • There was no indication from studies 202A, 202B, or 202C that brexanolone affected vital signs. However, one male subject in the thorough QT study experienced >1 min of apnea after exposure to brexanolone 150 µg/kg. • Brexanolone drug liking was comparable to alprazolam in human abuse potential studies.	there was a male subject who experienced apnea in a Phase 1 study. We do not know the consequences of continuing the infusion after the patient has lost consciousness. We do not feel that labeling can adequately mitigate these risks. We will approve brexanolone with a REMS to ensure that the infusion is monitored by a healthcare professional who can assess the patient and intervene (stop the infusion) if needed. Continuous pulse oximetry monitoring during the infusion will also be required. There was no signal for suicidal ideation and behavior in the studies and, because there is no indication that depressive symptoms recur for at least the month of follow-up, the usual boxed warning class language related to suicidal ideation and behavior will not be included. However, because studies were small, and we cannot rule out an effect, we will include modified suicidal ideation and behavior warning language in Section 5. Based on the human abuse potential studies, FDA will recommend DEA scheduling (schedule IV).

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application:

The patient experience data that was submitted as part of the application, include:			Section where discussed, if applicable
☒	**Clinical outcome assessment (COA) data**		**Section 8: Statistical and Clinical Evaluation of Efficacy**
	☒	**Patient reported outcome (PRO): EPDS, GAD-7, PHQ-9, BIMF, SF-36, HCRU**	**Section 8**
	□	Observer reported outcome (ObsRO)	
	☒	**Clinician reported outcome (ClinRO): HAM-D (Response/Remission), CGI-I**	**Section 8**
	□	Performance outcome (PerfO)	
□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
□	Patient-focused drug development or other stakeholder meeting summary reports		
□	Observational survey studies designed to capture patient experience data		
□	Natural history studies		
□	Patient preference studies (e.g., submitted studies or scientific publications)		
□	Other: (Please specify)		
Patient experience data that was not submitted in the application, but were considered in this review.			
□	Input informed from participation in meetings with patient stakeholders		
□	Patient-focused drug development or other stakeholder meeting summary reports		
□	Observational survey studies designed to capture patient experience data		
□	Other: (Please specify)		
□	Patient experience data was not submitted as part of this application.		

BIMF=Barkin Index of Maternal Functioning; CGI-I=Clinical Global Impression-Improvement; EPDS=Edinburgh Postnatal Depression Scale; GAD-7=Generalized Anxiety Disorder 7-item Scale; HAM-D=Hamilton Rating Scale for Depression PHQ-9=Patient Health Questionnaire (9-item); SF-36=Short-form 36.

X _____ APPEARS THIS WAY ON ORIGINAL

Tiffany R. Farchione, MD
Cross-Disciplinary Team Leader

2 Therapeutic Context

2.1. Analysis of Condition

PPD is a major depressive episode with onset during pregnancy or within 4 weeks of delivery. As with other forms of depression, it is characterized by sadness and/or anhedonia and may present with symptoms such as cognitive impairment, feelings of worthlessness or guilt, or suicidal ideation (see Table 1 for major depressive episode diagnostic criteria). Indeed, the most common cause of maternal death after childbirth in the developed world is suicide (Oates, 2003). A depressive episode at this time in a woman's life not only deprives her of the enjoyment of a new infant, but has serious effects on the maternal-infant bond and later infant development. Estimates place the prevalence of PPD in the United States at approximately 12% of births (Shorey et al., 2018).

Table 1. Diagnostic Criteria for a Major Depressive Episode.[a]

A	Five or more symptoms for 2 weeks (one of which must be either depressed mood or anhedonia)	1. Depressed mood most of the day nearly every day 2. Anhedonia most of the day nearly every day 3. Significant weight loss or gain 4. Insomnia or hypersomnia 5. Psychomotor agitation or retardation 6. Fatigue or loss of energy 7. Feelings of worthlessness or excessive guilt 8. Diminished ability to think or concentrate; indecisiveness 9. Recurrent thoughts of death; suicidal ideation or attempt
B	Symptoms cause clinically significant distress or functional impairment	
C	The episode is not attributable to the physiological effects of a substance or another medical condition	
D	The episode is not better explained by a psychotic illness	
E	There has never been a manic or hypomanic episode	

[a]American Psychiatric Association, 2013.

PPD is symptomatically indistinguishable from an episode of major depression. However, the timing of its onset has led to its recognition as a distinct illness.

Many hormones are neuroactive. Because of the changes in hormone concentrations during pregnancy, they have been attractive targets for PPD investigations. The concentration of allopregnanolone, an endogenous derivative of progesterone, increases during pregnancy, reaches a peak during the third trimester, then abruptly falls after delivery. As recently reviewed by McEvoy and colleagues (2018), allopregnanolone is a potent GABA-ergic regulator. At low concentrations, it acts as a positive allosteric modulator at synaptic and extrasynaptic $GABA_A$ receptors—at high concentrations, it can directly stimulate them without GABA. Whereas benzodiazepines increase chloride channel opening frequency and barbiturates increase the duration of chloride channel opening, allopregnanolone does both.

As allopregnanolone levels rise during pregnancy, $GABA_A$ receptors are down-regulated. Animal models have shown that the receptors return to previous concentrations within 48 hours of delivery. Because total allopregnanolone levels have not consistently correlated with PPD, it is possible the symptoms are more closely related to impairment in peripartum GABA receptor up- or down-regulation (or even changes in receptor subunits) and not necessarily to an abrupt decrease in allopregnanolone concentrations.

Per the Applicant, they had hypothesized that, in women experiencing PPD, returning the allopregnanolone concentration to that of the third trimester would ameliorate symptoms. Brexanolone dosing was, therefore, based on returning women to pre-delivery levels of allopregnanolone. The initial titration was meant for women to develop tolerance for the associated sedation. The taper was meant to prevent withdrawal symptoms from a GABA-active agent. Because the Applicant believed the dose was well-tolerated, they did not try to determine the minimally effective dose. Because the dose was effective, they did not try to determine whether higher doses would be more effective.

2.2. Analysis of Current Treatment Options

There are no drugs specifically approved to treat PPD. Drugs approved for the treatment of major depressive disorder (MDD) are used to treat PPD (see Table 2); however, efficacy data are sparse. Non-drug treatments, such as electroconvulsive therapy (ECT), repetitive transcranial magnetic stimulation (rTMS), and psychotherapy, are also used.

All available depression treatments show a delay in time-to-effect. Antidepressant drugs take approximately 4 weeks to demonstrate efficacy. Similarly, a course of ECT is twice per week for 4 or 5 weeks, rTMS is given daily for 4 to 6 weeks, and psychotherapy usually involves 8 to 20 weekly sessions.

Table 2. Drugs Approved for Treatment of Major Depressive Disorder.

Class	Drug	Initial Approval	Route of Administration
Monoamine oxidase inhibitor (MAOI)	isocarboxazid	1959	oral
	tranylcypromine	1961	oral
	phenelzine	1961	oral
	selegiline	2006	transdermal
Tricyclic Antidepressant (TCA)	desipramine	1964	oral
	protriptyline	1967	oral
	doxepine	1969	oral
	imipramine	1973	oral
	nortriptyline	1977	oral
	amitriptyline	1977	oral
	trimipramine	1979	oral
Tetracyclic	mirtazapine	1996	oral
Triazolopyridine	trazodone	1981	oral
Aminoketone	bupropion	1985	oral
Selective Serotonin Reuptake Inhibitor (SSRI)	fluoxetine	1987	oral
	sertraline	1991	oral
	paroxetine	1992	oral
	citalopram	1998	oral
	escitalopram	2002	oral
	vortioxetine	2013	oral
SSRI/5HT$_{1A}$ Partial Agonist	vilazodone	2011	oral
Serotonin-Norepinephrine Reuptake Inhibitor (SNRI)	venlafaxine	1997	oral
	duloxetine	2004	oral
	desvenlafaxine	2008	oral
	levomilnacipran	2013	oral

3 Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Brexanolone has not been approved or marketed in the United States.

3.2. Summary of Presubmission/Submission Regulatory Activity

On June 17, 2014, the Sponsor submitted Investigational New Drug (IND) application 122279 for brexanolone with the intention of providing documentation to support the initiation of a phase 2a study, entitled "An Open-Label Proof-of-Concept Study Evaluating the Safety, Tolerability, Pharmacokinetics, and Efficacy of Sage-547 Injection in the Treatment of Adult Female Patients

with Severe Postpartum Depression." The Division determined that the protocol was safe to proceed and sent the May Proceed letter on July 31, 2014.

Brexanolone was granted Breakthrough Therapy Designation on August 23, 2016 for the treatment of postpartum depression (PPD). The Breakthrough Therapy designation was based on the results of an open-label study (terminated early for efficacy after four patients had a large response) and a phase 2 randomized, double-blind, placebo-controlled study (Study 547-PPD-202A).

A Type B, IND Multidiscipline Guidance Meeting was held on November 2, 2016 with the Agency to discuss nonclinical and clinical development plans to support product approval.

On October 20, 2017, the Division communicated an Agreed Initial Pediatric Study Plan, which included plans to conduct a clinical study evaluating the efficacy, safety, (b) (4) of brexanolone in adolescent females (age 15 to less than 18 years) with (b) (4) PPD.

The Applicant submitted a Proposed Pediatric Studies Request on November 21, 2017. On March 20, 2018, the Division communicated a Written Request to the Sponsor that included a required randomized, double-blind, placebo-controlled, parallel-group study evaluating the efficacy and safety of brexanolone in adolescent females, 15 years to less than 18 years of age, with PPD.

The Sponsor met with the Division on January 18, 2018 for a pre-NDA meeting to discuss the data cut-off date for studies to be included in the NDA, content and format of the integrated summaries of safety and effectiveness, and content and search terms of the abuse liability package. The Sponsor then submitted the NDA on April 19, 2018.

3.3. Foreign Regulatory Actions and Marketing History

Brexanolone has not been approved or marketed in any other country.

4 Significant Issues from Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Three sites were selected for inspection (Table 3). Site 05 was selected because the site impacted efficacy results and there was no history of inspection. Site 39 was selected for high enrollment and because there was no history of inspection. Site 17 was selected because:

- The site impacted efficacy results

- There was high enrollment
- There was no history of inspection
- There were several major protocol violations
- OSI received a complaint that a subinvestigator pre-signed rating scales (before administering the instruments)

Table 3. Sites Selected for Inspection.

Site Number	Site Information	Study Participation and Enrollment[a]	Inspection Dates (2018)	Classification
05	David Grainger, MD Wichita, KS	547-PPD-202B n=5	August 27-30	No Action Indicated
17	Heather Harrison, DO Orem, UT	547-PPD-202B n=27 547-PPD-202C n=9	August 23-24, 27-31 September 05, 10-14, 17-19	Official Action Indicated
39	David J. Johnson, MD Owensboro, KY	547-PPD-202B n=24 547-PPD-202C n=27	July 09-13	Voluntary Action Indicated

[a]See section 7.1: *Clinical Effectiveness Studies* for more details.

Data collection for the phase 3 studies began on paper, but transitioned to using a tablet with an audio recording of the rating interviews. During the portion of the study when data was collected on paper, inspectors found poor record-keeping at Site 17 regarding the timing of interview assessments. Paper records were kept for 22 patients at this site; 7 of these patients had problematic timing for the primary efficacy assessment: the Hamilton Depression Rating Scale (HAM-D; Table 4).

Table 4. Patients with Assessment Timing Problems at Site 017.

Subject ID	Assessment Time	HAM-D Start Time	Next Assessment Start Time	Calculated Time to Complete HAM-D
(b) (6)	Hour 12	2240	2242	2 min
	Hour 4	1123	1125	3 min
	Hour 72	0826	0829	3 min
	Hour 36	1941	1943	2 min
	Day 21	1055	1057	2 min
	Hour 0	0705	0707	2 min
	Hour 12	2008	2010	2 min

The Site and Applicant's hypothesis regarding these irregularities was that the interview for the HAM-D provided information for several of the scales that were used and that the rater could be

moving between scales as the interview progressed. The OSI reviewer found the explanation inadequate because:

1. It is poor practice to move between scales during the interview,
2. The Applicant only hypothesized what happened rather than asking the rater (who still worked at the site) to make "a definitive statement regarding how he conducted the assessments" (OSI report, p. 5).

Once the assessments were recorded (audio and using a tablet), agreement between the Site 17 rater and a central rater was 97% for the primary efficacy endpoint (HAM-D at Hour 60).

Clinical Reviewer Comment: Based on my own prior experience conducting interviews for research studies, it is not uncommon to move between similar rating scales during an interview. Neither is it uncommon to put the date and time on the documents before starting the interview or after the interview as one is completing the forms. Coupled with the overall picture of efficacy in the three studies and the agreement between the Site 17 and central raters, I am comfortable accepting the data from Site 17.

4.2. Product Quality

The Office of Pharmaceutical Quality recommended approval of this application from a product quality perspective. The drug product is supplied in vials of 100 mg brexanolone in 20 ml of a sterile colorless preservative-free aqueous solution (5 mg/mL). The drug product is intended for dilution by a pharmacist prior to administration. The 60-hour infusion begins with a starting dose of 30 mcg/kg/h for 4 hours, which is increased to 60 mcg/kg/h for 20 hours, and further increased to 90 mcg/kg/h for 28 hours. The dose is then decreased to 60 mcg/kg/h for 4 hours, and finally to 30 mcg/kg/h for the last 4 hours.

In-use stability studies found that the diluted solutions can be stored for a maximum of 12 hours at room temperature as longer storage can support adventitious microbial growth. Studies found that the diluted product can be stored for up to 96 hours at refrigerated conditions prior to the 12 hours room temperature infusion. Therefore, the 60-hour infusion will generally require the preparation of five infusion bags. Additional bags will be needed for patients weighing ≥ 90 kg.

The original application proposed [redacted] (b) (4) single brexanolone concentration and by varying the infusion rate, as this was thought to be more in line with common practice.

As the drug substance is very water insoluble, a considerable quantity of a cyclodextrin solubilizer is employed, betadex sulfobutyl ether sodium. This is also known by one of its brand names, Captisol. Each vial contains 5 g of this excipient (250 mg/mL). The diluted drug product was found to be compatible with just one type of infusion tubing (polyolefin, non-DEHP, non-latex IV bag and the PVC, non-DEHP, nonlatex, no filter tubing system). An initial extractable study to on polyethylene-lined nitroglycerin infusion lines found significant levels of extractables. The compatible infusion bag and tubing are clearly identified in the labeling.

A 36-month drug product expiry period was found acceptable (refrigerated storage). Unused residual brexanolone will need to be discarded each day (i.e., should not be used for the next day's doses).

4.3. Clinical Microbiology

The label instructions indicate that the drug product can be administered via intravenous (IV) infusion at (b) (4). The drug product is to be diluted with sterile water for injection first and further diluted with 0.9% sodium chloride injection. In the original NDA submission, the Applicant provided in-use stability data that supported the storage of the reconstituted product up to (b) (4) hours under refrigerated conditions followed by 12 hours at room temperature conditions. As part of a quality information amendment (submitted December 13, 2018), the Applicant provided additional data that supported (b) (4) refrigerated storage conditions to up to 96 hours for the IV bag admixture. Longer refrigerated storage could simplify the preparation process which may reduce medications errors by allowing all bags for a single patient's 60-hour infusion period to be prepared at one time.

4.4. Devices and Companion Diagnostic Issues

Not applicable to this application.

5 Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

This application submitted by Sage Therapeutics is a 505(b)1 NDA for Zulresso (brexanolone). The proposed indication is for the treatment of postpartum depression (PPD). Brexanolone is a formulation of allopregnanolone in sulfobutyl ether beta-cyclodextrin (SBECD; Captisol) for intravenous (IV) administration. The maximum recommended human dose (MRHD) is 90 µg/kg/h (130 mg/day for a 60-kg body weight) with a dosing regimen consisting of a 4-hour dose titration at 30 µg/kg/h, a 20-hour dose titration at 60 µg/kg/h, a 28-hour maintenance period at 90 µg/kg/h, a 4-hour taper at 60 µg/kg/h, and a 4-hour taper at 30 µg/kg/h for a total of a 60-hour infusion.

Allopregnanolone is an endogenous neuroactive steroid and a metabolite of progesterone. Allopregnanolone and other neuroactive steroids bind with high affinity to $GABA_A$ receptors and act as $GABA_A$ receptor modulators. The binding site for neuroactive steroids on $GABA_A$ receptors is distinct from those of GABA, benzodiazepines, and barbiturates. Brexanolone (SAGE-547) was shown to potentiate GABA-mediated currents in mammalian cells expressing $\alpha_1\beta_2\gamma_2$ receptor subunits, $\alpha_4\beta_3\delta$ receptor subunits, and $\alpha_6\beta_3\delta$ receptor subunits of $GABA_A$ receptors and to inhibit binding of a GABA receptor antagonist to the picrotoxin/convulsant site on $GABA_A$ receptors. A pharmacodynamic drug interaction was observed between four GABAergic compounds (pentobarbital, midazolam, diazepam, and propofol) and brexanolone in vitro.

Brexanolone has low oral bioavailability in mice and rats and is highly protein bound in plasma. Brexanolone distributes to the brain rapidly in mice and rats (0.25 to 0.5 hr after IV infusion) with a greater exposure in brain compared to plasma. Brexanolone is extensively metabolized in rats and dogs and there are species differences for the metabolic profiles. In addition, a sex difference was observed for the metabolic profile in rats but not dogs. The three unique human metabolites M133, M136, and M137 (sulfate or glucuronide conjugates of C20-reduced forms of allopregnanolone) are not of toxicological concern since they are readily excreted, and no additional studies are needed.

General toxicology studies with up to 28-days of continuous IV administration in two species (rat and dog) were conducted to support acute use of brexanolone. General toxicities observed in rats and dogs that might have clinical relevance were sedative anesthesia, sedation, and convulsions. Sedative anesthesia was observed rapidly after single bolus IV doses of brexanolone in rats and dogs at 0.75- and 2-times, respectively, the MRHD based on body surface area, which were the lowest doses tested. Signs of sedation resulting in death or premature euthanizing due to poor clinical condition were observed in 5-day repeat dose toxicity studies in rats and dogs at 14- and 18-times, respectively, the exposures at the MRHD, but not at 10- and 9-times, respectively. Signs of sedation resulting in premature euthanizing due to poor clinical condition were also observed in the 14-day repeat dose toxicity study with continuous IV

administration in rats at 4-times the exposure at the MRHD, but not at 2-times. Signs of sedation were not observed in the 28-day repeat dose toxicity studies in rats and dogs at 5- and 6-times, respectively, the exposures at the MRHD. It should be noted that there was a lot of individual variability in the observed sedation in animals regardless of the dose level used across studies.

A convulsion after dose completion was observed in a single dog in each repeat dose toxicity study-two days after a 5-day infusion at 30 times the exposure at the MRHD, seven hours after a 14-day infusion at 7 times the exposure at the MRHD, and four days after a 28-day infusion at 3 times the exposure at the MRHD. The findings were not observed at 24 times the exposure at the MRHD in the 5-day study, 2 times the exposure at the MRHD in the 14-day study, and at an exposure equivalent to that at the MRHD in the 28-day. It is possible that the convulsions could be due to the rapid discontinuation of brexanolone, which is consistent with modulation of the $GABA_A$ receptor, because dosing was stopped without a taper in the 5-day and 14-day repeat dose toxicity studies; however, a taper of dose administration occurred over a 24-hour period for the 28-day study and the convulsion occurred four days after the end of the infusion. It is possible that a 24-hour taper period is too short for a 28-day continuous infusion. In addition, it appears that the longer the duration of dosing the lower the dose at which a convulsion occurs. However, in the 28-day study the convulsion occurred in a dog dosed with the mid dose, while there were no convulsions noted for dogs dosed with the high dose. Therefore, the observation of these seizures could not be predicted by the dose or the drug cessation method and thus could be dependent on individual variability between animals on how they respond to $GABA_A$ receptor modulation and how they manifest this effect after drug cessation. Because the duration of dosing clinically is only 60 hours (52 hours before the start of the taper period) with only 28 hours at the highest dose and with an 8-hour taper period at the end of dosing, convulsions are probably unlikely to occur clinically; however, there occurrence could not be totally ruled out.

Brexanolone was not genotoxic. Brexanolone was not assessed for carcinogenicity because the treatment of PPD with brexanolone infusion is considered acute.

Reproductive and developmental toxicity was observed with brexanolone administration in rats and rabbits. Effects on fertility were observed in female and male rats at 4- and 3-times, respectively, the exposures at the MRHD, but not at 1- and 0.8-times, respectively. Female rats showed signs of pseudopregnancy (prolonged estrous cycle, decreased mating and fertility indices, and increased days to mating) which was reversed or partially reversed after dosing stopped and had increased early resorptions and post implantation loss. Male rats had decreased mating and fertility indices; decreased conception rate and slight increase in days to mating; lower prostate, seminal vesicle, and epididymal weights; and decreased spermatozoa count. Fertility findings in male rats are not a concern clinically for this indication because the patient population is women. However, if additional indications are to be examined in the future in which males are included, the toxicity concerns for the male reproductive system should be reevaluated for relevance to humans.

Malformations were not observed in rats or rabbits at exposures up to 5- and 6-times, respectively, the MRHD. However, developmental and reproductive toxicities were observed in rat (decreased fetal weights) and rabbits (increased abortions, increased number of later resorptions, decreased number of live fetuses, increased pre- and postimplantation loss, and

decreased fetal weights) at 5- and 3- times, respectively the exposures at the MRHD, but not at 2- and 1.2-times, respectively. Fetal toxicity in the rabbit may be related to maternal decreased food consumption and decreased body weight gain/body weight loss that occurred at the same doses as the fetal toxicity. Decreased body weight gain and food consumption for dams were observed in the pre- and postnatal development study in rats during the lactation period which was associated with increased number of dead pups/litters at exposures 2-times the MRHD, but not at 0.8-times the MRHD. There was a decrease in pup viability between postnatal days (PND) 0 to 4 at exposures 5-times the MRHD, but not at 2-times the MRHD. A neurobehavioral deficit, characterized by slower habituation in the maximal startle response in the auditory startle test, was observed on postnatal day 55 in female offspring of dams dosed at 5-times the plasma levels at the MRHD, but not at 2-times the MRHD. Because the offspring were not exposed to test article at the time of testing, the effect on startle is a persistent effect. There were no findings of toxicity for pups after postnatal day 4 and no additional post-weaning pup development toxicity at 5-times the exposure at the MRHD.

Published animal studies have reported that administration of drugs that enhance GABAergic inhibition to neonatal rats caused widespread apoptotic neurodegeneration in the developing brain. The window of vulnerability to these effects in rats (PND 0 to 14) corresponds to the period of brain development that takes place during the third trimester of pregnancy in humans and may be up to three years of age. Although allopregnanolone is an endogenous neuroactive steroid, it is not clear if there is a threshold effect above which increased apoptotic neurodegeneration would occur. A statement was added to the brexanolone label in Section 8.1 based on the literature data for other drugs with a similar mechanism of action to address the potential risk to administering brexanolone during pregnancy. Additionally, a postmarketing requirement (PMR) to conduct an animal study to determine if these effects will be observed with brexanolone to allow for appropriate labeling and to reflect any risks to patients has been requested and accepted by the Applicant.

Brexanolone appears to have little-to-no local tolerance toxicity. Brexanolone is not expected to cause photosensitivity based on a low absorbance in the UV-visible spectrum range. In addition, brexanolone showed no sign of dermal irritation, skin sensitization, or hemolytic potential. Brexanolone showed signs of only minor eye irritation (reddening, discharge, and swelling of conjunctivae) in rabbits one-hour postdose that was resolved 24-hours postdose and that was lower than the irritation scores available for classification.

SBECD is an excipient with known animal toxicology findings including renal tubular vacuolation and foamy macrophages in the liver and lungs of rats and dogs. However, SBECD is an excipient present in approved drugs for IV administration at amounts much higher than will be used in the clinical formulation and for longer durations of dosing than will be used for this indication. Therefore, there are no clinically relevant concerns for the use of this excipient in this formulation at the levels used and for the proposed duration.

Recommendation: The Applicant has provided sufficient nonclinical safety information on brexanolone to support approval for short term use in PPD from the Pharmacology/Toxicology perspective.

5.2. Referenced NDAs, BLAs, DMFs

None

5.3. Pharmacology

Primary Pharmacology
Allopregnanolone is an endogenous neuroactive steroid. It is a metabolite of progesterone that is formed via 5-α reductase and 3-α hydroxy-steroid dehydrogenase in the corpus luteum of the ovary, adrenal cortex, and central nervous system (CNS) (Paul et al. 1992). It has been reported that endogenous concentrations of allopregnanolone measured in humans are at their highest in women during the third trimester of pregnancy and are approximately 159 nM (~50 ng/mL) at time of parturition (Luisi et al. 2000) with a large proportion produced by the placenta (Pasca et al. 2010). However, more recent publications report lower third trimester serum/plasma allopregnanolone levels ranging from 26 to 70 nM (8.15 – 22 ng/mL; Pennell et al. 2015; Gilbert Evans et. al. 2005; Paoletti et al. 2006; Parizek et al. 2005). Endogenous serum concentrations of allopregnanolone in women in the follicular phase of the menstrual cycle and men are approximately 0.8 nM (0.25 ng/mL; Genazzani et al. 1998).

Allopregnanolone binds stereo-selectively and with high affinity to GABA receptors. The GABA type A ($GABA_A$) receptor is the principal pharmacologic target of neuroactive steroids in the CNS (Paul et al. 1992). The binding site for neuroactive steroids on $GABA_A$ receptors is distinct from those of GABA, benzodiazepines, and barbiturates. Neuroactive steroids were originally examined for their sedative-anesthetic properties in the 1940s; however, because of problems with solubility, bioavailability, and pharmacodynamic profile, interest in steroid anesthetics declined (Paul et al. 1992).

Brexanolone potently inhibited [^{35}S]-TBPS (tert-butylbicyclophosphorothionate; a GABA receptor antagonist) binding to the picrotoxin/convulsant site on $GABA_A$ receptors with a Ki = 18 nM and an IC_{50} = 22 nM (Study No. SSN-403). Brexanolone potentiated GABA-mediated currents from recombinant human $GABA_A$ receptors; a concentration dependent enhancement of GABA-evoked currents was observed in mammalian cells expressing $\alpha_1\beta_2\gamma_2$ receptor subunits, $\alpha_4\beta_3\delta$ receptor subunits, and $\alpha_6\beta_3\delta$ receptor subunits of $GABA_A$ receptors (Table 5). Brexanolone inhibited overall firing of cultured cortical neurons from mouse with an EC_{50} = 1.4 μM (Study No. SSN-01730).

Table 5. Brexanolone (SAGE-547) potentiation of recombinant human $GABA_A$ Receptors.

SAGE-547 Lot Number[a]	Cell Type	Receptor Type	EC_{50} in nM	E_{max} (%)	Sage Study Number
SGE-102 lot not specified	Ltk	$\alpha_1\beta_2\gamma_2$	60	380	SSN-401
SGE-102 lot not specified	CHO	$\alpha_4\beta_3\delta$	80	418	SSN-402
SGE-00102-03-A	CHO	$\alpha_6\beta_3\delta$	155	624	SSN-1097-SGE-00102
SGE-00102-03-A	Ltk	$\alpha_1\beta_2\gamma_2$	290	642	SSN-01593
SGE-00102-10-A	Ltk; CHO	$\alpha_1\beta_2\gamma_2$; $\alpha_4\beta_3\delta$	234; 104	1749.3; 1483.1	SSN-01432
SGE-00102-11-A	Ltk; CHO	$\alpha_1\beta_2\gamma_2$; $\alpha_4\beta_3\delta$	528; 68	2905.8; 1135.5	SSN-01434

Abbreviations: α = alpha; β = beta; δ = delta; γ = gamma; CHO = Chinese hamster ovary cells; EC_{50} = half maximal effective concentration; E_{max} = maximum effect.
[a] SAGE-547 is equivalent to SGE-102 and SGE-00102.
Source: Applicant's Pharmacology Written Summary, p.11.

Consistent with the pharmacology of a $GABA_A$ receptor modulator, brexanolone had anticonvulsant properties (dose-dependently) in rodent seizure models, sedative effects, and altered rodent motor function in locomotor activity assays due to sedation (Study Nos. SSN-005.2, -009, -111, -197, -583, -591, -667, -714, -728, -734, -423, and -474). These findings are consistent with studies in the literature for allopregnanolone and consistent with findings of sedation/somnolence observed clinically and in animal general toxicology studies.

Because there is a potential for pharmacodynamic (PD) drug interactions between different classes of GABAergic drugs due to the multiple modulator binding sites present on $GABA_A$ receptors, PD drug interaction studies were conducted for brexanolone and four GABAergic compounds (pentobarbital, midazolam, diazepam, and propofol; Study Nos. SSN-647-SGE-00102, SSN-685-SGE-00102, SSN-686-SGE-00102, and SSN-1031, respectively). Synergism on the effect of $GABA_A$ receptor (either by lowering the EC_{50} or increasing the E_{max}, or both) was observed in vitro when brexanolone was co-applied with pentobarbital, midazolam, diazepam, and propofol (Table 6). Although an EC_{50} and E_{max} were not determined for the combination of diazepam and brexanolone, in the presence of 0.1 µM diazepam brexanolone modulated GABA-induced chloride currents (I_{GABA}) in a concentration-dependent manner. In addition, there was a 715% enhancement of I_{GABA} at the highest concentration of brexanolone tested (10 µM).

Table 6. Combination effects of brexanolone and other $GABA_A$ receptor modulators at $\alpha_1\beta_2\gamma_2$ $GABA_A$ receptors.

Sage Study Number	Treatment	$\alpha_1\beta_2\gamma_2$	$\alpha_1\beta_2\gamma_2$
		EC_{50} (nM)	E_{max} (%)
SSN-01593	0.01 to 10 µM SAGE-547[a]	290	642
SSN-686-SGE-00102	0.01 to 10 µM SAGE-547 [a] + 0.3 mM pentobarbital	108	979
SSN-1031	0.01 to 10 µM SAGE-547 [a] + 0.01 µM midazolam	321	1199
SSN-647-SGE-00102	0.01 to 10 µM SAGE-547 [a] + 0.1 µM diazepam	Not Determined	Not Determined
SSN-685-SGE-00102	0.01 to 10 µM SAGE-547 [a] + 0.1 µM propofol	76	437
SSN-685-SGE-00102	0.01 to 10 µM SAGE-547 [a] + 0.02-20 µM propofol (2:1 ratio of propofol:SAGE-547[a])	139	803

Abbreviations: α = alpha; β = beta; γ = gamma; EC_{50} = half maximal effective concentration; E_{max} = maximum effect.
[a] SAGE-547 is equivalent to SGE-102 and SGE-00102.
Source: Applicant's Pharmacology Written Summary, p.28.

Established Pharmacologic Class

The established pharmacologic class (EPC) for brexanolone was determined to be the following: neuroactive steroid gamma-aminobutyric acid (GABA) A receptor positive modulator. Although brexanolone is a $GABA_A$ receptor positive allosteric modulator, which is similar to the EPC for zolpidem (a non-benzodiazepine sedative that binds to the benzodiazepine modulatory site on $GABA_A$ receptors), it has been established in the literature that allopregnanolone binds to a different modulatory site on the $GABA_A$ receptor than the benzodiazepine modulatory site (Hosie et al. 2006). Therefore, the additional term "neuroactive steroid" is added because it is widely used in the literature and would be clinically meaningful to practitioners.

Metabolites of Brexanolone

The following three metabolites were identified as unique major human metabolites of brexanolone (sulfate or glucuronide conjugates of C20-reduced forms of allopregnanolone) and were examined for inhibition of [35^S]-TBPS binding (Study No. SSN-5191): M133 (SGE-03211), M136 (SGE-03212), and M137 (SGE0-3227). Binding affinities for the metabolites and brexanolone (for comparison) are shown in Table 7. M133 was the only metabolite for which an IC_{50} value could be calculated. In an in vitro assay using Ltk (leukocyte tyrosine kinase) cells, M133 was a weak negative modulator of I_{GABA} through $\alpha_1\beta_2\gamma_2$ receptor subunits (Table 8; Study No. SSN-05351).

Table 7. In vitro binding data for brexanolone and the brexanolone metabolites M133, M136, and M137.

Compound	IC50	Ki
Brexanolone	22 nM (7 ng/mL)	18 nM (6 ng/mL)
M133	310 nM (124 ng/mL)	250 nM (100 ng/mL)
M136	>9.5 µM	
M137	No inhibition up to 10 µM	

Table 8. Mean peak current effect of brexanolone metabolite, M133, in $\alpha_1\beta_2\gamma_2$ receptor subunits of $GABA_A$ receptors.

Concentration	Mean Peak Current Modulation
0.01 µM	-18.36%
0.1 µM	-22.12%
1 µM	-30.41%
10 µM	-59.80%

Secondary Pharmacology

Brexanolone receptor binding selectivity was assessed in two CEREP screening panels consisting of the same cellular and nuclear receptors, enzymes, and transporters (over 70 targets assessed; Study Nos. SSN-404 and SSN-1158-SGE-00102) and a second panel containing targets specifically of interest to assess abuse potential (Study No. SSN-01096). Brexanolone inhibited binding of the reference antagonist to progesterone and androgen receptors, to the $GABA_A$ receptor (Cl^- ion channel), and to the Sigma receptor (non-selective or Sigma 1 receptor) and enhanced binding of the reference agonist to the benzodiazepine site on the $GABA_A$ receptor (Table 9). Brexanolone did not have appreciable activity at other CNS receptors and transporters, including NMDA receptor and serotonin transporter and receptors (% inhibition ranged from -30.1 – 18.2).

Table 9. Summary of brexanolone-related displacement of receptor ligand binding by greater than 50%.

Sage Study Number	SAGE-547 Concentration (µM)	Inhibition (%) of Receptor Ligand Binding					
		Cl- ion channel (r)	BZD ion channel (r)	AR (h)	PR (h)	Sigma (h)	Sigma 1 (h)
SSN-404	10[a]	97.2	- 68.3	81.8	60.8	<50	NT
SSN-1158-SGE-00102	10[a]	96.8	-80.1	86.7	82.2	59.3	NT
SSN-01096	10[a]	NT	NT	NT	NT	NT	70.6

Abbreviations: AR = androgen receptor; BZD = benzodiazepine; Cl = chloride; PR = progesterone receptor
Source: (h) = human recombinant; (r) = rat cerebral cortex; NT= not tested
[a]Equivalent to 3185 ng/mL.

Source: Applicant's Pharmacology Written Summary, p.18.

In an in vitro screen for nuclear hormone receptors (Study No. SSN-405); brexanolone inhibited activity at the androgen and progesterone receptors, inhibited the beta estrogen receptor, increased activation of the progesterone receptors, and increased activity at the alpha estrogen receptor, all in a concentration-dependent manner (Table 10). The Applicant did not conduct further studies to determine IC50 values because the effects were modest and occurred at concentrations greater than clinical brexanolone plasma concentrations (approximately 2-, 9-, and 43-times for 0.4, 2, and 10 µM, respectively, the clinical concentration at steady-state).

Table 10. Summary or activity by brexanolone on nuclear receptor ligand binding.

Test Compound	Concentration (µM)[a]	Fold Increase in Activation		Activity Inhibition (%)		
		ERα (h)	PR (h)	AR (h)	ERβ (h)	PR (h)
SAGE-547	0.4	0.92	**12.61**	-2.5	6.2	23
	2	1.43	**28.21**	35	23	32
	10	**2.25**	**20.76**	**87**	**55**	**64**
DMSO	0.1%	1.0	1.0	0	0	0

AR = androgen receptor; DMSO = dimethylsulfoxide; ERα = estrogen receptor-α; ERβ = estrogen receptor-β; PR = progesterone receptor
Source: (h) = human recombinant
Bolded numbers represent those considered to be of possible biological significance
[a] 0.4, 2, and 10 µM are equivalent to 127, 637, and 3,185 ng/mL, respectively.
Source: Applicant's Pharmacology Written Summary, p.19.

Safety Pharmacology

CNS safety pharmacology was evaluated as part of the 14-day rat toxicology study using a Functional Observation Battery (FOB). Cardiovascular and respiratory safety pharmacology was evaluated as part of the 14-day dog toxicology study using electrocardiograms and indirect blood pressure measurements and a heated pneumotaches, respectively.

Study/Study No.	Findings
CNS (Rat)/SSN-605 (GLP)	No adverse findings were noted at exposures 5- to 6-times the clinical exposures at the MRHD.
hERG Assay/ SSN-634 (GLP)	IC50 > 6.6 μM (SAGE-547 insoluble in assay vehicle at higher concentrations), which is greater than 28-times the clinical exposures at the MRHD.
Cardiac Channel Inhibition/ SSN-406 (non-GLP)	IC50 > 30 μM for inhibition of Cav1.2, Nav1.5, hERG, Kv1.5, Kv4.3/KChIP2.2, KvLQT1/minK, Kir2.1, and HCN2 channels which is greater than 100-times the clinical exposures at the MRHD.
CV (Dog)/SSN-606 (GLP)	No test article-related effect on ECG parameters at exposures 6-times the clinical exposures at the MRHD.
Respiratory (Dog)/ SSN-606 (GLP)	No test article-related effect on respiratory parameters at exposures 7-times the clinical exposures at the MRHD. However, changes in respiration were noted in some rats and dogs under anesthesia or showing signs of sedation in acute and 14-day general toxicology studies.

5.4. ADME/PK

Type of Study	Major Findings
Absorption (Study Nos. SSN-062, SSN-060, SSN-383, SSN-675, SSN-01201, SSN-01429, SSN-01430)	Oral bioavailability is low for mice and rats (0.563% and 2.32%, respectively). **Table 11: PK of brexanolone in mice, rats, and dogs following single bolus IV administration** Parameter / Mice[a] / Rats[b] / Dogs[c] AUC_{last} (ng.h/mL) / 407 / 654 – 1200 / 331 $T_{1/2}$ (h) / 0.727 / 0.409 – 4 / 1.61 CL (L/kg/h) / 12.2 / 4.18 – 6.89 / 6.03 V_{ss} (L/kg) / 3.97 / 1.87 – 16 / 1.57 [a]dose = 5 mg/kg in 30% SBECD; [b]dose = 5 mg/kg in 15 – 30% SBECD; [c]dose = 2 mg/kg in 26% SBECD; AUC_{last}: area under the curve from zero to the time of the last quantifiable concentration; CL: clearance; $T_{1/2}$: terminal elimination half-life; V_{ss}: volume of distribution at steady-state *Note: Other routes of administration, including, intraperitoneal and subcutaneous were not included here because this review is focused on the clinical route of administration which is IV. However, oral bioavailability is included because of the potential for babies to be exposed through breastmilk. See* Table 78 *in Appendix for PK for oral administration in mice and rats.*

Type of Study	Major Findings
Distribution	
Protein Binding (Study Nos. SSN-408, SSN-01423, SSN-02178)	Brexanolone is highly bound to protein (>99%) with no apparent concentration dependent effect on binding. Brexanolone has a higher affinity for human serum albumin (HAS) than α1-acid glycoprotein (AAG; 99.75% bound vs. 54.02% bound). Brexanolone metabolites M133 and M136 are also highly bound to human plasma proteins (>99% and >98.5%, respectively) and do not show a concentration dependent effect on binding.
Brain Distribution (Study Nos. SSN-060, SSN-062, SSN-400, SSN-01201, SSN-01429, SSN-01430)	Brexanolone distributed to the brain in mice and rats rapidly (as soon as 0.25 to 0.5 hr after IV infusion) with a brain to plasma ratio ≥1 (range 1.87 to 3.03) following IV bolus administration and other routes of administration, including oral. After IV bolus (15 mg/kg) and a 2-hr infusion (8 mg/kg/h) in rat, brain to plasma ratio is 2.15 and CSF to plasma ratio is 0.00365.
In Vivo (Study No. SSN-01206)	^{14}C-SAGE-547-related radioactivity was extensively distributed in tissues and organs with peak radioactive concentration in plasma at 5 hours post start of infusion (the end of the continuous infusion) in rats. The highest radioactivity concentrations were found in gastrointestinal tract contents and bile. Radioactivity concentrations were observed in CNS tissues at higher than blood concentrations and in testis at low levels. ^{14}C-SAGE-547-related radioactivity was not associated with melanin-containing tissues.
Metabolism	
In Vitro (Study No. SSN-410, SSN-594)	Brexanolone was rapidly metabolized in mouse, rat, dog, monkey, and human hepatocytes. The major metabolic pathway involves oxidation of the 3-hydroxyl moiety of brexanolone to 3-ketone metabolite, followed by epimerization to form 3β-epimer brexanolone. Other metabolic pathways include hydroxylation, glucuronidation, hydroxylation followed by glucuronidation of brexanolone or its 3 β -epimer. These metabolites were formed in all species. CYP2C8, CYP2C9, CYP2C19, CYP3A4, UGT2B7, and UGT2B17 are involved in the metabolism of SAGE-547 in vitro.

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>In Vivo
Rat: 5-hour IV infusion of ^{14}C-SAGE-547 (Study No. SSN-01206)</td><td>Brexanolone was extensively metabolized in male and female rats to produce 84 radioactive components (See Figure 29 in Appendix for proposed metabolic pathway). Hydroxylation followed by glucuronidation was the predominant biotransformation pathway. The metabolite profiles were different for males and females; brexanolone accounted for 33% of total plasma radioactivity in males while it accounted for only 7% of total plasma radioactivity in females. Circulating metabolites of brexanolone accounted for approximately 12% and 43% of total plasma radioactivity in male and female rats, respectively.

Table 12: Mean blood and plasma PK parameters for total radioactivity following a 5-hour IV infusion in rats
<table><tr><th>Parameter</th><th>Male Blood</th><th>Male Plasma</th><th>Female Blood</th><th>Female Plasma</th></tr><tr><td>T_{max} (h)</td><td>5</td><td>5</td><td>5</td><td>5</td></tr><tr><td>C_{max} (ng eq/g)</td><td>172</td><td>277</td><td>387</td><td>665</td></tr><tr><td>$T_{1/2}$ (h)</td><td>8.02</td><td>8.45</td><td>15.11</td><td>11.1</td></tr><tr><td>AUC_{0-t} (ng eq.hr/g)</td><td>1225</td><td>2049</td><td>3621</td><td>6325</td></tr><tr><td>$AUC_{0-\infty}$ (ng eq.hr/g)</td><td>1355</td><td>2316</td><td>5330</td><td>8110</td></tr></table>T_{max} = time to reach C_{max}; C_{max} = maximal concentration observed; $T_{½}$ = half-life; AUC_{0-t} = area under the curve from 0 to T_{last}; $AUC_{0-\infty}$ = area under the curve from 0 to infinity; eq = equivalents ^{14}C-SAGE-547</td></tr>
<tr><td>Dog: 5-hour IV infusion of ^{14}C-SAGE-547 (Study No. SSN-871)</td><td>Brexanolone was rapidly and extensively metabolized in male and female dogs to produce 48 quantifiable radioactive components (See Figure 30 in Appendix for proposed metabolic pathway). Oxidation was the exclusive biotransformation mechanism identified in dogs. There was no substantive sex difference.

Table 13: Mean blood and plasma PK parameters for total radioactivity following a 5-hour IV infusion in dogs
<table><tr><th>Parameter</th><th>Male</th><th>Male</th><th>Female</th><th>Female</th></tr></table></td></tr>
</table>

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>Excretion
(Study No. SSN-675, SSN-01206, & SSN-871)</td><td>
<table>
<tr><th></th><th>Blood</th><th>Plasma</th><th>Blood</th><th>Plasma</th></tr>
<tr><td>T_{max} (h)</td><td>5</td><td>5</td><td>5</td><td>5</td></tr>
<tr><td>C_{max} (ng eq/g)</td><td>1090</td><td>1340</td><td>1030</td><td>1270</td></tr>
<tr><td>$T_{1/2}$ (h)</td><td>19.1</td><td>15.1</td><td>18.7</td><td>16</td></tr>
<tr><td>AUC_{0-t} (ng eq.hr/g)</td><td>18173</td><td>20355</td><td>18354</td><td>22859</td></tr>
<tr><td>$AUC_{0-\infty}$ (ng eq.hr/g)</td><td>19463</td><td>21210</td><td>19695</td><td>24072</td></tr>
</table>
T_{max} = time to reach C_{max}; C_{max} = maximal concentration observed; $T_{½}$ = half-life; AUC_{0-t} = area under the curve from 0 to T_{last}; $AUC_{0-\infty}$ = area under the curve from 0 to infinity; eq = equivalents ^{14}C-SAGE-547

Minimal amounts of brexanolone is excreted in urine, bile, or feces of rats and dogs. After an IV bolus administration of 5 mg/kg brexanolone (in 30% SBECD) to male rats, 0.27% of brexanolone was measured in urine and renal clearance was calculated as 0.0132 L/hour/kg. ^{14}C-SAGE-547-related radioactivity was excreted in rats primarily as hydroxylated SAGE-547 (brexanolone) metabolites in feces (79.3% in males and 69.2% in females), while excretion of radioactivity in urine was low (5.64% in males and 10.9% in females). ^{14}C-SAGE-547-related radioactivity was excreted in dogs as SAGE-547 dependent metabolites with similar amounts in urine and feces for male and female dogs (M: 43.3% in urine & 44.5% in feces; F:47.6% in urine & 45.2% in feces).</td></tr>
<tr><td>Drug Interaction
Transporters (Study Nos. SSN-01157, SSN-01311, & SSN-02081)</td><td>Brexanolone did not inhibit the BCRP, P-gp, or MRP-2 transporters at concentrations up to 25 µM (highest concentration tested) and the BSEP, MRP3, MRP4, MATE1, MATE2-K, OAT1, OAT3, OATP1B1, and OATP1B3 transporters up to 2 µM (highest soluble concentration). Brexanolone inhibited OCT1 and OCT2 in a concentration-dependent manner (OCT1: IC50 = 0.41 µM (130.6 ng/mL), OCT2: maximum relative inhibition = 45% at 2 µM (637.8ng/mL)). Brexanolone is unlikely a substrate for BCRP, MDR1, BSEP, OATP1B1, OATP1B3, and OCT1.

Brexanolone metabolites M133, M136, and M137 inhibited BCRP, BSEP, MDR1, MRP3, MRP4, OAT1, OAT3, OATP1B1, OATP1B3, and/or OCT1 transporters at concentrations up to 25 µM (see Table 79 in Appendix for summary of transporter inhibition for metabolites).</td></tr>
</table>

<table>
<tr><th>Type of Study</th><th>Major Findings</th></tr>
<tr><td>Enzyme Induction and Inhibition (Study Nos. SSN-409, SSN-411, SSN-412, SSN-01539, SSN-01924, SSN-01925, & SSN-02080)</td><td>Brexanolone inhibited CYP2C9 (IC_{50} = 0.41 µM & Ki = 0.256 µM) and UGT2B17 (IC50 = 1.7 µM) but did not substantially inhibit CYP1A2, 2C19, 2C8, 2D6, 3A4, or 2B6 isozyme activities or UGT1A1, 1A3, 1A4, 1A6, 1A9, 2B7, and 2B15 in human liver microsomes. (see Table 80 in Appendix for IC_{50} values).

In an in vitro cytochrome P450 induction assay, 30 – 50 µM concentrations of SAGE-547 induced CYP2B6 activity (2.5 – 4-times) but showed no effect on CYP1A2 and CYP3A. CYP2B6 mRNA was induced 2.8-times in one of three donor hepatocyte cultures, but CYP1A2, 3A4, 2C9, and UGT1A9 mRNA were unaffected by brexanolone treatment.

Brexanolone metabolites M133, M136, and M137 showed low potency inhibition at CYP isoforms tested (see Table 81 in Appendix for results).</td></tr>
<tr><td>TK data from general toxicology studies
<u>Rat</u>: 28-day continuous IV infusion (Study No. SSN-01272)
• Samples collected 24, 96, 240, 384, 528, and 672 hours after the start of the infusion on Day 1 and 2 and 24 hours after the end of infusion on Day 29
• TK parameters were estimated using Phoenix PK software.
• NOAEL is 60 mg/kg.

<u>Dog</u>: 28-day continuous IV infusion (Study No. SSN-01273)
• Samples collected 24, 168, 336, 504, and 672 hours after the start of the infusion on Day 1
• TK parameters were estimated for brexanolone and metabolite SGE-136 using Phoenix PK software.</td><td>Dose proportionality: approximately dose proportional
Sex differences: females slightly less than males

Table 14: TK of brexanolone in rats following 28-day continuous IV infusion
<table><tr><th>Parameter</th><th>Sex</th><th>10 mg/kg</th><th>30 mg/kg</th><th>60 mg/kg</th></tr><tr><td rowspan="2">AUC_{24h} (ng.h/mL)</td><td>M</td><td>1790</td><td>4890</td><td>9960</td></tr><tr><td>F</td><td>1410</td><td>4320</td><td>8110</td></tr><tr><td rowspan="2">C_{max} (ng/mL)</td><td>M</td><td>93.8</td><td>233</td><td>464</td></tr><tr><td>F</td><td>63.1</td><td>219</td><td>441</td></tr><tr><td rowspan="2">C_{ss} (ng/mL)</td><td>M</td><td>74.4</td><td>204</td><td>415</td></tr><tr><td>F</td><td>58.8</td><td>180</td><td>338</td></tr></table>$AUC_{24h} = AUC_{(0\text{-}672)}/28$; $C_{ss} = AUC_{(0\text{-}672)}/672$

Dose proportionality: approximately dose proportional
Sex differences: females slightly more than males at ≤36 mg/kg and slightly less than males at 72 mg/kg for brexanolone and females slightly less than males for metabolite SGE-136
Metabolite-to-parent ratio: 0.884 to 1.40

Table 15: TK of brexanolone in dogs following 28-day continuous IV infusion</td></tr>
</table>

Type of Study

- NOAEL is 12 mg/kg.

Major Findings

Parameter	Sex	12 mg/kg	36 mg/kg	72 mg/kg
AUC_{24h} (ng.h/mL)	M	1680	5760	12900
	F	1820	6960	11300
C_{max} (ng/mL)	M	89.3	310	708
	F	97.3	338	571
C_{ss} (ng/mL)	M	69.9	240	538
	F	75.9	290	471

$AUC_{24h} = AUC_{(0-672)}/28$; $C_{ss} = AUC_{(0-672)}/672$

Table 16: TK of brexanolone metabolite SGE-136 in dogs following 28-day brexanolone continuous IV infusion

Parameter	Sex	**12 mg/kg**	36 mg/kg	72 mg/kg
AUC_{24h} (ng.h/mL)	M	**2018**	6788	17970
	F	**1702**	6494	14080
C_{max} (ng/mL)	M	**99.7**	319	986
	F	**86**	345	745
C_{ss} (ng/mL)	M	**84.1**	282	749
	F	**70.9**	271	587

$AUC_{24h} = AUC_{(0-672)}/28$; $C_{ss} = AUC_{(0-672)}/672$

TK data from reproductive toxicology studies

Rat: embryo-fetal development (Study No. SSN-797)

- Samples collected before dosing started on GD 6 and 24, 96, 168, and 240 hours after the start of infusion on GD 6 and 1, 2, 4, and 24 hours after the end of infusion on GD 18.
- TK parameters were estimated using WinNonlin PK software
- NOAEL is 30 mg/kg.

Table 17: TK of brexanolone in pregnant rats

Parameter	15 mg/kg	**30 mg/kg**	60 mg/kg
AUC_{24h} (ng.h/mL)	2430	**4130**	9250
C_{max} (ng/mL)	129	**261**	467
C_{ss} (ng/mL)	101	**172**	385
$T_{1/2}$ (hr)	NC	**26.7**	11.2

NC: Not calculated; $AUC_{24h} = AUC_{(0-288)}/12$; $C_{ss} = AUC_{(0-288)}/288$

Rabbit: embryo-fetal development (Study No. SSN-825)

- Samples collected before dosing started on GD 7 and 1, 4, 8, 24, 96, 168, and 240 hours after the start of infusion on GD 7 and 0.5, 1, and 2 hours after the end of infusion on GD 20.

Table 18: TK of brexanolone in pregnant rabbits

Parameter	**7.5 mg/kg**	15 mg/kg	30 mg/kg
AUC_{24h} (ng.h/mL)	**2217**	4630	11400
C_{max} (ng/mL)	**108**	236	595
C_{ss} (ng/mL)	**88.5**	183	461
$T_{1/2}$ (hr)	**2.74**	2.39	1.68

$AUC_{24h} = AUC_{(0-314)}/13$; $C_{ss} = AUC_{(0-312)}/312$

Type of Study	Major Findings
• TK parameters were estimated using WinNonlin PK software. • NOAEL is 7.5 mg/kg	

C_{ss}: concentration at steady state; GD: gestational day

Pharmacology Reviewer Comment: Concas et al. reported plasma concentrations in rat: ~10 ng/mL during estrus; ~20 ng/mL GD 10, ~40 ng/mL GD 15 and 19, ~15 ng/mL GD 21 and PND 2. Concentrations in rat reproductive studies were 2.5- to 10-times the highest plasma concertation during pregnancy and was 1.6- to 17-times the concentration at the end of pregnancy and to PND 4.

5.5. Toxicology

Pharmacology Reviewer Comment: Although brexanolone-related findings are discussed in detail in the review below, vehicle- and procedure-related findings are only summarized. Further details regarding vehicle- and procedure-related findings can be found in the Nonclinical Pharmacology/Toxicology Section of the Appendix on page 179 and as noted in the review.

5.5.1. General Toxicology

A 28-Day Intravenous Infusion Toxicity Study of SAGE-547 in the Albino Rat Followed by a 28-Day Recovery Period/Study No. SSN-01272

Key Study Findings

- Increased body weight gain at doses ≥10 mg/kg/day for males during the first 7 days of the study and ≥30 mg/kg/day for females throughout the study compared to vehicle controls. Decreased body weight gain at 60 mg/kg/day for males for remainder of dosing and at doses ≥10 mg/kg/day for females for the first 7 days of the recovery period.
- Reduction in ovary weight in females dosed with ≥30 mg/kg, without correlating microscopic findings.
- SBECD (vehicle)-related findings in kidney (increased kidney weight, pink coloration, and vacuolation) and vacuolation in other organs.
- NOAEL is 60 mg/kg/day based on the absence of adverse effects at this dose, which is 5-times the human exposure (AUC24h = 1800 ng.h/mL; Css = 74.3 ng/mL) at the MRHD of 90 µg/kg/h.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	0 (saline), 0 (vehicle), 10, 30, 60 mg/kg/day Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration:	IV infusion
Formulation/Vehicle:	Solution/5 mM Citrate buffer, pH 6 ± 0.2 and 125 mg/mL SBECD diluted in 0.9% saline
Amount SBECD:	3000 mg/kg/day VC and HD; 500 mg/kg/day LD; 1500 mg/kg/day MD
Species/Strain:	Rat/Sprague-Dawley
Number/Sex/Group:	10/sex/group (main); 6/sex/group (recovery; no LD recovery group)
Age:	10 – 11 weeks old
Satellite groups:	3/sex/SC and VC and 6/sex/dose group (TK)
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	**Major findings**
Mortality	No test article or vehicle related. 12 deaths (9 due to inflammatory reaction or thrombosis associated with bacteria at infusion site; 1 accidental; 1 VC due to hepatocellular necrosis; 1 TK cause of death not determined; see Table 82 in Appendix)
Clinical Signs	No test article related effect. Vehicle-related decreased activity starting Day 4 and lasting for 7 days of the recovery period. Vehicle- or procedure-related limited usage of hindlimbs and/or hunched posture. See Table 83 in Appendix.
Body Weights	Males: Increases in body weight gain during first 7 days for all dose groups vs. VC (126, 126, & 132% for LD, MD, & HD, respectively). Decrease in body weight gain at HD for remainder of dosing vs. VC (71% for HD Day 7 to 28). Decreases in body weight gain for HD vs. VC for first 7 days of recovery period but gained similar amounts of weight for the remainder of recovery period. Females: Increases in body weight gain throughout the dosing period at MD and HD vs. VC with largest effect the first 7 days (160 & 193% for MD & HD, respectively, Day -1 to 7; 138 & 123% MD & HD, respectively Day 7 to 28). Decreases in body weight gain for all dose groups vs. VC for first 7 days of recovery period but gained similar amounts of weight for the remainder of recovery period.
Ophthalmoscopy	Unremarkable

Hematology	No test article or vehicle related effects. Changes noted for white blood cells, neutrophils, lymphocytes, monocytes, eosinophil, platelet, and reticulocyte counts were related to macroscopic and microscopic changes observed at the infusion site and are considered secondary to the infusion site reaction. Changes resolved after recovery period except in instances where evidence of inflammation was still present.
Clinical Chemistry	Unremarkable
Urinalysis	Unremarkable
Gross Pathology	No test article related effects. Vehicle-related findings in kidney (pale discoloration and enlargement; see Table 84 in Appendix). Findings at infusion site were observed in rats from all groups and are related to bacterial infections (see Table 85 in Appendix). Swelling was not observed at the end of the recovery period and the incidence of the remaining infusion site changes was lower following the recovery period.
Organ Weights	Decreased ovary weights for MDF and HDF vs. VC (-23 & 26%, respectively); these findings were reversible. Dose-dependent increase in kidney weights for all vehicle dosed groups vs. SC; this finding was still observed after recovery period, but with smaller magnitude. See Table 86 and Table 87 in Appendix.
Histopathology Adequate battery: Yes	No test article related effects. Vehicle-related findings (mostly vacuolation) were observed in the kidney, bladder, infusion site, lymph nodes, heart, bone femur synovium, and uterus and are consistent with known findings for cyclodextrins; incidence and severity of vehicle-related findings were decreased after the recovery period (Table 88 & Table 89 in Appendix). Findings related to the continuous infusion were observed across all groups (Table 90 & Table 91 in Appendix).
Toxicokinetics	See ADME/PK Section page 43.

SC: saline control; VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose

A 28-Day Study of SAGE-547 by Intravenous Infusion in Beagle Dogs with a 28-Day Recovery Period/Study No. SSN-01273

Key Study Findings

- A convulsion occurred 4 days after completing the 28-day infusion in one male dosed with 36 mg/kg.
- Increased severity of SBECD (vehicle)-related renal tubular vacuolation in males at ≥36 mg/kg and females at 72 mg/kg.
- SBECD (vehicle)-related findings included clinical signs, changes in hematology and clinical chemistry parameters, findings in kidney (increased kidney weight, pink coloration, and vacuolation), and increased organ weight and/or vacuolation in other organs.
- NOAEL is 12 mg/kg/day based on the convulsion after dosing completion in one 36 mg/kg dog and increased severity of SBECD-related renal tubular vacuolation at ≥36 mg/kg, which

is at the human exposure (AUC_{24h} = 1800 ng.h/mL; C_{ss} = 74.3 ng/mL) at the maximum recommended human dose of 90 µg/kg/h.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	0 (saline), 0 (vehicle), 12, 36, 72 mg/kg/day Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration:	IV infusion
Formulation/Vehicle:	Solution/5 mM Citrate buffer, pH 6 ± 0.2 and 125 mg/mL SBECD diluted in 0.9% saline
Amount SBECD:	3600 mg/kg/day VC and HD; 600 mg/kg/day LD; 1800 mg/kg/day MD
Species/Strain:	Dog/Beagle
Number/Sex/Group:	4/sex/group (main); 2/sex/group (recovery; no LD recovery group)
Age:	7 – 8 months old
Satellite groups/unique design:	No satellite groups. At the end of 28 days of continuous IV infusion at a rate of 2 mL/kg/h, the infusion rate was reduced to 1.5 mL/kg/h for 8 hours, then reduced to 1 mL/kg/h for 8 hours, then reduced to 0.5 mL/kg/h for 8 hours before dosing was stopped. Dogs that required surgical repairs of the catheter during the dosing period received anesthesia (propofol or sodium thiopental), antibiotics (benzathine penicillin G and procaine penicillin G), and Carprofen® (non-steroidal anti-inflammatory) and were allowed to fully recover prior to recommencing dose administration the following day. As a result of surgical repairs and elevated body temperature dose administration was interrupted for five dogs during the study. For more details see Appendix, page 192.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	No test article or vehicle related. Two deaths (both attributed to an inflammatory reaction; 1 VC due to poor clinical condition; 1 HDF due to severely swollen hind limb that precluded continuation of dosing; see Appendix, page 192 for further details.)
Clinical Signs	Test article-related clinical signs were limited to 1 MDM which had a non-sustained convulsion 4 days after the end of dose administration. Vehicle-related clinical signs included abnormal gait, decreased activity, lying on side, pale skin, weak, tremors, hunched posture, increased body temperature, reduced appetite, and limited usage of hindlimb/forelimb which generally occurred dose-dependently. See Appendix, page 194 for further details.
Body Weights	Unremarkable
Ophthalmoscopy	Unremarkable
ECG	Not performed.
Hematology	No test article related effects. Vehicle-related findings included decreased red blood cell count, platelet count, hemoglobin, and hematocrit and increased white blood cell count, neutrophil count, monocyte count, and fibrinogen vs. SC (see Table 94 in Appendix). There was no effect after the recovery period.
Clinical Chemistry	No test article related effects. Vehicle-related findings included increased alkaline phosphatase and decreased creatine kinase, albumin, A/G ratio, and calcium concentrations vs SC (see Table 95 in Appendix). There was no effect after the recovery period.
Urinalysis	Unremarkable.
Gross Pathology	No test article related findings. Vehicle-related findings in kidney (pale discoloration and enlargement) and iliac and mediastinal lymph nodes (enlargement; see Table 96 in Appendix). Findings at infusion site were observed in dogs from most groups and are related to inflammation and/or thrombosis at the infusion site (see Table 98 in Appendix). Vehicle-related and infusion site findings fully or partially recovered at the end of the recovery period (see Table 97 & Table 99 in Appendix).
Organ Weights	No test article related effects. Vehicle-related increased organ weight changes were observed in the kidney, liver, and spleen (see Table 100 in Appendix). Organ weight changes were not observed after the recovery period.
Histopathology Adequate battery: Yes	Test article- and vehicle-related findings in the kidney (Table 19). A vehicle dose-dependent increase in renal tubular

	vacuolation was observed with an increase in severity for test article groups at ≥36 mg/kg for males and 72 mg/kg for females; there was no test article-related effect and incidence and severity of vehicle-related findings decreased after the recovery period. Vehicle-related findings (all vacuolation) were observed in the bladder, brain, pituitary gland, bone femur synovium, stomach, GALT, heart, adrenal gland, lung, liver, spleen, epididymis, ovary, uterus, cervix, vagina, lymph nodes, and infusion site and are consistent with known findings for cyclodextrins; incidence and severity of vehicle-related findings were decreased or not present after the recovery period (Table 101 & Table 102 in Appendix). Findings related to the continuous infusion were observed across all groups (Table 103 in Appendix).
Toxicokinetics	See ADME/PK Section page 43 .

SC: saline control; VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose.

Table 19. Kidney microscopic findings in 28-day dog repeat dose study.

	Males					Females				
Group	1	2[b]	3	4	5	1	2[b]	3	4	5
SAGE-547 Dose (mg/kg/day)	0	0	12	36	72	0	0	12	36	72
Captisol Dose (mg/kg/day)	0	3600	600	1800	3600	0	3600	600	1800	3600
No. Animals Examined	4	4	4	4	4	4	3	4	4	3
Kidney (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; tubular	(0)[a]	(4)	(2)	(4)	(4)	(0)	(3)	(2)	(4)	(3)
Minimal	0	0	1	1	0	0	1	1	1	0
Mild	0	4	1	1	0	0	2	1	3	0
Moderate	0	0	0	2	4	0	0	0	0	3

Source: Applicant's Table, SSN-01273, p.46.

General toxicology; additional studies

Rat: A single dose IV study (slow bolus) at doses ≤50 mg/kg (Study No. SSN-600), a single slow bolus IV study at 20 mg/kg followed by a continuous IV infusion at doses of 8 – 12 mg/kg/h for 6 – 12 hours a day for 3 days (Study No. SSN-600), a 5-day continuous IV infusion study at doses ≤240 mg/kg/day (Study No. SSN-601), and a 14-day continuous IV infusion study at doses ≤96 mg/kg/day (Study No. SSN-605) were conducted in rat. Rapid anesthesia (within 1 min) was observed with a single bolus dose ≥10 mg/kg and the MTD for slow bolus administration was considered 30 mg/kg due to shallow respiration and death of one male dosed with 50 mg/kg within 5 minutes of the bolus dose. The response to a continuous infusion of 8 – 12 mg/kg/h following an anesthetic slow bolus dose (20 mg/kg) was variable and unpredictable and an MTD could not be determined; some rats showed wakefulness while other rats showed signs of lethargy/decreased activity, unsteady gait, abnormal breathing, and increased respiration and oxygen supplementation was required for some during the infusion. The MTD for the 5-day continuous IV infusion study was 120 mg/kg/day due to signs of sedation resulting in death or premature euthanizing due to poor clinical condition at doses ≥180 mg/kg/day. Tremors and twitches were also observed at doses ≥180 mg/kg/day. In the 14-day study, poor clinical condition (possibly due to sedation and included labored/shallow respiration, slight incoordination, decreased activity, ptosis, paleness of the whole body, and prostration) was

observed in one male and one female at 96 mg/kg/day resulting in early euthanasia of this dose group on Day 11 and one male at 48 mg/kg/day. The NOEL for poor clinical condition (possibly due to sedation) resulting in premature euthanizing was 18 mg/kg/day (AUC_{24} = 2910 ng.h/mL for males and 1790 ng.h/mL for females; C_{max} = 196 ng/mL for males and 111 ng/mL for females).

Dog: A single dose IV study (slow bolus) at doses ≤30 mg/kg (Study No. SSN-599), a single slow bolus IV study at 20 mg/kg followed by a continuous IV infusion at doses of 8 – 48 mg/kg/h for 8 to 10 hours (Study No. SSN-599), a 5-day continuous IV infusion study at doses ≤240 mg/kg/day (Study No. SSN-602), and a 14-day continuous IV infusion study at doses ≤ 72 mg/kg/day (Study No. SSN-606) were conducted in dog. Rapid anesthesia (within 1 min) was observed with a single bolus dose ≥7.5 mg/kg and the MTD for slow bolus administration was considered 20 mg/kg due to irregular breathing in 1/4 dogs at 30 mg/kg. The response to continuous infusion of 8 – 48 mg/kg/h following an anesthetic slow bolus dose (20 mg/kg) was variable and unpredictable (some dogs remained lightly anesthetized at doses that led to edema, while other dogs could not recover from anesthesia (1 dog remained deeply sedated for more than 5 hours after the end of the infusion) and were euthanized). The MTD for the 5-day continuous IV infusion study was 60 mg/kg/day due to signs of sedation resulting in premature euthanizing at doses ≥120 mg/kg/day. Tremors and shaking were observed at 240 mg/kg/day, and one dog with tremors and shaking had a convulsion two days after the 5-day infusion was completed. The NOAEL for the 14-day continuous IV infusion study was 36 mg/kg/day due to a convulsion that occurred in one female dosed with 72 mg/kg/day approximately 7 hours after dosing stopped on Day 15 (AUC_{24} = 3560 ng.h/mL for males and 3840 ng.h/mL for females; C_{max} = 201 ng/mL for males and 210 ng/mL for females).

Pharmacology Reviewer Comment: ***Sedative anesthesia is a concern clinically if there is a rapid administration of dose which might occur in instances of pump malfunction****. Consistent with the known pharmacology of neuroactive steroids as sedative anesthetics, slow bolus IV administration of doses ≥10 mg/kg in rat and ≥7.5 mg/kg in dog (0.75- and 2-times the MRHD based on body surface area, respectively), resulted in rapid anesthesia. In contrast, continuous IV infusions at a rate of 2 mL/kg/h at doses up to 60 mg/kg in rat and 72 mg/kg/day in dog (5- and 6-times the clinical exposure at the MRHD, respectively) did not generally show signs of sedation.*

A convulsion after dose completion was observed in a single dog in each repeat dose toxicity study—two days after a 5-day infusion at 240 mg/kg/day, seven hours after a 14-day infusion at 72 mg/kg/day, and four days after a 28-day infusion at 36 mg/kg/day (the mid dose). In the case of the convulsion in the 5-day and 14-day studies, dosing was stopped without a taper and the convulsions were thought to be due to the rapid discontinuation of brexanolone, which is consistent with modulation of the $GABA_A$ receptor and is observed with benzodiazepines. However, a taper of test article administration occurred over a 24-hour period for the 28-day study and the convulsion occurred 4 days after the end of the infusion. Therefore, it is not clear what the mechanism of these convulsions is. Nevertheless, because a convulsion was observed in each repeat dose study in dogs, a relationship to brexanolone cannot be ruled out. However, because the duration of dosing clinically is only 60 hours (only 28 hours at the highest dose), convulsions are probably unlikely to occur clinically; however, this could not be totally ruled

out.

SBECD is not a concern clinically for a 60-hour infusion. *In the repeat-dose studies conducted in support of this application, SBECD (the vehicle)-related toxicity increased with duration of dosing in rats and dogs. In addition, there appeared to be an increase in severity of kidney toxicity with the addition of brexanolone after 28-days of dosing in dogs, which was not observed after 14-days of dosing. Because the duration of dosing clinically is only 60 hours, the increase in vehicle related findings with increasing duration of dosing are not relevant to humans. In addition, SBECD is an excipient with known animal toxicology findings and is used in marketed drugs for IV administration at much greater amounts than will be administered for brexanolone (*Table 20*).*

Table 20. Comparison of the amount of SBECD administered by IV for the approved drugs amiodarone and voriconazole with brexanolone based on a human body weight of 60 kg.

	Amiodarone	Voriconazole	Brexanolone
Drug: SBECD	1.8 mg:18 mg	200 mg:3200 mg	5 mg:250 mg
Amount SBECD on Day 1	10 g	11.5 g	4.0 g
Amount SBECD for duration of dosing	7.2 g/day for 14 – 21 days	7.7g/day for minimum 14 days	6.5 g on Day 2 2.2 g on Day 3
Cumulative Dose SBECD	101 – 151 g	119 g	12.7 g

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Bacterial Revers Mutation Test/Study No. SSN-635

Key Study Findings

- SAGE-547 was negative for mutagenicity in bacterial cells in a valid Ames test.

GLP compliance: Yes

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

Study is valid: Yes

In Vitro Assays in Mammalian Cells

SAGE-547 In Vitro Micronucleus Test in Human Lymphocytes/Study No. SSN-63

Key Study Findings:

- SAGE-547 was negative for clastogenicity in human lymphocytes in a valid in vitro micronucleus assay.

GLP compliance: Yes

Test system: Human lymphocytes in whole blood culture; doses ≤ 55 µg/mL in DMSO +S9 and ≤ 27.5 µg/mL in DMSO -S9

Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

SAGE-547: In Vivo Rat Micronucleus Assay/Study No. 637

Key Study Findings:

- SAGE-547 was not clastogenic in a valid in vivo micronucleus assay at plasma concentrations 34-times clinical exposure.
- Mean plasma concentrations of SAGE-547 at 0.25 hours after a slow bolus injection of 30 mg/kg was 3315 ng/mL.

GLP compliance: Yes
Test system: rat, bone marrow micronuclei; two slow bolus intravenous injections of 7.5, 15, and 30 mg/kg spaced 24 hours apart; bone marrow was collected 24 hours after the second dose
Study is valid: Yes

5.5.3. Carcinogenicity

Because brexanolone use for PPD is considered a short-term treatment (2.5 days), carcinogenicity studies were not conducted.

5.5.4. Reproductive and Developmental Toxicology

Fertility and Early Embryonic Development

Study of Fertility and Early Embryonic Development to Implantation of SAGE-547 by Intravenous Infusion in Female Rats/Study No. SSN-01271

Key Study Findings

- A dose-dependent increase in body weight gain at doses ≥10 mg/kg/day compared to controls during the premating period and a non-dose-dependent increase in body weight gain from gestational day 0 to 7 which correlated with increased food consumption at 60 mg/kg. A dose-dependent reduction in body weight gain at doses ≥10 mg/kg/day compared to controls after dosing stopped from gestational day 7 to 13 which correlated with decreased food consumption at ≥30 mg/kg.

- Brexanolone administration to female rats resulted in a prolongation of the estrous cycle at 60 mg/kg, decreased mating and fertility indices at ≥30 mg/kg, increased days to mating at ≥10 mg/kg, and increased early resorptions and post implantation loss at 60 mg/kg. These effects reversed or partially reversed after dosing was stopped at 60 mg/kg.

- The NOAEL is 60 mg/kg for maternal toxicity and 10 mg/kg for fertility and reproductive function which is 4.5-times and 0.8-times, respectively, the human exposure (AUC24h = 1800 ng.h/mL; Css = 74.3 ng/mL) at the MRHD.

Conducting laboratory and location [redacted] (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 10, 30, and 60 mg/kg/day Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration:	IV infusion
Formulation/Vehicle:	Solution/5 mM citrate buffer, pH 6 ± 0.2, and 125 mg/mL of SBECD in 0.9% Sodium Chloride

Amount SBECD:	3000 mg/kg/day for VC and HD; 500 mg/kg/day for LD; 1500 mg/kg/day for MD
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	22 females/group
Satellite groups:	None
Study design:	Females were dosed starting 14 days before mating, during mating, and until Gestation Day (GD) 7 and were necropsied on GD 13. Males were not dosed. Unmated females in 0, LD, and MD groups were dosed until completion of the 17-day mating period. Duration of dosing was 23 – 37 days. Because of the low number of HD females that mated, HDF that remained unmated after 9 days of cohabitation were no longer dosed from Study Day 24 and were retained for a recovery period of 4 weeks (Days 24 – 52) to determine reversibility. HDF were mated a second time until they mated or Day 66 for females that did not mate. Rats that failed to mate were necropsied 8 days after the end of the mating period.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	None
Clinical Signs	No test article related effects.
Body Weights	Premating period: A dose-dependent increase in body weight gain for dosed females vs. VC (125, 150, & 213% for LD, MD, and HD, respectively) which correlated with a 15% increase in food consumption for HDFs vs. VC. Gestation: A non-dose-dependent increase in body weights and body weight gain for dosed females vs. VC from GD 0 to 7 (dosing period; 144, 100, & 121% for LD, MD, and HD, respectively). A dose-dependent decrease in body weight gain for dosed females vs. VC from GD 7 to 13 (dosing stopped; 78, 67, & 26%, for LD, MD, and HD, respectively) which correlated with a dose-dependent decrease in food consumption for MDF and HDF vs. VC (89 and 80%, respectively).
Necropsy Findings	No test article-related effects.
Fertility and Pregnancy Parameters	Estrus cycle: An increased incidence of HDF with prolonged periods in diestrus (≥ 4 days; 5/22 controls, 7/22 LD, 3/22 MD, and 11/22 HD) during the premating period that continued into the mating period. During the recovery period, the number of HDF with

	prolonged diestrus and/or abnormal cycles diminished and by 9 days postdose, effects on estrous cycles were no longer observed. However, after the initiation of the second cohabitation period after the recovery period, 5/17 HDFs showed prolonged periods in diestrus. Mating and Fertility: A dose-dependent decrease in mating and a dose-dependent increase in the mean number of days to mating (Table 21). Following the 4-week recovery period, mating and fertility indices remained lower than VC (not a concurrent control) and at the low end or lower than the HCR. There was no effect on the conception rate. Pregnancy: In HDFs that mated during the dosing period, the number of early resorptions and post implantation loss were greater vs. VC and the values were outside the HCR (Table 22). However, because of the low number of HDF that mated, these differences did not reach statistical significance. After the 4-week recovery period, the number of early resorptions and post implantation loss was within HCR. Although, the preimplantation loss for HDF was greater than concurrent VC, it was within the HCR; therefore, the preimplantation loss was not considered test article-related. There was no effect on numbers of corpora lutea, implantation sites, and live embryos.
Toxicokinetics	Concentrations of brexanolone in rat plasma samples on Day 7 2-hours post syringe change: LD: 67.9 ng/mL; MD: 287 ng/mL; HD: 428 ng/mL

VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose, GD: gestation day, HCR: historical control range

Table 21. Mating and fertility indices in female rat fertility and early embryonic development study.

Data Subset	Mean Days to Mating	Mating Index (%)	Fertility Index (%)
HCD	2.1 – 4.6	73.9 – 100	62.5 – 100
HCD – Infusion Route	2.3 – 3.6	81.8 - 100	80 - 100
Group 1 (vehicle control)	2.4	100	100
Group 2 (10 mg/kg/day)	3.7	95.5	95.5
Group 3 (30 mg/kg/day)	4.3	59.1**	54.5***
Group 4 (60 mg/kg/day) – End of dosing	6.0*	22.7***	22.7***
Group 4 (60 mg/kg/day) - Recovery	2.2	64.7	64.7

HCD = Test Facility's historical control data for all routes of administration combined, unless otherwise indicated
* $p \leq 0.01$ (Dunn)
** $p \leq 0.01$ (Fisher's)
*** $p \leq 0.001$ (Fisher's)
Source: Applicant's Table, No. SSN-01271, p.33.

Table 22. Pregnancy parameters in female rat fertility and early embryonic development study.

Parameter	0 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg	60 mg/kg R	HCD
Corpora lutea/rat	16.8	18.7	18.9	18.8	19.8	
Implantations/rat	15.4	17.2	17.5	16.8	18.5	
Preimplantation loss	8.73%	8.46%	6.97%	11.05%	6.82%	3.3–20.8%
Early resorptions	**1**	**1.6**	**1.2**	**2.5**	**1.4**	**0.4–1.6%**
Dead embryos	0	0	0	0	0	
Post implantation loss	**6.33%**	**9.03%**	**6.86%**	**15.93%**	**7.01%**	**2.6–10.9 %**
Live embryos/litter	14.4	15.6	16.3	14.3	17.1	

R = Recovery; HCD = Historical Control Data
Source: Applicant's Table, Study No. SSN-01271, pp.35 and 63-66.

Pharmacology Reviewer Comment: ***Fertility findings in female rats are not a concern clinically for a 60-hour infusion.*** *The findings of increased body weight gain, estrous cycle changes, decreased mating and fertility indices, and increased days to mating suggest that the female rats are pseudopregnant. This is consistent with allopregnanolone exposure being highest during the 3rd trimester of pregnancy in humans. In addition, these effects are reversed or partially reversed after dosing was stopped at the high dose. Also, it should be noted that rats were dosed for a considerably longer period than humans will be (14 days prior to mating, through mating, and to GD7 compared to 2.5 days in humans).*

A Fertility and Early Embryonic Development to Implantation Study with SAGE-547 by Intravenous Infusion in Male Rats/Study No. SSN-01274

Key Study Findings

- Twelve premature deaths occurred that are most likely not brexanolone or SBECD (vehicle) related; however, the cause of death of four rats could not be determined.
- Brexanolone administration to male rats resulted in decreased mating and fertility indices at ≥30 mg/kg; decreased conception rate and slight increase in days to mating at 45 mg/kg; lower prostate, seminal vesicle, and epididymal weights at ≥30 mg/kg, with non-adverse decreases in seminal vesicle and epididymal weights at 10 mg/kg; and decreased spermatozoa count at ≥30 mg/kg.
- SBECD (vehicle)-related findings in kidney (discoloration and enlargement) and in testis, epididymis, and infusion site (vacuolation of macrophages).
- The NOAEL is 10 mg/kg for fertility and reproductive function which is 0.8-times the human exposure (AUC_{24h} = 1800 ng.h/mL; C_{ss} = 74.3 ng/mL) at the MRHD.

Conducting laboratory and location [redacted] (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 10, 30, and 45 mg/kg/day
Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration: IV infusion

Formulation/Vehicle:	Solution/5 mM citrate buffer, pH 6 ± 0.2, and 125 mg/mL of SBECD in 0.9% Sodium Chloride
Amount SBECD	2250 mg/kg/day for vehicle and HD; 500 mg/kg/day for LD; 1500 mg/kg/day for MD
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	22 males/group
Satellite groups:	None
Study design:	Males were dosed starting 4 weeks before mating, during mating (14 days), and until termination on day 42 to 44 (41 to 43 days of continuous infusion). Females were not dosed.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	Twelve animals were found dead or euthanized prematurely due to poor clinical condition (Table 104 in Appendix): 1 VC, 1 LD, 5 MD, and 5 HD. Cause of death for most was vascular/perivascular inflammation at the infusion site that was sometimes associated with the presence of bacteria. Cause of death for 3 MD and 1 HD were undetermined because there was no evidence of moderate to severe vascular inflammation and no bacteria at the infusion site; however, clinical signs prior to their unscheduled euthanasia were similar to the other unscheduled euthanasia except for 1 MD which had no clinical signs noted prior to death.
Clinical Signs	Decreased activity, prominent backbone, limited usage/swollen limbs and/or paws, dehydration, thinness, and/or partly closed eyes were observed in rats of all groups, including VC, but at a slightly higher incidence at HD.
Body Weights	Increased body weight gain at HD (171%) vs. VC during the first week of dosing; however, body weight gain was similar for the remainder of dosing and body weights were similar to VC on Day 42 (end of dosing).
Necropsy findings	Increased incidence of small prostate and seminal vesicles at MD and HD, which correlated with decreased prostate and seminal vesicle weights vs. VC (Table 23 & Table 24). Seminal vesicle weights were decreased at the LD vs. VC; however, these were not considered adverse because the changes were small and mating and fertility were unaffected at LD. Epididymis weights were decreased at all doses vs. VC; however, these were not considered adverse at the LD because the changes were small and sperm parameters and mating and fertility were not affected. Vehicle-related findings were observed in kidney, testis, epididymis,

	and infusion site (see Table 105 in Appendix) and macroscopic findings at the infusion site were observed in all groups (see Appendix page 204).
Fertility and Pregnancy Parameters	Decreased mating and fertility indices at MD and HD and conception rate at the HD vs. VC (Table 25). Although the mating and fertility index were less than VC at the MD, values were within HCR. Males that had not mated in the first 10 days were given a new mating partner for the last 4 days of the mating period. 6/19 HD rats did not mate with their first partner; 2/6 HD rats mated with their second partner, but only one produced a pregnancy. 4/17 MD rats did not mate with their first partner; 2/4 MD rats mated with their second partner and produced a pregnancy. All VC and LD mated within the first 10 days. The mean number of days to mating at the HD was slightly greater (3.1 days) vs. VC (2.6 days). A statistically significant decrease in cauda epididymis weight at all doses, which was associated with a dose-related decreasing trend for spermatozoa count at MD and HD (Table 26). This correlated with decreased epididymis weights; however, there was no histopathological correlate. There was no test article-related effect on other sperm parameters evaluated, including motility and abnormal spermatozoa. The number of implantation sites, live embryos, dead embryos, early resorptions, and pre-and post-implantation loss were comparable for dosed males mated with untreated females vs. VC.
Toxicokinetics	Concentrations of brexanolone in rat plasma samples on Day 14 2-hours post syringe change: LD: 132 ng/mL; MD: 202 ng/mL; HD: 351 ng/mL

VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose GD: Gestation Day, HCD: Historical Control Data

Table 23. Gross pathology findings in male rat fertility study.

	Males			
Group	**1**	**2**	**3**	**4**
SAGE-547 Dose (mg/kg/day)	**0**	**10**	**30**	**45**
Captisol Dose (mg/kg/day	**2250**	**500**	**1500**	**2250**
No. Animals Examined	**21**	**21**	**17**	**17**
Prostate (No. Examined)	(21)	(21)	(17)	(17)
Small	1	1	7	5
Seminal vesicle (No. Examined)	(21)	(21)	(17)	(17)
Small	2	0	5	9

Source: Applicant's Table, Study No. SSN-01274, p.39.

Table 24. Organ weight changes in male rat fertility study.

	Males			
Group	**1**	**2**	**3**	**4**
SAGE-547 Dose (mg/kg/day)	**0**	**10**	**30**	**45**
Captisol Dose (mg/kg/day)	**2250**	**500**	**1500**	**2250**
No. Animals per Group	**21**	**21**	**17**	**17**
Prostate (No. Weighed)[a]	(21)	(21)	(17)	(17)
Absolute value	-	-12	**-30**	**-31**
% of body weight	-	-11	**-32**	**-30**
Seminal vesicle (No. Weighed)	(21)	(21)	(17)	(17)
Absolute value	-	**-19**	**-42**	**-48**
% of body weight	-	**-19**	**-43**	**-47**
Epididymis L/R[b] **(No. Weighed)**	(21)	(21)	(17)	(17)
Absolute value	-	**-11**/-10	**-10**/-10	**-14**/-6
% of body weight	-	**-12**/-11	**-12**/-12	**-12**/-3

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.
[b] L/R = Left/Right

Source: Applicant's Table, Study No. SSN-01274, p.40.

Table 25. Fertility parameters in male rat fertility study.

	Mating Index (%)[a]	**Fertility Index (%)**[a]	**Conception Rate (%)**
Group 1/ Vehicle Control	100/100	100/100	100
Group 2/ 10 mg/kg/day	100/100	100/100	100
Group 3/ 30 mg/kg/day	88.2/ 76.4	88.2/76.4	100
Group 4/ 45 mg/kg/day	78.9*/ 68.4	57.9***/52.6	73.3*
Historical Control Range	73.9 to 100%	62.5 to 100%	75.0 to 100%

[a] The first value is for the 14 day mating period, the second value is for the first 10 days of the mating period. Statistics are not available for the mating and fertility indices presented for the first 10 days of mating.

* - $P \leq 0.05$ ** - $P \leq 0.01$ *** - $P \leq 0.001$ (Fisher's)

Source: Applicant's Table, Study No. SSN-01274, p.38.

Table 26. Summary of sperm evaluation in male rat fertility study.

	Males			
Group	**1**	**2**	**3**	**4**
SAGE-547 Dose (mg/kg/day)	**0**	**10**	**30**	**45**
Captisol Dose (mg/kg/day)	**2250**	**500**	**1500**	**2250**
No. Animals per Group	**21**	**21**	**17**	**17**
Sperm Count (No. Evaluated)	(21)	(21)	(17)	(17)
Mean (millions per gram of Cauda Epididymis)	725.9	737.7	672.8	645.6
Difference from control group mean[a]	-	2	-7	-11
Cauda Epididymis Weight (No. Weighed)	(21)	(21)	(17)	(17)
Mean (g)	0.287	**0.260**	**0.243**	**0.248**
Difference from control group mean[a]	-	**-10**	**-15**	**-14**

[a] All values expressed as percent difference of control group means.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Source: Applicant's Table, Study No. SSN-01274, p.41.

Pharmacology Reviewer Comment: Fertility findings in male rats are not a concern clinically because the patient population is women. However, if additional indications should be examined in the future in which males would be included, the toxicity concerns should be reexamined. Although there are SBECD (vehicle)-related findings in the testis and epididymis, they are most likely due to the proximity of the infusion site to the male reproductive organs. Brexanolone in SBECD is being chronically infused in the vena cava of rat as a femoral indwelling catheter. Therefore, it is unlikely to be relevant to humans. In addition, SBECD is used at much greater amounts in other approved drugs than will be administered for brexanolone (see Table 20, page 52).

Embryo-Fetal Development

A Continuous Intravenous Infusion Embryo/Fetal Development Study of SAGE-547 in the Rat/Study No. SSN-797

Key Study Findings

- Dose-dependent increase in body weight gain and food consumption early in the dosing period (GD 6 to 9) and decreased food consumption and body weight gain following the end of dosing (GD 18 to 21) at ≥30 mg/kg with no effect on body weight.
- Decreased fetal weights (5%) at 60 mg/kg compared to controls.
- The NOAEL is 60 mg/kg for maternal toxicity and 30 mg/kg for fetal development which is 5-times and 2-times; respectively, the human exposure (AUC_{24h} = 1800 ng.h/mL; C_{ss} = 74.3 ng/mL) at the MRHD.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing:	0, 15, 30, and 60 mg/kg/day Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration:	IV infusion
Formulation/Vehicle:	Solution/5 mM citrate buffer, pH 6 ± 0.2, and 125 mg/mL of SBECD in 0.9% Sodium Chloride
Amount SBECD:	3000 mg/kg/day for VC and HD; 750 mg/kg/day for LD; 1500 mg/kg/day for MD
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	22 females/group
Satellite groups:	2 controls and 8/dose group (TK)
Study design:	Pregnant rats were dosed from gestational day (GD) 6 to 17 (infusion pump stopped on morning of GD 18). The fetuses were delivered by C-section on GD21 and examined.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major findings
Mortality	No test article related effect. One control TK (pregnant) euthanized in poor condition on GD 14 with signs of abnormal respiration and discharge from vulva. The cause of death was not determined.
Clinical Signs	No test article related effect.
Body Weights	Between GD 6 and 9, body weight gain was increased for HD vs. controls (↑81%). Once dosing stopped (GD 18 to 21), body weight gain was dose-dependently decreased at MD and HD vs. controls (↓21 and 51%, respectively) and HD weighed 18.7 g less than controls on GD 21. Effects on body weight gain correlated with food consumption (increased between GD 6 and 12 and decreased between GD 18 to 21).
Necropsy Findings	No test article related effect. Procedure related findings observed at the infusion site and findings secondary to the procedure related findings observed in the iliac lymph node (See Appendix, page 205 for details).
Cesarean Section Data	Fetal weights were decreased 5% at the HD compared to controls (Table 27). There was a statistically significant, dose-dependent decrease in the sex ratio; however, the biological significance of this finding is unclear since there was no effect on dead fetuses, post implantation loss, or number of resorptions. In addition, there was no effect on sex ratio in the rat pre- and post-natal development study.
Offspring Data	No test article-related effect (see Table 106 in Appendix).
Toxicokinetics	See ADME/PK Section page 44.

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

Table 27. Cesarean section findings from rat embryo-fetal development study.

Parameter	0 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
Mean live fetuses/litter	12.7	12.6	12.2	13.6
Post Implantation Loss (%)	3.9	4.72	8.31	5.31
Mean Sum of Resorptions	0.5	0.6	1.1	0.7
Mean Early Resorptions	0.5	0.6	1.1	0.6
Mean Late Resorptions	0	0	0	0.1
Mean sex rate (% males)	**57**	**49**	**45***	**44***
Mean fetus weight (g)	**6.086**	**6.234**	**6.150**	**5.758****
Mean uterus weight (g)	108.8	106.3	104.6	106.8

*p≤0.05; **p≤0.01

A Continuous Intravenous Infusion Embryo-fetal Development Study of SAGE-547 in Rabbits/Study No. SSN-825

Key Study Findings

- Maternal toxicity occurred at ≥15 mg/kg based on clinical signs of sedation and reduced food consumption and body weight gain and/or body weight loss during dosing and after dosing stopped.
- Fetal toxicity occurred at ≥15 mg/kg based on increased abortions and number of late resorptions at ≥15 mg/kg and decrease in the number of live fetuses and increases in pre-and postimplantation loss at 30 mg/kg. Fetal weights were decreased 13 and 21% at 15 mg/kg and 30 mg/kg, respectively, correlating with delayed ossification in the hyoid and pubic bones.
- The NOAEL is 7.5 mg/kg for maternal toxicity and fetal development which is 1.2-times, the human exposure (AUC24h = 1800 ng.h/mL; Css = 74.3 ng/mL) at the maximum recommended clinical dose.

Conducting laboratory and location: (b) (4)
GLP compliance: Yes

Methods

Dose and frequency of dosing: 0, 7.5, 15, and 30 mg/kg/day
Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration: IV infusion
Formulation/Vehicle: Solution/5 mM citrate buffer, pH 6 ± 0.2, and 125 mg/mL of SBECD in 0.9% Sodium Chloride
Amount SBECD 3000 mg/kg/day for VC and HD; 750 mg/kg/day for LD; 1500 mg/kg/day for MD
Species/Strain: Rabbit/New Zealand White
Number/Sex/Group: 22/group
Satellite groups: 2 controls and 4/dose group for TK
Study design: Pregnant rabbits were dosed from gestational day (GD) 7 to 19 (infusion pump stopped on morning of GD 20). The fetuses were delivered by C-section on GD 29 and

examined.

Dosing was based on a preliminary dose range finding study conducted in pregnant rats. The study is summarized in the Appendix, page 206.

Deviation from study protocol affecting interpretation of results: No

Observations and Results

Parameters	Major findings
Mortality	One LD rabbit was found dead on GD12, cause of death was pericarditis and was not test article related. 1 HD euthanized on GD15 in poor clinical condition, stopped eating on GD7 till euthanized. 1 VC, 4 MD, and 7 HD aborted and euthanized (between GD14 and 27).
Clinical Signs	Dose-dependent decrease in activity with onset occurring earlier at MD and HD than at the LD and a dose-dependent increase in incidence of reduced appetite. Other pharmacologic effects observed at the HD include abnormal gait, uncoordinated, headtilt, decreased muscle tone, and/or lying on side.
Body Weights	Dose-dependent decrease in body weight gain during dosing period (↓17, 31, 38% at LD, MD, HD, respectively, vs. VC) and continuing after dosing stopped from GD20 to 29 (↓22 30% at LD & MD, respectively, vs. VC). There was a 5% body weight loss from GD16 to 26 at HD. Although HD rabbits gained most of this lost weight back from GD26 to 29, HD rats still weighed 10% less than controls on GD29. Decreased body weight gain and body weight loss correlated with reduced food consumption. Eight HD rabbits had prolonged periods (≥3 days) of little to no food consumption (<70 g) during the dosing period and 13 MD and 14 HD rabbits had prolonged periods of little to no food consumption after dosing stopped.
Necropsy Findings	A small increased incidence of pale discoloration of the liver (0, 0, 1, & 3 for VC, LD, MD, & HD, respectively) and heart (0, 3, & 3 for VC, MD, & HD, respectively) and firmness at the infusion site (2, 2, 4, & 5 for VC, LD, MD, & HD, respectively) at the MD and HD; however, no histopathological evaluation was performed so significance of findings is unclear.
Cesarean Section Data	A dose-dependent increase in the incidence of abortions at the MD and HD, which is at or above the HCR. Many of the rabbits that aborted had prolonged periods of almost no food consumption (<20 g/day) prior to aborting. An increase in the number of late resorptions were observed at the MD and HD. A decrease in the number of live fetuses and an increase in pre- and postimplantation loss were

	observed at the HD. One MD and HD rabbit had total litter resorption. Fetal weights were decreased 13 and 21% at the MD and HD, respectively, vs VC. See Table 28.
Offspring Data	A statistically significant increase in the overall incidence of skeletal variants at the MD (42% MD vs. 22% VC fetuses), the fetal incidence of incomplete hyoid bone ossification at MD (13% MD vs. 4% LD fetuses), and the litter and fetal incidences of incomplete ossification of the pubic bone at MD and HD (9% MD and HD vs. 0% VC fetuses and 19% MD and 33% HD vs. 0% VC litters). However, the values remained within the historical control range (hyoid bone: 0 – 44% affected fetuses; pubic bone: 0 – 44% affected litters, 0 – 10% affected fetuses). These findings may indicate a delay in ossification possibly due to the lower fetal weights at the MD and HD. For a summary of offspring data see Table 109 & Table 110 in Appendix.
Toxicokinetics	See ADME/PK Section page 44.

VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose; GD: gestational day; HCR: historical control range

Table 28. Cesarean section findings from rabbit embryo-fetal development study.

Parameter	0 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg	HCR
Total pregnancy rate	22/22	22/22	22/22	22/22	
Mean corpora lutea	10	9.6	9.8	10	8.4 – 12.3
Mean implantation sites	9.2	8.7	8.9	8	5.7 – 10.6
Preimplantation loss (%)	**6.83**	**10.13**	**8.25**	**20.77***	3.5 – 38.5
Postimplantation loss (%)	**3.01**	**8.77**	**12.86**	**19.85***	0.6 – 29.1
Mean live fetuses	**8.9**	**7.8**	**7.9**	**6.3***	4.9 – 10
Mean total resorptions	0.3	0.9	1.1	1.8	0 – 2.4
Mean early resorptions	0.2	0.5	0	0.4	0 – 1.7
Mean late resorptions	**0**	**0.4**	**1.1***	**1.3***	**0 – 1.4**
Abortions	**0**	**0**	**4**	**7**	**0 – 4**
Mean sex rate (% males)	44.24	50.66	41.04	50.63	37.4 – 63.5
Mean fetus weight (g)	42.12	41.49	36.62*	33.21*	36.9 – 49.6
Mean uterus weight (g)	516.4	476.2	455.9	348.2	

HCR = Historical Control Range; * $p \leq 0.05$

Prenatal and Postnatal Development

A Continuous Intravenous Infusion Pre and Postnatal Study of SAGE-547 in the Rat/Study No. SSN-01263

Key Study Findings

- Maternal toxicity (F_0 dams) occurred at ≥30 mg/kg based on body weight loss from postnatal day (PND) 0 to 4, decrease body weight gain from PND 4 to 10, and decreased food

consumption during the lactation period at 60 mg/kg and increased number of dead pups/litter at birth and fewer live pups/litter at birth at ≥30 mg/kg.
- Pup developmental toxicity (F_1 generation) occurred at 60 mg/kg based on decreased pup viability between PND 0 and 4 with a resulting smaller litter sizes.
- A neurobehavioral deficit, characterized by slower habituation in the maximal startle response in the auditory startle test, was observed in F1 generation females of dams dosed with 60 mg/kg.
- Learning, locomotor activity, sexual development, mating, and fertility were not affected by brexanolone.
- The NOAEL for maternal toxicity is 10 mg/kg and for pup and post-weaning developmental is 30 mg/kg, which is 0.8-times and 2-times, respectively, the human exposure (AUC_{24h} = 1800 ng.h/mL; C_{ss} = 74.3 ng/mL) at the MRHD.

Conducting laboratory and location:	(b) (4)
GLP compliance:	Yes

Methods

Dose and frequency of dosing:	0, 10, 30, and 60 mg/kg/day Continuous (24 hours/day at a rate of 2 mL/kg/h)
Route of administration:	IV infusion
Formulation/Vehicle:	Solution/5 mM citrate buffer, pH 6 ± 0.2, and 125 mg/mL of SBECD in 0.9% Sodium Chloride
Amount SBECD:	3000 mg/kg/day for vehicle and HD; 500 mg/kg/day for LD; 1500 mg/kg/day for MD
Species/Strain:	Rat/Sprague Dawley
Number/Sex/Group:	24 females/group
Satellite groups:	None
Study design:	Pregnant rats (F_0 Dams) were dosed from gestational day (GD) 6 to Postnatal Day (PND; also referred to as lactation day) 21, 22, or 23. Blood was collected from 5 F_0 Dams/group on PND4 and PND20, from 5 F_1 Generation Litters/group on PND4, and 5 F_1 Generation Litters/group (targeting 1/sex/litter) on PND20 2 – 3 hours post syringe change for TK.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Generation	Major Findings
F0 Dams	Mortality: 8 dams were found dead or were preterminally euthanized with undetermined cause of death (Table 111 in Appendix); infusion site masses could be a contributing factor. One VC, 1 LD, and HD euthanized due to signs of dystocia. Two VC, 4 LD, 3 MD, and 3 HD euthanized because there were no remaining live pups on PND 0 or 1. Two VC, 2 MD, and 1 HD euthanized early on GD26 because they failed to deliver pups; 1 VC was not pregnant and

	remaining were pregnant but no live fetuses in utero (total resorption or implant site scars observed with the latter suggesting they had littered during the night and cannibalized their litter). Body weight: During gestation, dosed rats had increased body weight gain vs. VC with the largest effect from GD6 to 9 at the HD (↑110 and 117% for MD and HD, respectively). At the end of gestation, HD group had a 10% increase in body weight gain vs. VC, which correlated with an 11% increase in food consumption. During lactation, body weight loss was observed at the HD from PND0 to 4 and decreased body weight gain was observed at the LD from PND 4 to 10, the MD from PND 0 to 10, and the HD from PND4 to 10 (Table 29); but, by PND 21, body weights were similar across all groups. At the HD this correlated with a 15% decrease in food consumption from PND 0 to 4 and an overall decrease in food consumption by 11% during the lactation period. Uterine Content: The gestation length was comparable for all groups and to the HCR (21.3 to 22 days). Gestation index was below the HCR for all groups, including VC (Table 30); the LD group had the highest gestation index suggesting the finding may be vehicle-related (the LD had the lowest level of vehicle). There was a dose-related decrease in the live birth index and the live birth index was below the HCR for all dose groups, while the VC group was within range (Table 30). There was a dose-dependent decrease in live pups/litter at birth. Although the number of dead pups/litter at birth was within the historical control range at the LD, there is a clear dose-response, so I cannot discount this finding as not being test article-related at the LD. There was no effect on sex ratio (% males) as had been observed in the embryo-fetal development study. Necropsy: There were no test article-related macroscopic findings; however, there were vehicle-related findings in the kidney (pale kidneys) consistent with findings from the rat general toxicology study. Other findings were procedure related (see Appendix, page 210). Toxicokinetics: Plasma concentrations for test article in F_0 Dams on PND4 and 20 are shown in Table 113 in Appendix.
F1 Generation	Survival: Pup survival to PND4 was lower at all doses vs. VC; however, there was no dose response at the LD and MD and only the HD group was below the HCR (Table 31). Decreased food consumption and body weight loss noted for HD dams between PND0 and 4 may have contributed to decreased milk production, affecting viability. There was no effect on survival index (PND 7 and 14) and lactation index (PND 21). Effects on survival at the HD correlated with a decrease in total litter size at the HD that was 25% less than VC on PND 0, 35% less than VC on PND 4, and 12% less than VC for the rest of the lactation period (Table 32). Clinical Signs: On PND 0 or 1, 2 males and 1 female pups from a HD litter

	were cold to touch and 2 of these pups had empty stomachs and were found dead or missing (presumed cannibalized) the same or next day. On PND 2, 3 males and 5 female pups from a HD litter were cold to touch; however, they survived to PND 4. Body Weight: No test article-related effect on mean body weight at birth for male and female pups. There was a small, non-significant decrease in body weight gain for male and female pups from PND 4 to 10 (7 – 10%) at the HD vs. VC; however, there was no effect for the remainder of the lactation period and body weights and body weight gain were similar across groups on PND 21. Physical development: There were no test article-related effects on development in the F1 generation (pupillary closure, visual placing, and vaginal opening and preputial separation). Neurological assessment: The linear time contrast (slope of habituation) for maximum startle was statistically significantly shallower (i.e., habituation slower) for F_1 generation females from HD dams in the auditory startle test with a trend at the MD on PND 55 (Table 33). At the time of testing in the auditory startle test the rats are not exposed to test article; therefore, the effect on startle is persistent. There was no effect on average startle response and the time of maximum startle for females and no differences were observed for F1 generation males. There was a non-statistically significant decrease in locomotor activity (10 – 33%) for F_1 generation rats from HD dams in a Home Cage Motor Activity System on PND 60. There was no test article-related effect on learning and memory in the Cincinnati Water Maze between PND 60 and 70. Reproduction: There was no test article-related effect on estrous cyclicity (number of estrus cycles and average length of cycles), reproductive performance (mean number of days to mating, mating index, fertility index, and conception rate), and ovarian and uterine parameters (mean number of corpora lutea, implantations, pre- and post-implantation loss, and live fetuses) in F_1 generation rats. Necropsy: There was no test article-related external or internal findings noted in pups that were euthanized or found dead between PND 0 and 7. There was no test article-related macroscopic findings in adult F_1 generation rats. Test article was not detected in most F1 generation litters on PND 4 and in all litters on PND 20. The 2 litters which had test article detected on PND 4 could be due to experimental error.

VC: vehicle control; LD: low dose; MD: mid dose; HD: high dose; HCR: historical control range

Table 29. Mean maternal body weight and body weight gain for F_0 dams during lactation in the rat pre- and postnatal development study.

Dose (mg/kg/day)	Body Weight (g)				Gain (g/% control)	
	PND 0	PND 4	PND 10	PND 21	PND 0 - 4	PND 4 -10
0	295	306	326	345	11/--	20/--
10	296	309	326	338	13/116	17/85
30	302	311	326	345	9/82	15/75
60	307	305	321	346	-2/--	16/80

Table 30. Effects on maternal performance at birth in the rat pre- and postnatal development study.

Group	Gestation Index (%)	Live Birth Index (%)	No. Live Pups at Birth/Litter[a]	No. Dead Pups at Birth/Litter	No. of Dead Pups (Litters Affected)
1/ Vehicle control	73.9	88.2	11.7	0.3	5 (3)
2/ 10 mg/kg/day	83.3	83.4	10.9	0.7	15 (6)
3/ 30 mg/kg/day	75.0	80.0	10.6	1.2	24*** (5)
4/ 60 mg/kg/day	79.2	74.7	9.3	1.2	22*** (5)
Historical Control Range	88.9 to 100	84.8 to 95.5	11.3 to 16.4	0 to 1.0	-

*** $P \leq 0.001$ (Fisher's).

[a] Includes litters with only dead pups, and no live pups, i.e., number of live pups at birth in a litter can be 0.

Source: Applicant's Table, Study No. SSN-01263, p.55.

Table 31. Mean survival data in F_1 generation rats in the rat pre- and postnatal development study.

Survival (%)	0 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg	HCR
Birth (Live Birth Index)	88.2	83.4	80	74.7	84.8 – 95.5
PND 4 (Viability Index)	98.65	89.51	94.52	80.50	91.1 – 100
PND 7 (Survival Index)	100	99.34	100	98.53	95.5 – 100
PND 14 (Survival Index)	100	98.68	100	99.22	94.9 – 100
PND 21 (Lactation Index)	100	98.68	100	99.22	94.1 – 100

PND = Postnatal Day; HCR = Historical Control Range

Table 32. Mean litter size in F_1 generation rats in the rat pre- and postnatal development study.

Litter Size	0 mg/kg	10 mg/kg	30 mg/kg	60 mg/kg
PND 0	12.4	11.5	11.8	9.3
PND 4	12.2	10.7	11.1	7.9
PND 7	8	7.9	7.8	7.1
PND 14	8	7.9	7.8	7.1
PND 21	8	7.9	7.8	7.1

PND = Postnatal Day

Table 33. Maximum startle for F1 generation female rats in the pre- and postnatal development study.

F1 Generation Adults - Day 55 (±2) Post Partum
Females
Maximum Startle (voltage)

Group 1 - Vehicle control
Group 2 - SAGE-547 10 mg/kg/day
Group 3 - SAGE-547 30 mg/kg/day
Group 4 - SAGE-547 60 mg/kg/day

Group	Summary Information	Trial 1-10	11-20	21-30	31-40	41-50	Mean Level	Linear Time Contrast
1	Mean	294.99	271.59	264.90	245.19	236.04	262.54	-144.30
	SD	116.88	90.22	101.28	82.16	80.59	88.43	158.52
	N	16	16	16	16	16	16	16
2	Mean	316.81	301.69	269.53	263.56	255.40	281.40	-160.96
	SD	72.72	57.38	78.50	80.10	89.29	71.13	122.60
	N	19	19	19	19	19	19	19
3	Mean	263.44	241.27	233.96	228.45	211.24	235.67	-117.21
	SD	79.54	81.48	83.08	93.15	90.98	82.39	95.25
	N	18	18	18	18	18	18	18
4	Mean	322.86	302.82	302.19	301.21	303.48	306.51	-40.37 A
	SD	77.63	79.55	78.06	90.54	92.04	81.78	90.69
	N	15	15	15	15	15	15	15

Significantly different from control group (Group 1) value: A - $P \leq 0.05$ B - $P \leq 0.01$ C - $P \leq 0.001$ (Dunnett)
D - $P < 0.05$ E - $P < 0.01$ F - $P < 0.001$ (Dunn)

Source: Applicant's Table, Study No. SSN-01263, p.136.

Pharmacology Reviewer Comment: Published animal studies have reported that administration of drugs that enhance GABAergic inhibition to neonatal rats caused widespread apoptotic neurodegeneration in the developing brain (Ikonomidou et al. 2000, Bittigau et al. 2002, Turski and Ikonomidou 2012). The window of vulnerability to these changes in rats (PND 0 to 14) corresponds to the period of brain development that takes place during the third trimester of pregnancy in humans and may be up to three years of age. In 2016 followed by an update in 2017, the Agency issued a Drug Safety Communication to add a warning about using general anesthetics and sedatives (drugs that antagonize NMDA receptors or potentiate GABA signaling) in pregnant women because of the concerns about apoptotic neurodegeneration in the developing brain. Because brexanolone has $GABA_A$ receptor activity and there is no data for brexanolone, we added a statement to the brexanolone label (Section 8.1) based on the literature data for other drugs with a similar mechanism of action to address the potential risk to administering brexanolone during pregnancy. Even though an effect might be predicted for a nursing baby based on the effected age range in humans, this is not an issue for this product since the amount released in milk is very minimal and the drug has a very low oral bioavailability. In addition, a PMR has been requested for a study to determine if these concerns are relevant for brexanolone and to allow labeling to appropriately reflect the risk to patients.

5.5.5. Other Toxicology Studies

Local Tolerance
Study Nos. RPT57495.01, SSN-01392, SSN-01393, and SSN-01394 were reviewed by Dr. Julie Frank.

***UV-Vis Absorbance Profile of SAGE-547 (Study No. RPT57495.01; Non-GLP)*:** There was some absorbance noted from 208 to 210 nm and 240 to 320 nm (maximum absorbance at 285 to 290 nm). However, the molar extinction coefficient at these wavelengths was below 1000 L mol^{-1} cm^{-1} and there was no absorbance in the spectrum from 330 to 720 nm indicating that brexanolone is not photosensitive in the UV-visible region.

***SAGE-547: An Acute Dermal Irritation/Corrosion Study in Rabbits (Study No. SSN-01392; GLP)*:** Dermal administration of 500 mg brexanolone for four hours to three rabbits showed no signs of irritation (edema, erythema, or eschar) up to 72 hours post dose.

***SAGE-547: An Acute Eye Irritation/Corrosion Study in Rabbits (Study No. SSN-01393; GLP)*:** Ocular administration of 100 mg brexanolone to three rabbits resulted in reddening of the conjunctivae and discharge was observed 1-hour postdose in all three rabbits and chemosis (swelling) of the conjunctivae was observed in one rabbit 1-hour postdose. There were no findings 24-hours postdose. Because the severity of the irritation observed was lower than the irritation scores available for classification, brexanolone is not considered an ocular irritant.

***SAGE-547: A Skin Sensitization Study (Buehler Method) in Guinea Pigs (Study No. SSN-01394; GLP)*:** Dermal administration of 100% brexanolone for 3 weekly 6-hour exposures (on Days 1, 8, and 15) to guinea pigs did not elicit a skin reaction or contact dermatitis. A brexanolone challenge 2 weeks following the last induction exposure (Day 29) also did not elicit a skin reaction or contact dermatitis.

***Hemolytic Potential of SAGE-547 Injection on Human Whole Blood (Study No. SSN-638; Non-GLP)*:** Brexanolone (5 mg/mL SAGE-547 in 250 mg/mL SBECD) at concentrations of 0.166 and 1.66 mg/mL did not cause hemolysis in fresh human whole blood from two separate donors.

Impurity Qualification
Starting material, reagents, process intermediates, process impurities, and potential process impurities were adequately evaluated for mutagenicity potential by in silico evaluation (Study Nos. INDS-SG2016-01, INDS-SG2016-02, INDS-SG2016-03, and (b) (4)-17-005). (b) (4) which is predicted to be negative) was identified as positive for mutagenic potential in bacteria by (b) (4), but weight-of-evidence of closely-related reference structures resulted in an overall negative prediction. A negative prediction was confirmed by an internal evaluation by the CDER/OTS/OCP/DARS Chemical Informatics Program.

In addition, (b) (4) were identified as plausible

mutagens by Derek analysis based on the presence of an aromatic nitro group (b) (4) which is a class 1 compound with evidence of mutagenic and carcinogenic activity. (b) (4) was determined to be negative in a valid AMES assay (see Appendix page 211); therefore, (b) (4) is considered a class 4 compound. The remaining (b) (4) containing compounds are also considered negative for mutagenic potential because the structural alert for these compounds was the same that was assessed in the AMES assay conducted with (b) (4) and determined to be negative. It should be noted that (b) (4) contain (b) (4) groups compared to (b) (4). However, it does not appear that (b) (4) represent a greater risk for mutagenicity than (b) (4) because the in silico mutagenicity results for (b) (4) were the same for (b) (4) (plausible in DEREK and negative in Leadscope), the chemical environment for

The Applicant is proposing specification limits for impurities (b) (4) in the drug substance of NMT (b) (4) based on qualification in a 14-day general toxicology study in rat in which these impurities were tested neat. There were no test article-related findings for (b) (4) at doses up to 0.15 mg/kg/day for 14-days (see Appendix page 211). In addition, (b) (4) are predicted to be negative for mutagenicity by QSAR.

The Applicant is proposing specification limits for impurities (b) (4) in the drug product of NMT (b) (4) and (b) (4) respectively. Both impurities are considered qualified. (b) (4) general toxicity study to be at exposures similar to brexanolone (see Table 16 in ADME/PK section) and was predicted to be negative for mutagenicity by QSAR. (b) (4) was present in the batch of brexanolone used for dog at (b) (4)% and the HED at the NOAEL is (b) (4) mg/kg (b) (4) mg for 60 kg person) and is predicted to be negative for mutagenicity because (b) (4) which was predicted to be negative in QSAR and was negative in the battery of genotoxicity assays.

Unique Major Human Metabolite Qualification

Three metabolites present in human plasma at levels greater than 10% of drug related radioactivity were identified in the human ADME study conducted with ^{14}C-SAGE-547: M133 (SGE-03211), M136 (SGE-03212), and M137 (SGE-03277). M133 was observed at 27.6% of total drug-related exposure and M136 and M137 (these metabolites could not be reliably resolved chromatographically) were 30.5% of total drug-related exposure. These metabolites were not detected in rats or dogs. All three metabolites are either sulfate (M133 and M136) or glucuronide (M137) conjugates of C20-reduced forms of allopregnanolone (5α-pregnan-3α,20α-diol or 5α-pregnan-3β,20α-diol). Although M133, M136, and M137 are not present in rats or dogs and were not covered in toxicology studies, they are likely to be water soluble since they are Phase II conjugates and thus readily excreted. In addition, M133, M136, and M137 have previously been reported in the literature to be present in the plasma and breast milk of female humans (Axelson et al. 1983 and Sahlberg et al. 1986).

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6 Clinical Pharmacology

6.1. Executive Summary

The clinical pharmacology of brexanolone is supported by eight clinical studies, including a mass balance study, organ impaired studies, drug-interaction study, thorough QT study, abuse potential study, oral bioavailability study, and lactation study.

The proposed dose is 90 mcg/kg/h with a dosing regimen as a one-time continuous intravenous (IV) infusion over a total of 60 hours (2.5 days) as follows:
• Initiate with a dose of 30 mcg/kg/h and infuse for 4 hours
• Increase dose to 60 mcg/kg/h and infuse for 20 hours
• Increase dose to 90 mcg/kg/h and infuse for 28 hours
• Decrease dose to 60 mcg/kg/h and infuse for 4 hours
• Decrease dose to 30 mcg/kg/h and infuse for 4 hours prior to completion of therapy

The proposed trade name is ZULRESSO.

Recommendations
The Office of Clinical Pharmacology has determined that there is sufficient clinical pharmacology information provided in the NDA package to support a recommendation of approval for brexanolone. The key review issues with specific recommendations and comments are summarized below:

Key Review Issues	Relevant Clinical Studies	Reviewer Recommendations and Comments
Is the proposed general dosing regimen acceptable?	Phase 2 study-202A Phase 3 studies-202B and 202C	The proposed general dosing regimen is supported by 3 independent clinical studies conducted in PPD patients. The proposed regimen utilizing a 90 µg/kg/h maximum dose level demonstrated efficacy in moderately- and severely-depressed PPD patients in 3 clinical trials (202A, 202B, 202C), was well-tolerated, and presented no clinically-relevant safety issues. An alternate treatment regimen utilizing a 60 µg/kg/h maximum dose level was utilized in just a single trial, Trial 202B, and demonstrated efficacy as well. However, the 90 µg/kg/h regimen has demonstrated efficacy in more trials, greater number of patients, and across a wider range of disease severity than the 60 µg/kg/h regimen. In addition, targeting 90 µg/kg/h and reducing to 60 µg/kg/h in case of tolerability issues is logistically preferable to the alternative of targeting 60 µg/kg/h and

		increasing to 90 µg/kg/h in case of inadequate effectiveness. Details are provided in Section 6.3.2 (Clinical Pharmacology Questions)
Are any dose adjustments required for organ impaired patients?	Hepatic impairment study (#103) Renal impairment study (#104)	A dedicated hepatic impairment study demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in hepatic impaired subjects (mild, moderate and severe) compared to normal healthy subjects. Thus, no dose adjustment of brexanolone is recommended for hepatic impaired PPD patients. A dedicated renal impairment study demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in renal impaired subjects (severe) compared to normal healthy subjects. However, SBECD (solubilizing excipient in brexanolone formulation) exposures were significantly higher in renal impaired subjects. Therefore, it is recommended that caution should be used in patients with moderate and severe renal impairment due to potential accumulation of SBECD. Serum creatinine levels should be closely monitored in these patients. Use is not advised in patients with end stage renal disease (ESRD) with eGFR of < 15 mL/min/1.73 m^2. The detailed analysis is provided in Section 6.3.2 (Clinical Pharmacology Questions)
Is it safe for the child to breast feed from patients getting the brexanolone infusion?	Lactation study (#108)	A dedicated open-label lactation study was conducted to assess the amount of brexanolone that is getting transferred into the breast milk of lactating mothers during the standard dosing regimen of brexanolone infusion for 60 hrs. Corresponding PK analysis for brexanolone in milk and plasma demonstrated that it gets transferred into the breast milk of nursing mothers. However, due to the low oral bioavailability, the net relative infant dose via the breast milk is likely to be very low

		compared to the dose to PPD patients. Therefore, based on extremely low levels of brexanolone in milk and potentially low levels of SBECD in milk, we recommend that it is acceptable for patients to continue breast-feeding during the infusion. The detailed analysis is provided in Section 6.3.2 (Clinical Pharmacology Questions)

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Brexanolone is a positive allosteric modulator of $GABA_A$ receptors that is chemically identical to endogenous allopregnanolone. The precise mechanism of action of brexanolone in the treatment of PPD is not fully understood. Dysregulation of GABA signaling and receptor trafficking, including hypofunction, is thought to be associated with PPD. The rapid onset of PPD symptom relief with brexanolone is thought to be mediated through positive allosteric modulation of both synaptic and extra-synaptic $GABA_A$ receptors resulting in a durable state change in $GABA_A$ receptor activity. The binding site for brexanolone on $GABA_A$ receptors is distinct from CNS depressants such as benzodiazepines and alcohol. The following is the summary of clinical PK features of brexanolone
Brexanolone is administered as a continuous IV infusion and it demonstrates a 2-compartment bi-exponential PK profile. It has a linear dose-proportional PK from 30 mcg/kg/h to 270 mcg/kg/h. PK variability is low with inter-subject variability of 21% CV.

Absorption: Brexanolone is administered as a continuous IV infusion over 60 h. It has low oral bioavailability of <5%.

Distribution: The volume of distribution for brexanolone is approximately 3 L/kg, suggesting extensive distribution into tissues. Plasma protein binding is greater than 99%.

Elimination: Brexanolone is rapidly cleared with total plasma clearance of approximately 1 L/h/kg. Following intravenous administration of brexanolone, plasma concentrations decline biexponentially with a terminal half-life of approximately 9 hours.

Metabolism: In humans, brexanolone is eliminated via biotransformation. Brexanolone is extensively metabolized via non-CYP based pathways via three main routes- Aldo-ketone reductase (AKR's), glucuronidase (UGT's) and sulfation (SULT's). Because brexanolone is extensively metabolized by multiple enzymes (non-CYP based) and via several metabolic pathways, it is unlikely that brexanolone would be a substrate for a metabolic drug interaction with another concomitant drug.

Following IV administration of [14C]-labeled brexanolone to healthy subjects, many radiolabeled metabolic products were recovered. Three major circulating metabolites observed were M133, M136 and M137 which were found to be pharmacologically inactive and do not contribute to the overall efficacy of brexanolone.

Excretion: Following IV administration of [14C]-labeled brexanolone, there were comparable amounts of drug-related radioactivity in urine (42%) and feces (47%). Urinary excretion of unchanged radiolabeled brexanolone was negligible (less than 1% of administered dose appeared in urine).

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The recommended maximum dose is 90 mcg/kg/h administered as a continuous intravenous infusion over 60 hours (2.5 days) as follows:

- Increasing doses: 30 mcg/kg/h infused for 4 hours, followed by 60 mcg/kg/h infused for 20 hours
- Maximum maintenance dose: 90 mcg/kg/h infused for 28 hours
- Decreasing doses: 60 mcg/kg/h infused for 4 hours, followed by 30 mcg/kg/h infused for 4 hours

For patients who do not tolerate 90 mcg/kg/h, infusion can be interrupted until the symptoms resolve or a lower dose of 60 mcg/kg/h may be considered.

Therapeutic Individualization

Hepatic Impairment: A dedicated hepatic impairment study demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in hepatic impaired subjects (mild, moderate and severe) compared to normal healthy subjects. Thus, no dose adjustment of brexanolone is recommended for hepatic impaired PPD patients.

Renal Impairment: A dedicated renal impairment study demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in renal impaired subjects (severe) compared to normal healthy subjects. However, SBECD (solubilizing excipient in brexanolone formulation) exposures were significantly higher in renal impaired subjects. Therefore, it is recommended that caution should be used in patients with moderate and severe renal impairment due to potential accumulation of SBECD. Serum creatinine levels should be closely monitored in these patients. Use is not advised in patients with end stage renal disease (ESRD) with eGFR of < 15 mL/min/1.73 m^2.

Postpartum depression patients receiving concomitant medicines: Though brexanolone is extensively metabolized, the major in vivo biotransformation occurs by ketoreduction (via AKR enzymes) and conjugation with a sulfate (via SULT's) or glucuronide (via UGT's). Because

brexanolone is extensively metabolized by multiple non CYP enzymes and via several metabolic pathways, it is unlikely that brexanolone would be a substrate for a metabolic drug interaction with any other concomitant drug. No dedicated study was done to assess the potential of drug interaction of brexanolone as a victim drug, However, PK analysis from clinical studies demonstrated no effect on PK of brexanolone when concomitant medicines such CYP inhibitors, AKR inhibitors, hormonal contraceptives etc. were taken. Thus, no dose adjustment of brexanolone is recommended for PPD patients receiving concomitant medicines.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Brexanolone is an allosteric modulator of $GABA_A$ receptors and chemically identical to the endogenous metabolite of progesterone, allopregnanolone. The brexanolone drug product is a sterile, clear, colorless, 5 mg/mL solution formulated with SBECD. It is provided in a single use vial, diluted prior to use and administered intravenously as a 60-hour continuous infusion.

ZULRESSO is indicated for the treatment of postpartum depression (PPD). There is currently no approved product for PPD.

The proposed dose is 90 mcg/kg/h with a dosing regimen as a continuous intravenous (IV) infusion over a total of 60 hours (2.5 days) as follows:

- Initiate with a dose of 30 mcg/kg/h and infuse for 4 hours
- Increase dose to 60 mcg/kg/h and infuse for 20 hours
- Increase dose to 90 mcg/kg/h and infuse for 28 hours
- Decrease dose to 60 mcg/kg/h and infuse for 4 hours
- Decrease dose to 30 mcg/kg/h and infuse for 4 hours prior to completion of therapy

Pharmacology	
Mechanism of Action	Brexanolone is a positive allosteric modulator of $GABA_A$ receptors that is chemically identical to endogenous allopregnanolone. The precise mechanism of action of brexanolone in the treatment of PPD is not fully understood. Dysregulation of GABA signaling and receptor trafficking, including hypofunction, is thought to be associated with PPD. The rapid onset of PPD symptom relief with brexanolone is thought to be mediated through positive allosteric modulation of both synaptic and extra-synaptic $GABA_A$ receptors resulting in a durable state change in $GABA_A$ receptor activity. The binding site for brexanolone on $GABA_A$ receptors is distinct from CNS depressants such

	as benzodiazepines and alcohol.
Active Moieties	Brexanolone is the only active moiety. Though there are many circulating metabolites, all the major circulating metabolites were determined to be "inactive".
QT Prolongation	Thorough QTc study demonstrated that there was no prolongation of QT interval and there was no correlation of exposure with QTc prolongation.
General Information	
Bioanalysis	Brexanolone concentrations were measured using validated LC/MS/MS methods. A summary of the method validation reports is included as appendix.
Drug exposure at steady state following the therapeutic dosing regimen	A population PK model developed with data from multiple studies in subjects with PPD demonstrated steady state levels as follows: - At 60 mcg/kg/h = 54 ng/mL - At 90 mcg/kg/h = 79 ng/mL
Dose Proportionality	Brexanolone PK is dose-proportional from 30 mcg/kg/h to 270 mcg/kg/h.
Accumulation	No accumulation is anticipated
Absorption	
1. Brexanolone is to be administered as an IV infusion. However, an absolute BA study demonstrated <5% oral BA in adults. 2. Brexanolone has low inter-subject PK variability (21% between subjects)	
Distribution	
1. Volume of Distribution: 3L/kg. 2. Plasma Protein Binding: >99%	
Elimination	

1. Clearance: Brexanolone has a mean total plasma systemic clearance of 1 L/h/kg.
2. Mean Terminal Elimination half-life: 9 hrs.
3. Primary metabolic pathway(s): Following IV administration of [14C]-labeled brexanolone, there were comparable amounts of drug-related radioactivity in urine (42%) and feces (47%). Urinary excretion of unchanged radiolabeled brexanolone was negligible (less than 1% of administered dose appeared in urine).
4. Metabolism: In humans, brexanolone is eliminated via biotransformation. Brexanolone is extensively metabolized via non-CYP based pathways via three main routes- Aldo-ketone reductase (AKR's), glucuronidase (UGT's) and sulfation (SULT's).
5. Transporter: Brexanolone and the 3 major circulating metabolites are unlikely to inhibit the transporters (BCRP, OAT1, OAT3, OCT2, OATP1B1, or OATP1B3) at clinically relevant concentrations.
6. Inhibitor/Inducer to CYP enzymes: In vitro studies suggest that brexanolone and 3 major circulating metabolites are unlikely to inhibit or induce any key CYP enzymes at clinically relevant concentrations except CYP2C9. A dedicated drug interaction study with Phenytoin (2C9 substrate) demonstrated that brexanolone had no effect at clinically relevant dose.

6.3.2. **Clinical Pharmacology Questions**

6.3.2.1. Is the proposed general dosing regimen for brexanolone appropriate?

Yes.

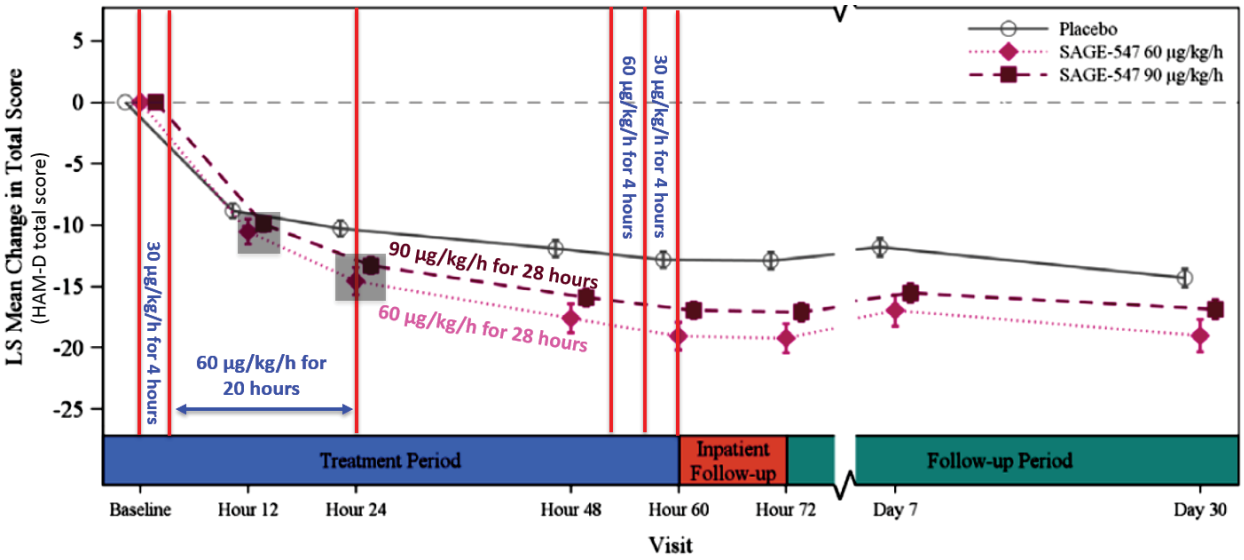
The proposed dose for brexanolone is 90 µg/kg/h with a dosing regimen as a continuous intravenous (IV) infusion over a total of 60 hours (2.5 days) as follows:

- Initiate with a dose of 30 µg /kg/h and infuse for 4 hours
- Increase dose to 60 µg/kg/h and infuse for 20 hours
- Increase dose to 90 µg/kg/h and infuse for 28 hours
- Decrease dose to 60 µg/kg/h and infuse for 4 hours
- Decrease dose to 30 µg/kg/h and infuse for 4 hours prior to completion of therapy

The proposed dose and dosing regimen are identical to the one used in 3 independent short-term efficacy and safety studies (study # 202A, 202B and 202C). All 3 studies demonstrated statistically significant changes in primary endpoint (change from baseline in HAM-D score at 60 h). Additionally, efficacy was demonstrated both in patients with severe PPD (i.e., baseline HAM-D >26) and patients with moderate PPD (i.e., baseline HAM-D 20-25).

The 60 µg/kg/h dose level performed numerically better than 90 µg/kg/h in terms of the primary efficacy endpoint (HAM-D assessed at hour 60; end of infusion). However, the 60 µg/kg/h dose level appears to present greater reduction in HAM-D score than 90 µg/kg/h at hours 12 and 24, time points when patients in the two groups are receiving identical dosages (see **Figure 2**).

Figure 2. Least Squares Mean Change (± Standard Error) in HAM-D Total Score Over Time for Pooled Key Efficacy Studies (Full Analysis Set).



Source: Applicant's Summary of Clinical Efficacy, p.59.

As the brexanolone terminal elimination half-life is approximately 9 hours, brexanolone elimination is expected to be nearly complete at approximately 45 hours (5-half-lives) after infusion cessation (i.e., by Day 5 after infusion start). However, a difference in the mean HAM-D score between the 60 µg/kg/h arm and the 90 µg/kg/h arm is still apparent at Hours 72, day 7, and Day 30 (over 27 days after the infusion ends). Overall, these findings suggest that there were other unknown factors that affected the HAM-D scores differently in the patients in the 60 µg/kg/h arm than the 90 µg/kg/h arm.

Topline safety information suggests that there is no apparent relationship between AE risk and dose or exposure. For example, AE rates in PBO, 60 µg/kg/h, and 90 µg/kg/h arms were 5.6%, 21.1%,12.7% for sedation and 7.5%, 15.8%, 12.7% for dizziness.

Overall, both infusion regimens appear to present acceptable safety and efficacy profiles. However, the proposed dose of 90 µg/kg/h has been assessed in a larger number of subjects over a wider range of PPD severities than 60 µg/kg/h. In addition, the experience with the 90 µg/kg/h dose has been replicated in three separate studies. Also, it is more practical to target the high dose and reduce dose if AEs occur than to target the low dose and increase due to inadequate therapeutic benefit (because the full benefit is not observed until the end of the 60-hour infusion). Hence, we agree with the Applicant's proposed general dosing regimen.

6.3.2.2. Is an alternate dosing regimen required for patient sub-populations based on intrinsic factors (i.e., age, weight, organ impairments etc.)?

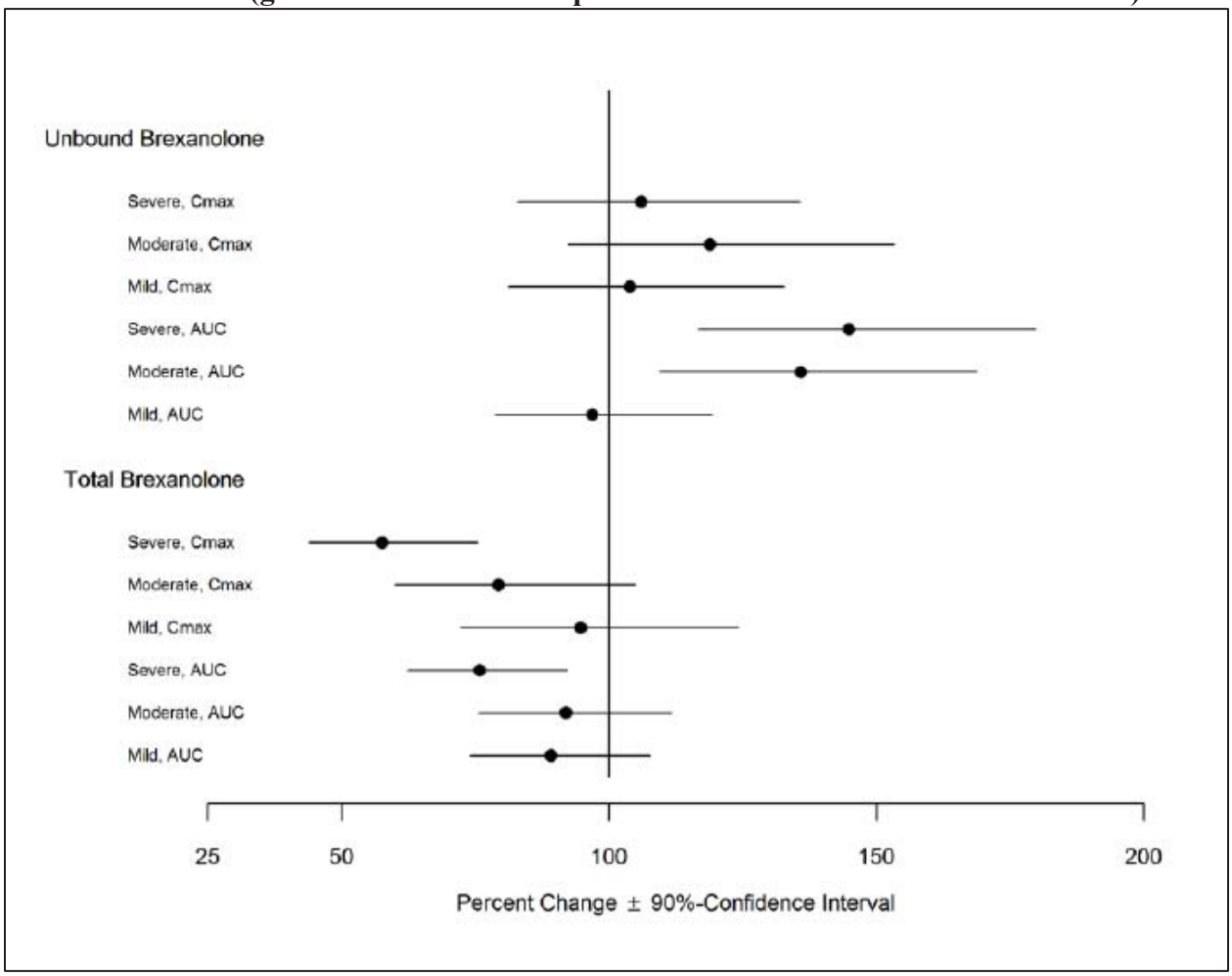
No.

Effect of age, sex and Weight/BMI: All patients being treated for PPD are women and are generally in a narrow age range (child-bearing potential). Age and sex were not found to be

significant as covariates impacting the PK of brexanolone based on the population PK analysis. The body weight was found to be a covariate impacting the PK of brexanolone and therefore, the proposed dosing regimen of brexanolone includes administration of the dose on a per kilogram of body weight basis.

Effect of Hepatic Impairment: A dedicated hepatic impairment study (study # 103) demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in hepatic impaired subjects (mild, moderate and severe) compared to normal healthy subjects. Thus, no dose adjustment of brexanolone is recommended for hepatic impaired PPD patients.

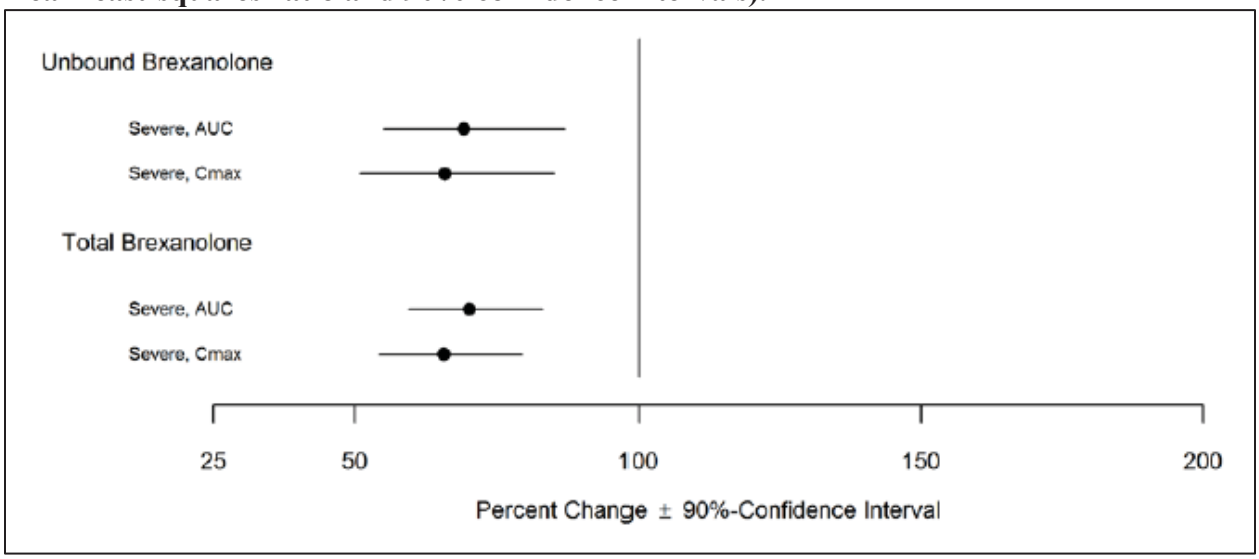
Figure 3. Effect of Varying Degrees of Hepatic Impairment on Brexanolone Pharmacokinetics (geometric mean least-squares ratio and 90% confidence intervals).



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Effect of Renal Impairment: A dedicated renal impairment study demonstrated that brexanolone exposures (i.e., Cmax and AUC) were generally similar in renal impaired subjects (severe) compared to normal healthy subjects. Thus, a marginal 30% reduction in exposure of brexanolone is not clinically relevant and no dose adjustment is recommended.

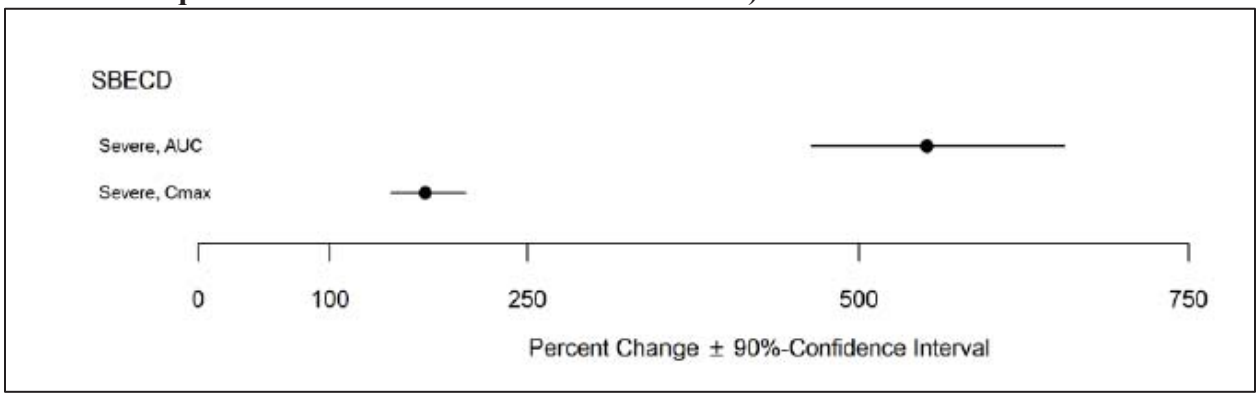
Figure 4. Effect of Severe Renal Impairment on Brexanolone Pharmacokinetics (geometric mean least-squares ratio and 90% confidence intervals).



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

However, SBECD exposures were significantly higher in subjects with renal impairment. Given that SBECD is eliminated by glomerular filtration, its clearance was expected to decrease in patients with renal impairment. Systematic exposure, based on Cmax and AUCinf, for SBECD was 1.7X and 5.5X fold higher, respectively, in severe renal impaired subjects compared with the normal renal function cohort.

Figure 5. Effect of Severe Renal Impairment on SBECD Pharmacokinetics (geometric mean least-squares ratio and 90% confidence intervals).



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

SBECD has historically been established as a safe and effective component of IV drugs requiring solubilization. The use, applicability, and tolerability of SBECD in humans has been reviewed extensively because there are seven FDA approved medicines that include SBECD in their IV formulations.

Table 34. FDA approved products containing SBECD.

Brand Name	Generic Name	NDA (approval year)
Nexterone	Amiodarone	022325 (2008)
Carnexiv	Carbamazepine	206030 (2016)
Kyprolis	Carfilzomab	202714 (2012)
Evomela	Melphalan	207155 (2016)
Noxafil	Posaconazole	205596 (2014)
Vfend	Voriconazole	21267 (2002)
Baxdela	Delafloxacin	208610 (2017)

Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Additionally, several of the FDA approved products are also known to (a) have much higher daily exposure to SBECD than brexanolone and (b) have exposure over a longer duration i.e., several days, contrasted with a shorter duration for brexanolone.

Table 35. Daily SBECD Doses in Patients with Severe Renal Impairment for Approved SBECD Containing Products, and for Brexanolone.

Product	SBECD dose, mg/day (assuming 60kg patient)	Duration
Baxdela IV (200 mg BID)	3200	5-14 days
VFEND IV (Loading dose: 6 mg/kg BID in first 24h)	11520	Day 1 exposure
VFEND IV (Maintenance dose: 4 mg/kg BID)	7680	At least 7 days (total)
Brexanolone (reaching maximum 90 µg/kg/h infusion)	5040 (averaged over 60h regimen)	60 hours or 2.5 days
Brexanolone (reaching maximum 60 µg/kg/h infusion)	4032 (averaged over 60h regimen)	60 hours or 2.5 days

Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Therefore, brexanolone levels are anticipated to be generally similar in renal impaired patients, but SBECD levels are expected to be significantly higher in severe renal impaired subjects. Given the experience with the other approved products with similar or higher SBECD amounts, our labelling recommendation for brexanolone is consistent with those other products (i.e., use with caution in moderate and severe renal impaired patients with close monitoring of the serum creatinine levels and not recommended in ESRD patients).

6.3.2.3. Should patients receiving brexanolone treatment continue breast feeding?

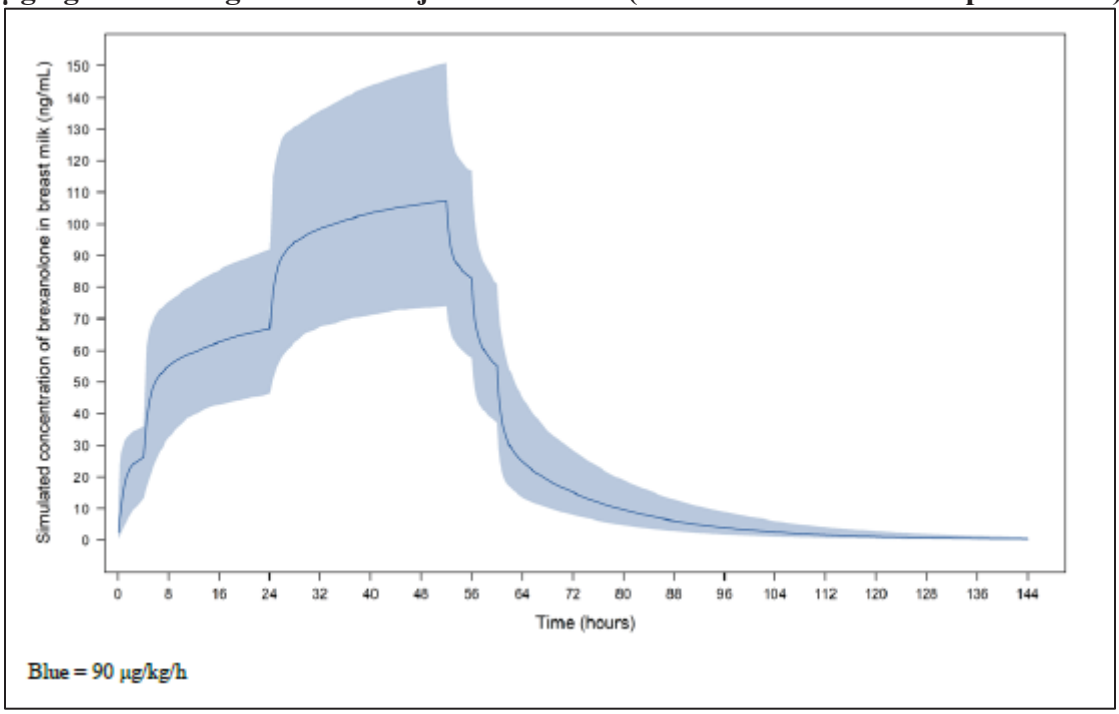
Yes, we recommend that it is acceptable for patients to continue breast-feeding during the infusion.

Study CLP-108 was a dedicated lactation study designed to evaluate the concentration of brexanolone in breast milk of healthy adult lactating women who are being administered

brexanolone IV infusion. The subjects were given a continuous IV infusion of brexanolone identical to the proposed dosing regimen (i.e., for 60 hrs with a maintenance infusion rate of 90 mcg/kg/h). Breast milk was pumped and collected predose and at least every 12 hours throughout the Treatment Period and thereafter through the Day 7 Visit. Corresponding blood samples were also collected from pre-dose, during the infusion and up to 7 days. Plasma and breast milk samples were analyzed for concentrations of brexanolone utilizing validated LC-MS/MS method.

Detectable levels of brexanolone were observed in milk. The brexanolone concentration ratio (i.e., concentration in milk: concentration in plasma) was approximately 1.4. A population PK model was developed to characterize brexanolone levels in milk.

Figure 6. Model-Predicted Exposures of Brexanolone in Milk Following the Proposed 90 µg/kg/h Dose Regimen for Subjects with PPD (Median and 5th to 95th percentiles).



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

To assess the worst-case scenario of the relative infant dose, the applicant utilized the highest observed maternal range of plasma AUC values from 24 to 48 hours, when the maximum 90 µg/kg/h infusion was given**.**

Table 36. Applicant's calculation of Maximum Brexanolone Relative Infant Dose (RID).

	Plasma AUC_{24-48}, ng.h/mL	Milk AUC_{24-48}[a], ng.h/mL	Average Brexanolone in Milk[b], ng/mL	Daily Dose in Milk[c], ng/kg/day	RID[d],%
Maximum	3410	4638	193.2	28980	1.342

[a] Computed as 1.36*Milk AUC
[b] Milk AUC divided by 24 hours
[c] Based on feeding rate of 150 mL/kg/day
[d] Computed as infant dose divided by maternal dose (90 µg/kg/h*24 h) *100% (Bennett 1996)

Source: Applicant's Table, Summary of Clinical Pharmacology Studies.

Because of the relative imprecision of the applicant's estimate of the milk:plasma concentration ratio and assumptions regarding the calculation of average brexanolone in milk, the review team performed a sensitivity analysis utilizing observed milk concentration (Appendix). The calculated %RID when using observed maximum milk concentration of 254 ng/mL was 1.76%, which is consistent with the estimate provided by the applicant.

Brexanolone is dosed as an IV infusion to lactating mothers, whereas brexanolone will get delivered orally (not IV) to a child who is breast-fed. The oral bioavailability of brexanolone is known to be <5% in adults. It is unknown how the oral bioavailability from breast milk in neonates or infants compares to the oral bioavailability in adults from the Applicant's oral solution formulation. If neonates or infants have comparable oral bioavailability from milk to adults with the oral brexanolone formulation (i.e., <5%), effective relative infant dose could be <0.05% to 0.1%

Although the amount of brexanolone dose delivered (via milk) to the infant is negligible, the study did not capture the exact amounts of circulating metabolite(s) of brexanolone or the excipient SBECD getting transferred to the infant. SBECD is known to be eliminated primarily via renal filtration and is also known to impact renal function. However, SBECD levels are expected to be low in breast milk due to its physico-chemical properties (i.e., high molecular weight of 2200 Da, extremely hydrophilic, multiple negative charges at neutral pH making it poorly permeable to cross the epithelial layer of mammary tissues) as well clinical PK properties (very low V_{ss}, high clearance and short $T_{½}$ of 2 hr) limiting its potential distribution into milk. Additionally, there is clinical data demonstrating that neonates treated intravenously with voriconazole (which contains SBECD levels up to 336 mg/kg/day) for multiple days does not result in any safety issues.

Therefore, based on extremely low levels of brexanolone in milk and potentially low levels of SBECD in milk, we recommend that it is acceptable for patients to continue breast-feeding during the infusion.

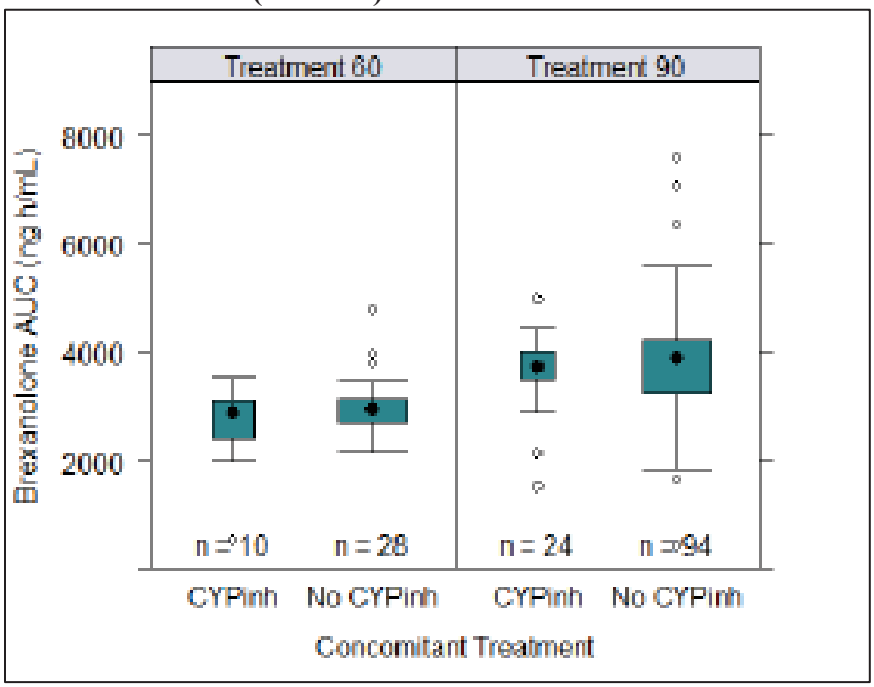
6.3.2.4. Is clinically relevant drug-drug interaction anticipated in patients receiving brexanolone and other comedications?

No. The potential for any clinically relevant drug-drug interactions with brexanolone is low.

Brexanolone and the three major circulating metabolites (M133, M136 and M137) were all assessed for their in vitro inhibition potential towards the key metabolizing enzymes (i.e., CYP450's and UGT's) as well as the key efflux and influx transporters (i.e., Pgp, BCRP, OATP's, OAT's, OCT's etc.) and determined to be non-inhibitors, except some inhibition of CYP2C9 enzyme. A drug interaction study was conducted using the CYP2C9 substrate phenytoin and brexanolone had no effects on the PK of phenytoin. Additionally, brexanolone was determined to be a non-inducer for the key CYP450 enzymes. These results clearly suggest that brexanolone is unlikely to have any clinically relevant drug interactions as a perpetrator.

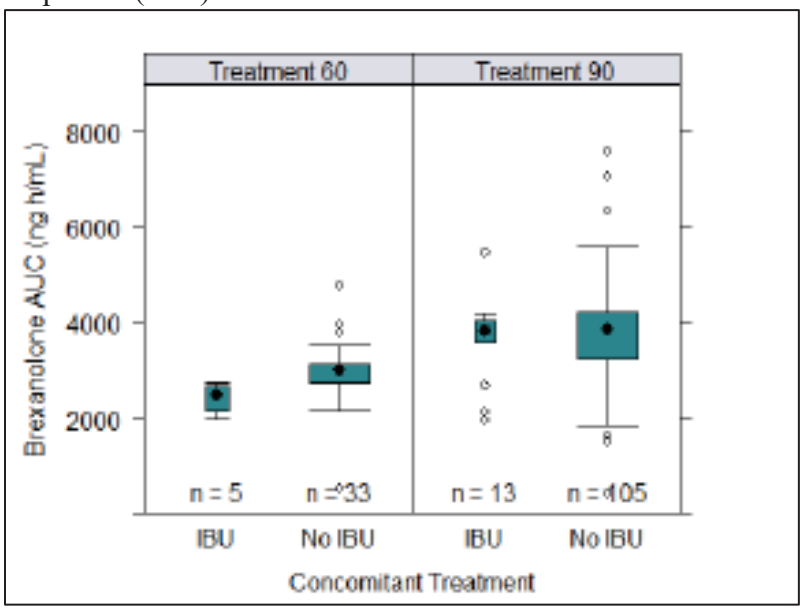
The human mass balance and metabolite identification study demonstrated that brexanolone is extensively metabolized via non-CYP based pathways and is bio-transformed majorly via three main routes: Aldo-ketone reductase (AKR), glucuronidase (UGT) and sulfation (SULT). Contrary to CYP pathways, these enzymatic pathways are usually not inhibited readily by exogenous drugs. Concomitant dosing of other medicines was allowed in the clinical studies conducted for brexanolone. Population PK analysis performed for concomitant dosing with AKR inhibitors, CYP inhibitors, or hormonal contraceptives demonstrated that brexanolone concentrations remained unchanged in presence of these co-medications. Additionally, the concentration of brexanolone remained essentially unchanged in subjects with hepatic impairment (who might have some level of overall enzymatic inhibition of the liver due to their hepatic impairment). All these results suggest that brexanolone is unlikely to have any clinically relevant drug interactions as a victim.

Figure 7. Box Plots of Brexanolone AUC at 60 and 90 µg/kg/h in the presence and absence of CYP inhibitors (CYPinh).



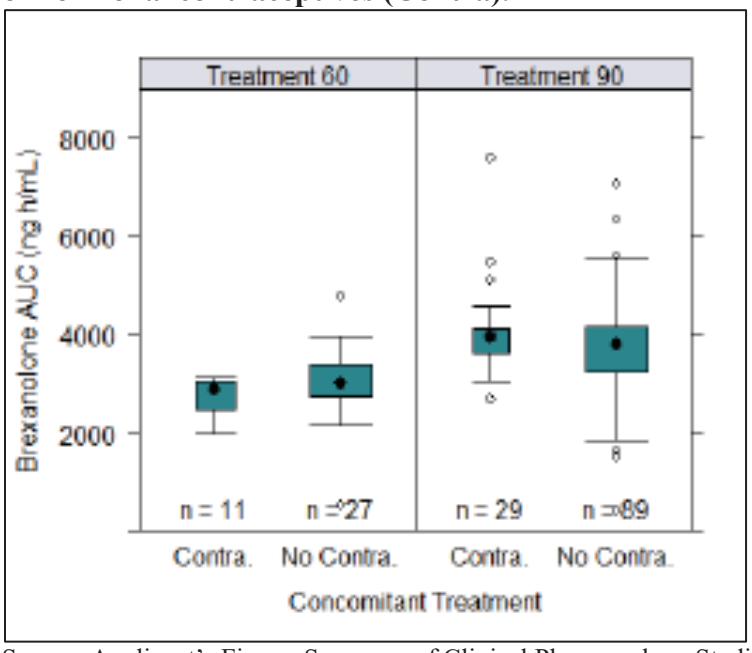
Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Figure 8. Box Plots of Brexanolone AUC at 60 and 90 µg/kg/h in the presence and absence of ibuprofen (IBU)—A clinical AKR inhibitor.



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Figure 9. Box Plots of Brexanolone AUC at 60 and 90 µg/kg/h in the presence and absence of hormonal contraceptives (Contra).



Source: Applicant's Figure, Summary of Clinical Pharmacology Studies.

Therefore, we believe the potential for any clinically relevant drug-drug interactions with brexanolone either as a victim or as a perpetrator is low.

APPEARS THIS WAY ON ORIGINAL

X	X
Primary Reviewer	Team Leader
Praveen Balimane, PhD Mike Bewernitz, PhD Xiaolei Pan, PhD	Hao Zhu, PhD Kevin Krudys, PhD
DCP-1 Director Mehul Mehta, PhD	

7 Sources of Clinical Data and Review Strategy

7.1. Clinical Effectiveness Studies

Studies submitted by the Applicant and evaluated for efficacy are listed in Table 37. (A complete Table of submitted studies can be found in Section 10.1: *Safety Review Approach*.) These studies were randomized, double-blind, and placebo-controlled and were completed in the United States.

Table 37. Studies Evaluated for Efficacy.

Study 547-PPD-	Phase	NCT Number	Population Studied (Baseline HAM-D)	Centers Enrolling Patients	Placebo	Brexanolone	Total N
202A	2	02614547	Severe PPD (≥26)	4	11	10	21
202B	3	02942004	Severe PPD (≥26)	32	43	79[a]	138
202C	3	02942017	Moderate PPD (20 to 25)	32	53	51	108

[a]Brexanolone 60 µg/kg/h, n=38; brexanolone 90 µg/kg/h, n=41.

Studies 202A, B, and C used one "umbrella" protocol. Brexanolone dosing was as previously described (90 µg/kg/h; Section 1.1: *Product Information*). Important individual study differences were:

- Study 202B included an additional brexanolone arm (60 µg/kg/h). In the 60 µg/kg/h group, dosage titration was as follows: 30 µg/kg/h for 4 hours, 60 µg/kg/h for 52 hours, 30 µg/kg/h for 4 hours.
- Studies 202A and 202B enrolled patients with severe PPD; Study 202C enrolled patients with moderate PPD.

Effects were observed over time for the first 60 hours. The primary endpoint for the 202 Studies was change from baseline in HAM-D at 60 hours (i.e., the end of the infusion). The secondary endpoint for Studies 202B and C was the change from baseline in HAM-D at 30 days.

Compliance with Good Clinical Practices

The Applicant attested to compliance with good clinical practice for all three efficacy studies in accordance with the International Conference on Harmonisation (ICH) guidelines and with 21 CFR parts 50, 56, and 312.

Data Quality and Integrity

The submission contains all required components of the electronic common technical document (eCTD). The overall quality and integrity of the application appear to be acceptable. Requests for additional information from the applicant throughout the review process were addressed in a timely fashion.

Financial Disclosure

See Section 22.2: *Financial Disclosure.*

7.2. Review Strategy

The biostatistics and clinical review teams determined that the three randomized, double-blind, placebo-controlled studies would be reviewed for efficacy. (See Section 1.3: *Risk/Benefit Assessment* for the discussion of balancing the safety findings against clinical need and the effectiveness for this product.) The biostatistics review team conducted an independent analysis that confirmed the Applicant's reported results.

8 Statistical and Clinical Evaluation of Efficacy

8.1. 547-PPD-202A

8.1.1. Study Design

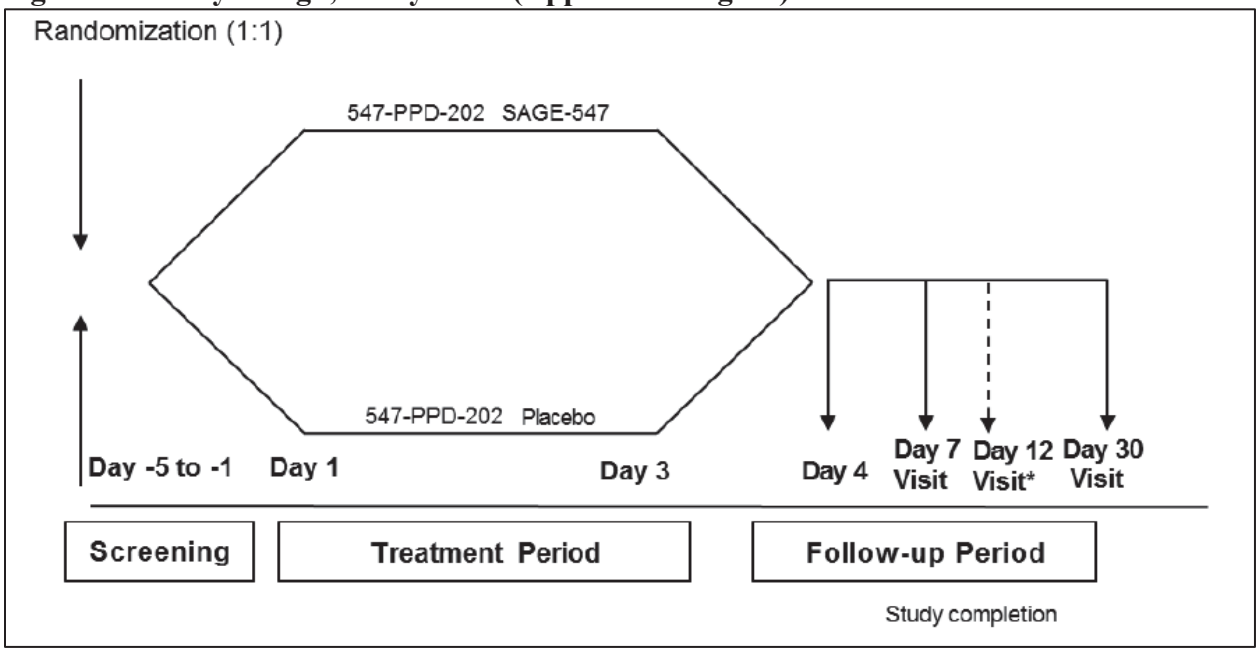
Overview and Objective

The primary objective of 202A was to determine if brexanolone, infused intravenously for 60 hours, reduced depressive symptoms in patients with PPD compared to placebo as assessed by the change from baseline in the 17-item Hamilton Depression Rating Scale (HAM-D) total score.

Trial Design

This was a multicenter, randomized, double-blind, parallel-group, placebo-controlled study of the efficacy, safety, and PK of brexanolone in adult females diagnosed with severe PPD (defined as a baseline HAM-D score greater than 26). Eligible patients were randomized in 1:1 ratio to receive 60 hours of IV treatment with either brexanolone or placebo. See Figure 10 for a schematic representation of the study design. HAMD total score was scheduled to be assessed at baseline and at Hours 1, 2, 4, 8, 12, 24, 36, 48, 60, 72 and on Days 7 and 30.

Figure 10. Study Design, Study 202A (Applicant's Figure).



Source: Applicant's Clinical Study Report, Figure 1.

Study Assessments and Endpoints

The primary efficacy endpoint was change from baseline in HAM-D total score at the end of the treatment period (at 60 hours). There was no secondary endpoint for this study. The HAM-D is a 17-item rating scale with 8 items scored 0 to 2 and nine items scored 0 to 4. The HAM-D was not modified to remove items that might not change in 60 hours (e.g., weight).

Secondary outcomes included the Barkin Index of Maternal Functioning (BIMF), Clinical Global Impression-Improvement (CGI-I), Edinburgh Postnatal Depression Scale (EPDS), Generalized Anxiety Disorder 7-item Scale (GAD-7), a rating of healthcare resource utilization (HCRU), Patient Health Questionnaire 9-item (PHQ-9), and the Short-form 36 (SF-36).

The BIMF is a patient reported outcome scale BIMF covers a broad range of functional areas (self-care, infant care, mother-child interaction, psychological well-being of mother, social support, management, adjustment). This new application of maternal functional status is a robust construct where the physical and mental health of the mother is essential to optimal functioning. Each item is rated on a scale of 0 (strongly disagree) to 6 (strongly agree) , and subscales are drawn from these items.

The CGI-Severity (CGI-S) item uses a 7-point Likert scale to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis. Considering total clinical experience, a patient is assessed on severity of mental illness at the time of rating 1 = normal, not at all ill, 2 = borderline mentally ill, 3 = mildly ill, 4 = moderately ill, 5 = markedly ill, 6 = severely ill, and 7 = extremely ill.

The EPDS is a patient-rated depressive symptom severity scale specific to the perinatal period. The EPDS total score will be calculated as the sum of the 10 individual item scores.

The GAD-7 is a patient-rated depressive symptom severity scale (Spitzer 2006). Scoring for GAD-7 generalized anxiety is calculated by assigning scores of 0, 1, 2, and 3 to the response categories, respectively, of "not at all sure," "several days," "over half the days," and "nearly every day." GAD-7 total score for the seven items ranges from 0 to 21, where a score of 0 to 4 = minimal anxiety, 5 to 9 = mild anxiety, 10 to 14 = moderate anxiety, and 15 to 21 = severe anxiety.

Subject-reported healthcare resource utilization data, including baseline diagnosis history, baseline antidepressant treatment history, and healthcare visits, inpatient visits, and medication use was collected at screening and on Day 30 of follow-up (or at early termination).

The PHQ-9 is a patient-rated depressive symptom severity scale. To monitor severity over time for newly diagnosed patients or patients in current treatment for depression, patients may complete questionnaires at baseline and at regular intervals thereafter. Scoring is total based on responses to specific questions, as follows: "not at all" = 0; "several days" = 1; "more than half the days" = 2; and "nearly every day = 3."

The Medical Outcomes Study Short Form-36 (SF-36v2) is a 36-item measure of health status that has undergone validation in many different disease states (Ware 2007). The SF-36 covers eight health dimensions including four physical health status domains (physical functioning, role participation with physical health problems [role-physical], bodily pain, and general health) and four mental health status domains (vitality, social functioning, role participation with emotional health problems [role-emotional], and mental health). In addition, two summary scores, physical component summary (PCS) and mental component summary (MCS), are produced by taking a weighted linear combination of the eight individual domains. The SF-36v2 is available with two recall periods: the standard recall period is 4 weeks and the acute recall period is 1 week. This study will use the acute version, which asks patients to respond to questions as they pertain to the past week. Higher SF-36 scores indicate a better state of health. The SF-36 requires approximately 10 minutes to complete and can be self-administered or completed by interview in person or by telephone.

Safety assessments included the Columbia Suicide Severity Rating Scale (C-SSRS) and the Stanford Sleepiness Scale (SSS; Study 202A only). See Section 10: *Review of Safety* for more information on these scales.

See Table 38 for the schedule of study assessments.

Table 38. Schedule of Assessments for 547-PPD-202 Umbrella Protocol.

Assessments	Screening	Treatment													Follow-up						
	D -7 to -1	D1							D2				D3		D3		D7	D12[a]	D14[b]	D21[b]	D30
		H0	H2	H4	H8	H12	H18	H24	H30	H36	H42	H48	H54	H60	H66	H72					
History and Physical	X															X	X				
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X
EKG	X											X					X				
Pulse Oximetry[a]		X	X	X	X	X	X	X	X	X	X	X	X	X							
Clinical Labs	X															X	X				
Pregnancy Test[c]	X	X																			X
C-SSRS		X						X						X		X	X		X	X	X
HAM-D	X	X	X	X	X	X		X		X		X		X		X	X		X	X	X
CGI-S	X	X																			
CGI-I			X	X		X		X		X		X		X		X	X		X	X	X
MADRS	X	X						X				X		X		X	X		X	X	X
BIMF, SF-36[b]		X															X		X	X	X
EPDS, GAD-7, PHQ-9		X												X			X		X	X	X
HCRU	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
SSS[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					

[a]Study 202A only (pulse oximetry not recorded on case report forms).
[b]Studies 202B and C only.
[c]Serum at screening; urine all other times.

Statistical Analysis Plan

The statistical plan was finalized before the data were unblinded. The Efficacy Population included all randomized subjects who started the infusion of study drug and had a valid baseline HAM-D assessment and at least one post-baseline HAM-D assessment. This analysis population was used for all efficacy analyses.

The change from baseline in HAM-D total score at all post-baseline visits was analyzed using a mixed effects model for repeated measures (MMRM), the model included center, treatment, baseline HAM-D total score, assessment time point, and time point-by-treatment as explanatory variables. Center was planned to be treated as a random effect, while all other explanatory variables were treated as fixed effects. However, due to the fact that several sites had a small number of subjects enrolled, center was removed from the final MMRM models. An unstructured covariance structure was used to model the within-subject errors. The Kenward-Roger approximation was used to estimate denominator degrees of freedom. The primary comparison was between SAGE-547 and placebo treatment groups at the 60-hour time point. Model based point estimates (i.e., LS means, 95% confidence intervals, and p-values) were reported for each time point. In case of convergence issues, other covariance structures were to be used, including autoregressive (AR (1)), compound symmetry, and variance components with each model fit to find the covariance structure with the best fit. No missing data would be imputed. There was no planned method for handling missing data. There was no planned subgroup analysis.

An interim analysis of the placebo group was planned to be conducted by an independent statistician for sample size re-estimation purposes when at least 16 subjects had completed HAM-D efficacy assessments through 60 hours. Based on the interim observed placebo response rate only, the independent statistician would communicate one of the following messages back to the Applicant: (1) “No adjustment to the sample size is required” or (2) “Increase the sample size by 5 subjects per group.” Because the Applicant would be kept uninformed of any unblinded information during and after the interim analysis, and there was no analysis of drug effect, no statistical adjustment would be made to the level of significance for any hypothesis testing at the end of the study.

Biometric Reviewer Comment: The Statistical Analysis Plan (SAP) was not submitted during the IND stage. Therefore, FDA did not review the SAP. FDA was concerned about the original plan of treating center as a random effect because it was not clear if the center effect followed a normal distribution.

Protocol Amendments

The 202A protocol was amended twice as follows (only substantive changes presented):

- December 22, 2015
  - added optional breastmilk PK sampling
  - ended 90 µg/kg/h infusion at Hour 52 and added the 8-hour taper (previously, the 90µg/kg/h infusion ran until Hour 60)
  - pulse oximetry assessment was changed from collection at specific timepoints to

continuous monitoring (checked every 2 hours) during the infusion

- June 30, 2016
  - Parts 202B and C were added to the existing 202A protocol

Clinical Reviewer Comment: None of the amendments altered study integrity. Continuous pulse oximetry improved study safety; however, values were not recorded on case report forms nor submitted with the NDA. The taper was in response to convulsions seen in animal studies when large brexanolone doses were withdrawn. There were no convulsions in any of the human studies.

8.1.2. Study Results

Patient Disposition

This trial was conducted in four centers in the United States. A total of 23 subjects were screened; two were screening failures, leaving 21 subjects (10 SAGE-547, 11 placebo) randomized and treated. All 21 subjects completed the study and were included in the planned analyses.

Protocol Violations/Deviations

There were no protocol violations. Eight brexanolone and seven placebo patients had minor protocol deviations (e.g., missing an assessment at a certain time point). One brexanolone and two placebo patients had major protocol deviations.

- Subject (b) (6); brexanolone 90 μg/kg/h: Initially believed to be taking 0.5 mg clonazepam at baseline. After the start of the infusion, it was discovered that she was taking 6 mg clonazepam daily. The clonazepam was discontinued during the infusion, restarted at 2 mg on Day 4, and discontinued again on Day 9.
- Subject (b) (6); placebo: A pump error caused the infusion to stop at Hour 21. It was restarted without incident at Hour 24.
- Subject (b) (6); placebo: The infusion was interrupted for localized edema from Hour 46 to Hour 48.5.

Table of Demographic Characteristics

All subjects (100%) were female. A summary of the other demographic data is presented in Table 39.

Table 39. Demographic Characteristics, Study 202A.

Characteristic	Placebo (n=11)	Brexanolone 90 µg/kg/h (n=10)
Age, years		
Mean (SD)	28.8 (4.6)	27.4 (5.3)
Median	28	27
Min, Max	22, 36	20, 40
Race, n (%)		
AA/Black	6 (55%)	7 (70%)
White	5 (45%)	3 (30%)
Ethnicity, n (%)		
Hispanic	0	0
Non-hispanic	11 (100%)	10 (100%)
Height, cm		
Mean (SD)	161.7 (6.7)	162 (7.1)
Median	162	164
Min, Max	151, 174	153, 175
Weight, kg		
Mean (SD)	77.0 (22.3)	86.7 (28.8)
Median	73.5	76.5
Min, Max	53.3, 122.6	49.7, 130.7
BMI, kg/m^2		
Mean (SD)	29.3 (7.8)	32.7 (9.9)
Median	28.2	30.5
Min, Max	21.0, 45.0	20.4, 47.1

Clinical Reviewer Comment: This was a very small, Phase 2 study and cannot be expected to adequately represent the diversity of the U.S. population. However, African-American/black patients were well-represented.

Other Baseline Characteristics

Concomitant antidepressant and benzodiazepine medications were allowed. See Table 40 for antidepressant use at baseline in Study 202A.

Table 40. Antidepressant Use at Baseline, Study 202A.

Antidepressant	Placebo (n=11)	Brexanolone 90 µg/kg/h (n=10)
Any antidepressant[a]	3	3
Bupropion	2	1
Citalopram	1	0
Escitalopram	1	0
Fluoxetine	2	1
Nortriptyline[b]	1	0
Sertraline	2	2
Trazodone[b]	1	1
Venlafaxine	2	0

[a]Several patients were on multiple antidepressants at the start of the study.
[b]Recorded as used for insomnia.

Treatment Compliance and Rescue Medication Use

Because the study drug was administered as an infusion in a monitored setting, compliance was 100%.

"Rescue antidepressant use" (Table 41) was defined as either of the following at Day 4 or later:

- Initiation of a new antidepressant
- Any increase in dose for a medication previously taken at a stable, lower dose

The Applicant defined antidepressant medications as those with an "[Anatomical Therapeutic Chemical] ATC 3 code N06A or N05A, or with indication containing terms depression, postpartum depression, major depression, PPD, MDD, or mood."

Table 41. Rescue Antidepressant Use, Study 202A.

Antidepressant	Placebo (n=11)	Brexanolone 90 µg/kg/h (n=10)
Initiation of New Antidepressant or Increase in Antidepressant Dose	3[a]	3

[a]One patient had initiation of nortriptyline for sleep.

Clinical Reviewer Comment: All six patients who met the criteria for use of a "rescue antidepressant" were also taking antidepressants at baseline. Put another way, all subjects taking an antidepressant at baseline were thought to require some change to their regimen after exposure to study drug. However, there was no difference between brexanolone and placebo groups.

Efficacy Results: Primary Endpoint

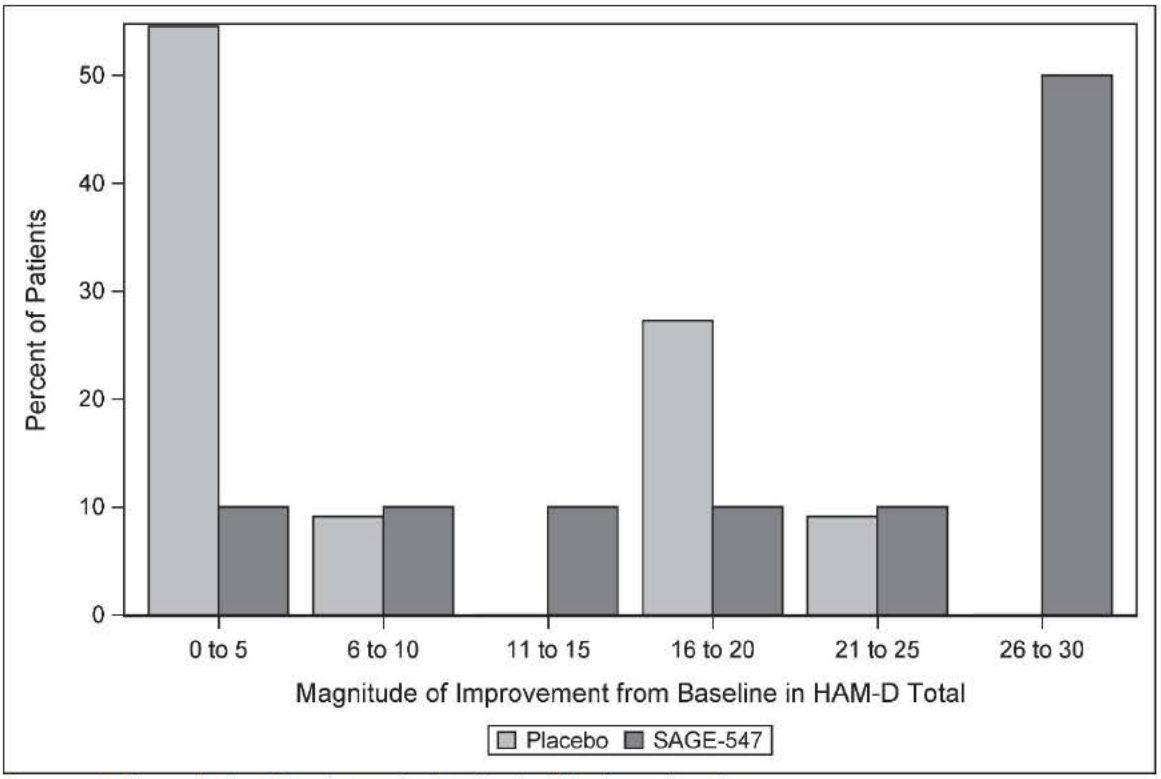
The single, primary efficacy endpoint was change from baseline in HAMD total at Hour 60. The primary analysis result is summarized in Table 42. There were no missing data for the primary endpoint. Figure 11 displays the histogram of the magnitude of improvement from baseline in HAM-D total at Hour 60.

Table 42. Primary Analysis Results on Change-from-Baseline in HAM-D Total at Hour 60, Study 202A.

	Placebo (n=11)	**Brexanolone 90 µg/kg/h** (n=10)
Mean score at Baseline (SD)	28.8 (1.99)	28.1 (1.29)
Mean Score at Hour 60 (SD)	19.7 (9.59)	7.5 (8.72)
LS Mean Change from Baseline (SE)	-8.8 (2.80)	-21.0 (2.94)
Placebo -subtracted Difference (95% CI)		-12.2 (-20.8, -3.7)
P-value		0.008

Source: Biostatistics Reviewer's Analysis (dqshamd.xpt).

Figure 11. Histogram of the Magnitude of Improvement from Baseline in HAM-D Total at Hour 60, Study 202A.

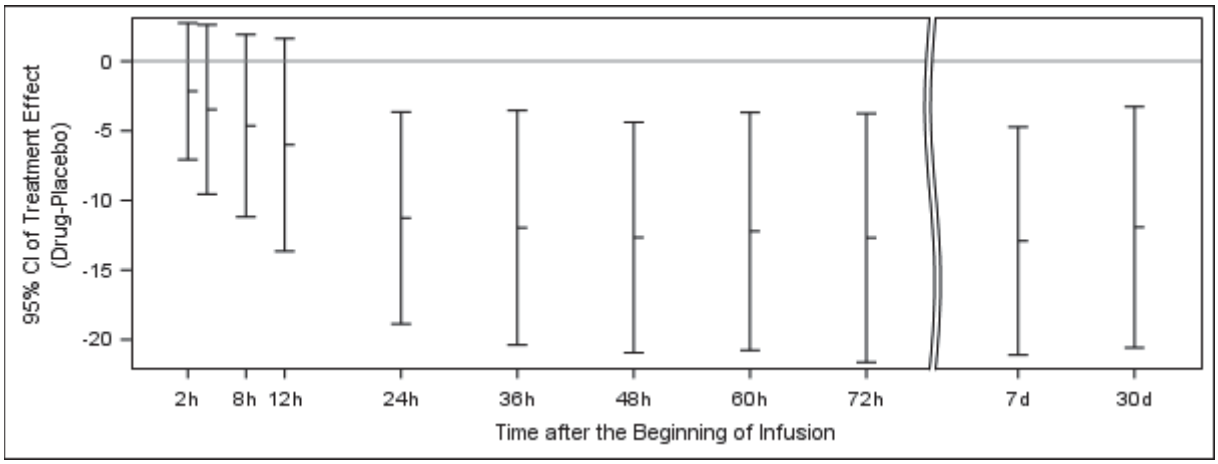


Source: Biostatistics Reviewer's Analysis (dqshamd.xpt).

The time course of the treatment effect is graphically presented in Figure 12 and Figure 13. There was a numerically greater change from baseline for the SAGE-547 group at all time points

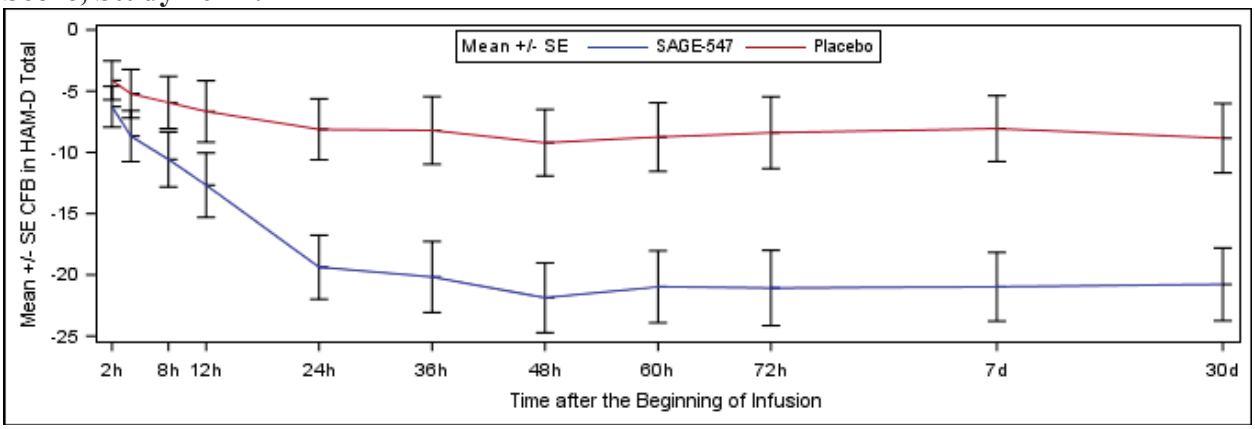
through Day 30 and the effect appeared maximal at 24 to 36 hours, after which it appeared stable for 30 days.

Figure 12. Least Squared Mean Difference and 95% Confidence Interval of Treatment Effect Over Time, Study 202A.



Source: Biostatistics Reviewer's Analysis (dqshamd.xpt).

Figure 13. Least Squared Mean (±SE) Change-from-Baseline over Time in HAM-D Total Score, Study 202A.



Source: Biostatistics Reviewer's Analysis (dqshamd.xpt).

A sample-size re-estimation analysis was conducted by an independent CRO statistician when 16 subjects had completed HAM-D efficacy assessments through Hour 60. As a result of this analysis, the independent statistician communicated that no increase in the planned sample size was required. As there was no analysis of drug effect and Sage was kept blinded to the data and uninformed of the interim results (i.e., response rates) until final database lock, no statistical adjustment was made to the level of significance for hypothesis testing at the end of the study.

This trial was conducted in the United States. All subjects were adult females. Because of the small sample size of the study, no subgroup analysis by age, gender and race was performed.

Efficacy Results: Secondary Endpoints

Study 202A included no prespecified secondary endpoints.

Dose and Dose Response

Study 202A did not explore dose response.

Durability of Response with Continued Administration

Brexanolone is delivered as a one-time infusion. The Applicant did not investigate durability of response with continued administration.

Persistence of Effect

Study 202A did not prespecify any secondary endpoints. Nevertheless, the placebo-adjusted least squared mean change for the HAM-D at Day 30 was -11.9 (SE=4.1; p=0.0095).

8.2. 547-PPD-202B

8.2.1. Study Design

Overview and Objective

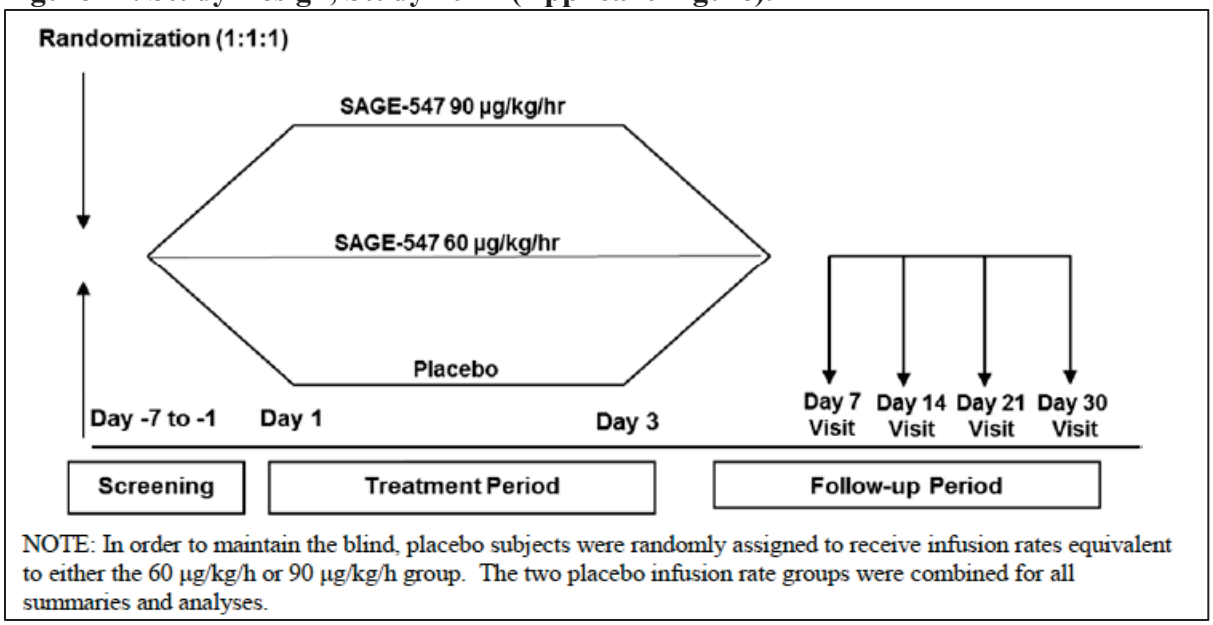
The primary objective was to determine if SAGE-547 infused intravenously at up to 90 μg/kg/h for 60 hours reduces depressive symptoms in subjects with severe PPD compared to placebo injection, as assessed by the change from baseline in HAM-D total score, and to compare two doses of SAGE-547, 90 μg/kg/h and 60 μg/kg/h.

Trial Design

The trial design was similar to the design of Trial PPD-202A with the principal difference being the inclusion of an additional dose arm and stratification by antidepressant use at baseline, as suggested by FDA. Follow-up visits at Day 14 and 21 were added by amendment in January 2017, but earlier patients were not assessed at these time points. The sample sizes at these two visits were, therefore, smaller than the sample sizes at the other visits (i.e., Days 7, 30). Subjects were randomly assigned to one of three treatment groups (brexanolone 60 μg/kg/h, brexanolone 90 μg/kg/h, or placebo) in a 1:1:1 ratio. To achieve a 1:1:1 balance among the treatment groups while maintaining the blind, all subjects were randomized in a 2:2:1:1 to (90 μg/kg/h: 60 μg/kg/h: placebo for 90 μg/kg/h: placebo for 60 μg/kg/h) and the two placebo groups were then combined for analysis.

This trial was conducted entirely in the United States. The trial design is presented in Figure 14. HAM-D total score was scheduled to be assessed at baseline and Hours 1, 2, 4, 8, 12, 24, 36, 48, 60, 72 and Days 7, 14, 21, and 30 (see Table 38). Entry criteria were a diagnosis of PPD starting during the third trimester or within 4 weeks of delivery and a HAM-D score of ≥ 26.

Figure 14. Study Design, Study 202B (Applicant Figure).



Source: Clinical Study Report Figure 1.

Study Assessments and Endpoints

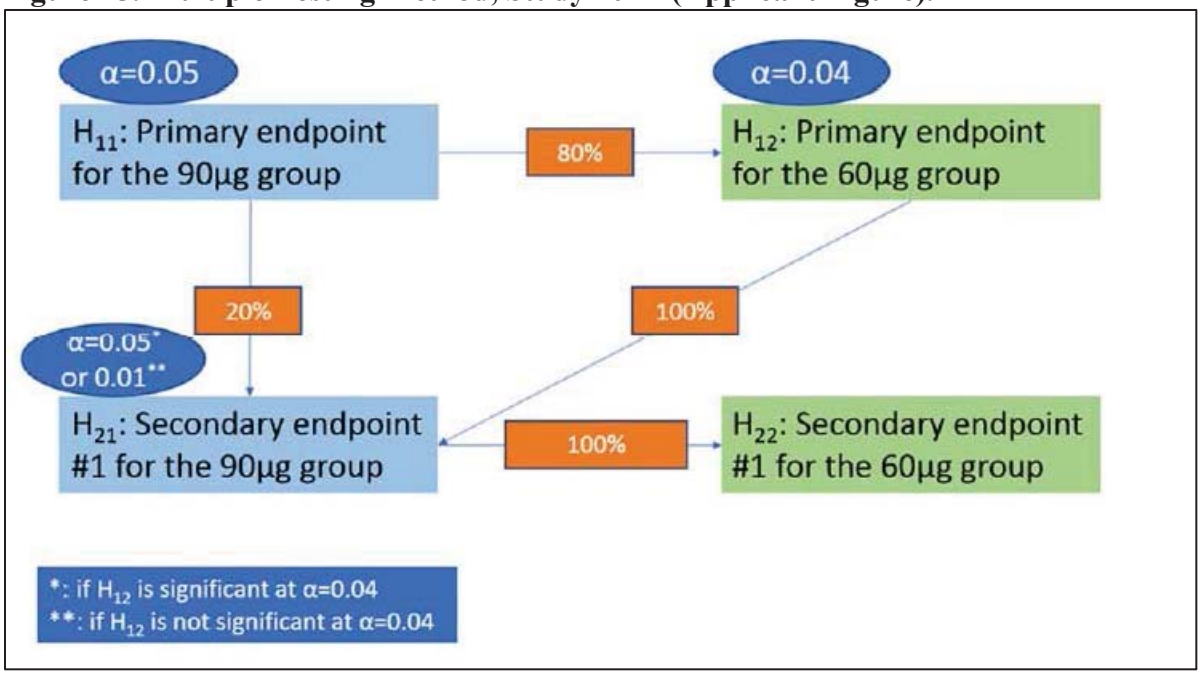
The change from baseline in HAM-D total score at the end of the treatment period (Hour 60) was the primary efficacy endpoint. The change from baseline in HAM-D total score at Day 30 was the pre-specified secondary efficacy endpoint.

Statistical Analysis Plan

The statistical analysis plan (SAP) was evaluated in the statistical review dated 12/13/2016 in DARRTS under IND 122279. The statistical plan was finalized before data lock. The Efficacy Set included all randomized subjects who started the infusion of study drug and who had a valid baseline HAM-D assessment and at least one postbaseline HAM-D assessment. This analysis population was used for all efficacy analyses. All the post-baseline assessments of the efficacy population were used in all efficacy analysis. There was no interim analysis. Subgroup analyses by age, race, baseline antidepressant use, baseline BMI, onset of PPD within 4 weeks of delivery, and family history of PPD were planned. A sensitivity analyses based on Missing Not at Random (MNAR) to handle missing data was pre-specified.

Change from baseline to each postbaseline assessment time point in HAM-D total score was analyzed using MMRM. The model included the change from baseline at each visit time point as the dependent variable, center (pooled), treatment, baseline antidepressant use, baseline HAM-D total score, visit time point, and visit time point-by-treatment interaction terms as explanatory variables. All explanatory variables including pooled center were treated as a fixed effect in the primary analysis. To control the type I error rate for conducting multiple comparisons, the approach in Figure 15 was utilized.

Figure 15. Multiple Testing Method, Study 202B (Applicant Figure).



Source: Clinical Study Report Figure 2.

Protocol Amendments

The 202B protocol was amended twice as follows (only substantive changes presented):

- January 31, 2017
  - removed the Stanford Sleepiness Scale, added the SF-36 and HCRU
  - Clarified follow-ups (7, 12, and 30 days for 202A; 7, 14, 21, 30 days for 202B and 202C)
  - Stratified enrollment by baseline antidepressant use
  - Removed pulse oximetry assessment
  - Removed the breast milk PK sub-study from the PPD-202 Umbrella Protocol

- March 16, 2017
  - Shortened requirement for pumping and discarding breastmilk based on data and advice from FDA (from 12 to 7 days)

Clinical Reviewer Comment: None of the amendments altered study integrity. It is unfortunate that we have no pulse oximetry data from the studies.

8.2.2. Study Results

Patient Disposition

This trial was conducted in 32 centers in the United States. One hundred thirty-eight subjects were randomized into the study, 122 of whom received study drug (43 placebo, 38 brexanolone 60 µg/kg/h, and 41 brexanolone 90 µg/kg/h). Of the subjects who received study drug, nine discontinued the study early (one placebo, three brexanolone 60 µg/kg/h, five brexanolone 90 µg/kg/h). No subject withdrew from the study because of an AE. The ITT population contains all

122 randomized subjects who received study drug. Of the 122 subjects, 119 (97.5%) had a primary efficacy endpoint assessment (Hour 60) and 113 (92.6%) had the pre-specified secondary endpoint assessment (Day 30).

Protocol Deviations

There were no protocol violations (i.e., a protocol deviation that reduces the quality or completeness of the data, makes the informed consent document inaccurate, or impacts a subject's safety, rights, or welfare).

All patients in the brexanolone 90 µg/kg/h arm (n 41), 37 patients in the brexanolone 60µg/kg/h arm, and 33 patients in the placebo arm had protocol deviations. Most were considered minor (e.g., an assessment performed out of window). Four brexanolone 90 µg/kg/h patients and two brexanolone 60 µg/kg/h patients had more serious protocol deviations.

- Subject (b) (6) brexanolone 90 µg/kg/h: Patient's baseline HAM-D score was 25, but she was randomized to 202B (202B was for patients with a HAM-D of ≥ 26; 202C was for patients with a HAM-D of 20 to 25).
- Subject (b) (6) brexanolone 90 µg/kg/h: Patient's infusion was calculated based on the wrong weight (79 kg versus actual weight of 53 kg).
- Subject (b) (6); brexanolone 90 µg/kg/h: Patient's coagulation labs were not processed by the lab.
- Subject (b) (6) brexanolone 90 µg/kg/h: Patient's antidepressant dose was not stable in the 14 days prior to screening (patient had discontinued bupropion 19 days before infusion).
- Subject (b) (6) brexanolone 60 µg/kg/h: Patient's screening urinalysis was not done.
- Subject brexanolone 60 µg/kg/h: Patient's baseline HAM-D score was 23, but she was randomized to 202B (202B was for patients with a HAM-D of ≥ 26; 202C was for patients with a HAM-D of 20 to 25).

Clinical Reviewer Comment: I do not believe these deviations would have had a significant effect on the study's efficacy results. The mis-assignment of patients with "moderate" rather than "severe" PPD to the Study did not confound the interpretation of the results.

Table of Demographic Characteristic

All subjects (100%) were female. The study was conducted entirely in the United States. A summary of the other demographic data is presented in Table 43.

Table 43. Demographic Characteristics, Study 202B.

Characteristic	Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
Age, years			
Mean (SD)	27.2 (6.1)	27.7 (6.5)	27.5 (6.1)
Median	27	27	27
Min, Max	18, 42	18, 42	19, 42
Race, n (%)			
AA/Black	15 (35%)	12 (32%)	8 (19%)
Am Indian/Alaskan Native	1 (2%)	0	0
Asian	0	0	1 (2%)
Native Hawaiian/Pacific Islander	0	0	1 (2%)
White	27 (63%)	25 (66%)	29 (70%)
Other	0	1 (3%)	2 (5%)
Ethnicity, n (%)			
Hispanic	7 (16%)	3 (8%)	7 (17%)
Non-hispanic	36 (84%)	35 (92%)	34 (83%)
Height, cm			
Mean (SD)	165.4 (8.0)	164.1 (6.5)	164.3 (6.7)
Median	164.4	165.0	163.0
Min, Max	145.0, 180.3	147.3, 178.5	149.8, 180.3
Weight, kg			
Mean (SD)	81.8 (23.4)	87.1 (20.8)	80.7 (20.5)
Median	74.9	85.5	82.7
Min, Max	48.1, 142.3	48.5, 134.7	52.2, 125.0
BMI, kg/m^2			
Mean (SD)	29.9 (8.2)	32.3 (7.4)	29.8 (7.1)
Median	28.6	31.7	29.3
Min, Max	17.9, 51.7	20.2, 48.0	19.0, 50.7

Clinical Reviewer Comment: The 2015 U.S. Census data reports 77% of the population is white, 13% is African American/black, and 6% is Asian; Hispanics make-up 18% of the population. The Study's enrollment does not reflect the exact racial and ethnic make-up of the country, but (for a study of its size) the Applicant did well, enrolling at least one Alaskan Native/American Indian and one Native Hawaiian/Pacific Islander as well as a substantial African-American/black population. There were minor differences in proportions of patients of different races and ethnicities between arms, but I do not feel this represents a significant problem in interpreting the study's results. The mean/median ages appear to well-represent the population of interest (i.e., not skewed to younger or older mothers).

Differences in weight/BMI between arms are not likely to affect results because brexanolone dosing is weight-based, but the mean weights between the brexanolone 60 and 90 µg/kg/h arms were not statistically different (t-test 1.4; p=0.17).

Other Baseline Characteristics

See Table 44 for baseline characteristics of interest in Study 202B. Table 43 includes medications with potential antidepressant action being taken at baseline.

Table 44. Baseline Patient Characteristics, Study 202B.

Characteristic	Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
Prior antidepressant treatment, n (%)	14 (33%)	15 (40%)	13 (32%)
Onset of PPD, n (%)			
Third Trimester	14 (33%)	10 (26%)	8 (20%)
Within 4 weeks of delivery	29 (67%)	28 (74%)	33 (80%)
Previous episodes of depression, n (%)	12 (28%)	8 (21%)	7 (17%)
1	3 (7%)	4 (11%)	2 (5%)
2	1 (2%)	0	0
3	0	0	1 (2%)
>3			
Previous episodes of PPD, n (%)			
Yes	16 (37%)	12 (32%)	10 (24%)
Severity of Depression at Baseline			
HAM-D, Mean (SD)	28.6 (2.5)	29.0 (2.7)	28.4 (2.5)
EPDS, Mean (SD)	21.7 (3.0)	21.7 (3.4)	19.9 (3.7)

Clinical Reviewer Comment: The groups were fairly well-matched at baseline. Although there were numerical differences in the proportions of patients who were diagnosed within 4 weeks of delivery, the difference between the brexanolone 90 µg/kg/h and placebo groups was not significant (Chi square=1.8, p=0.18). The mean HAM-D at baseline indicates that, on average, the patients' PPD was, indeed, severe (defined by the Applicant as ≥ 26).

Table 45. Antidepressant Medication Use at Baseline, Study 202B.[a]

Class	Medication	Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
At least one antidepressant		14 (33%)	14 (37%)	13 (32%)
SNRI	Duloxetine	0	0	1 (2%)
	Venlafaxine	0	0	1 (2%)
SSRI	Citalopram	0	1 (3%)	0
	Escitalopram	1 (2%)	2 (5%)	0
	Fluoxetine	2 (5%)	2 (5%)	1 (2%)
	Paroxetine	2 (5%)	0	0
	Sertraline	7 (16%)	7 (18%)	9 (22%)
TCA	Clomipramine	0	0	1 (2%)
Other Antidepressants	Bupropion	1 (2%)	4 (11%)	1 (2%)
	Mirtazapine	0	0	2 (5%)
	Trazodone	2 (5%)	1 (3%)	1 (2%)
	Vortioxetine	1 (2%)	0	0
Other Drugs	Aripiprazole	1 (2%)	0	1 (2%)
	Cariprazine	0	0	1 (2%)
	Quetiapine	1 (2%)	1 (3%)	1 (2%)

[a]Patients could be taking more than one of these drugs.

Clinical Reviewer Comment: One-third of the 202B patients were on an antidepressant at the start of the trial. The enrolled sample is representative of patients both already taking a psychotropic medication and those not already taking a psychotropic medication. However, patients were on a wide variety of medications and it is impossible to say how brexanolone interacts with any individual drug or class.

Treatment Compliance and Rescue Medication Use

Because the study drug was administered as an infusion in a monitored setting, compliance was 100%.

Table 46 presents rescue antidepressant use in the study.

Table 46. Rescue Antidepressant Use, Study 202B.

Rescue Antidepressant	Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
Initiation of New Antidepressant or Increase in Antidepressant Dose, n (%)	3 (7%)	4 (11%)	5 (12%)

Efficacy Results: Primary Endpoint and Prespecified Secondary Endpoints

A summary of statistical significance for the primary and prespecified secondary efficacy

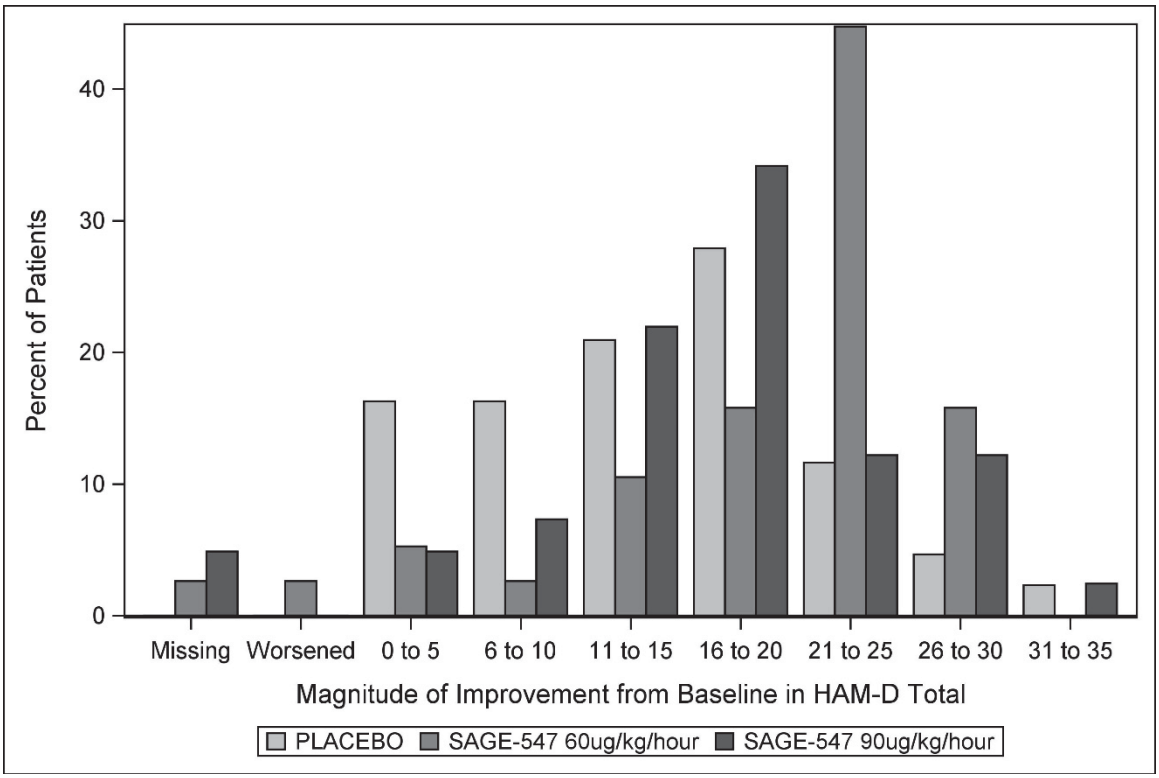
endpoints according to the pre-specified testing procedure is summarized in Table 47. All prespecified comparisons were considered statistically significant based on the testing procedure. No sensitivity analysis was performed because of the negligible level of missing data. Figure 16 and Figure 17 display the histograms of the magnitude of improvement from baseline in HAM-D total at Hour 60 and Day 30, respectively.

Table 47. Primary Efficacy and Secondary Efficacy Results, Study 202B.

Timepoint		Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
Hour 60	Mean score at Baseline (SD)	28.6 (2.54)	29.0 (2.70)	28.4 (2.47)
	Mean Score at Hour 60 (SD)	14.6 (7.55)	9.2 (7.01)	10.7 (5.78)
	LS Mean Change from Baseline (SE)	-14.40 (1.15)	-19.5 (1.23)	-17.7 (1.19)
	Placebo-subtracted Difference (95% CI)		-5.5 (-8.8, -2.2)	-3.7 (-6.9, -0.5)
	P-value (unadjusted)		0.0013	0.0252
	Significance (MCP-adjusted)		Yes	Yes
Day 30	Mean Score at Baseline (SD)	28.6 (2.54)	29.0 (2.70)	28.4 (2.47)
	Mean Score at Day 30 (SD)	14.7 (9.46)	9.1 (7.97)	11.0 (8.34)
	LS Mean Change from Baseline (SE)	-13.8 (1.32)	-19.5 (1.44)	-17.6 (1.40)
	Placebo-subtracted Difference (95% CI)		-5.6 (-9.5, -1.8)	-3.8 (-7.6, -0.0)
	P-value (unadjusted)		0.0044	0.0481
	Significance (MCP-adjusted)		Yes	Yes

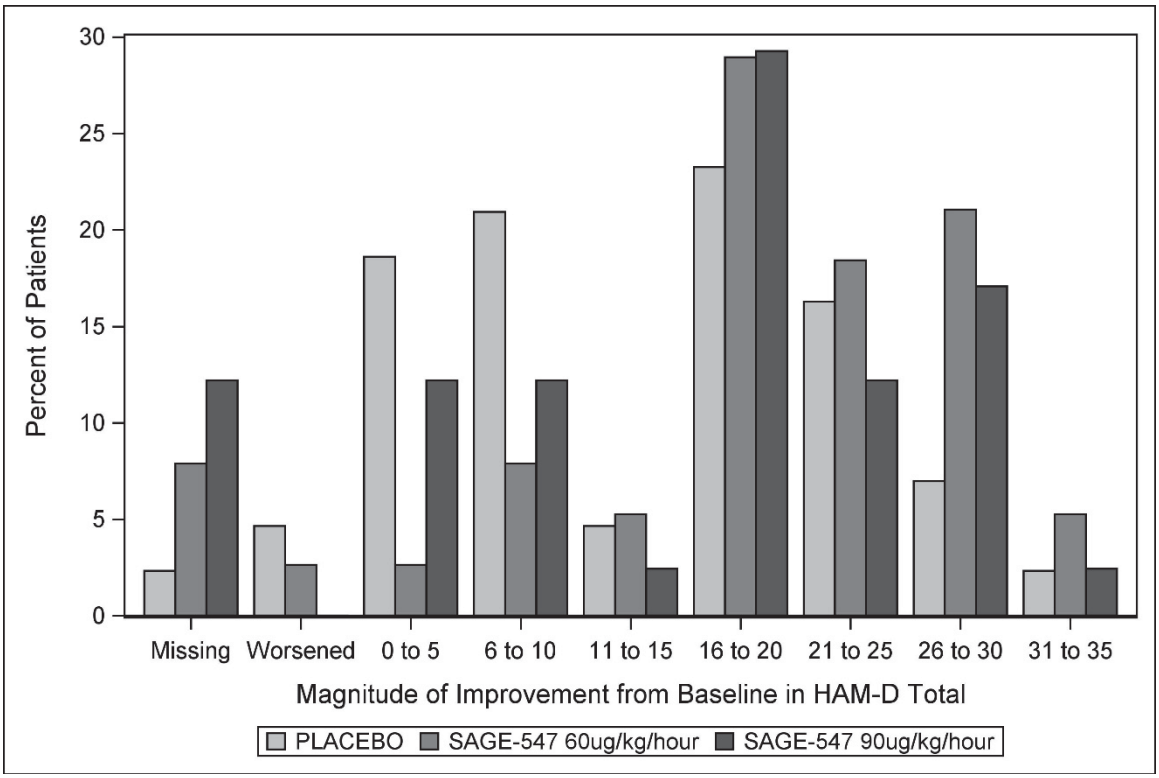
MCP=Multiple comparison procedures.
CI were not adjusted with multiplicity.
Source: Biostatistics Reviewer's Analysis (adqspri.xpt).

Figure 16. Histogram of the Magnitude of Improvement from Baseline in HAM-D Total at Hour 60, Study 202B.



Source: Biostatistics Reviewer's Analysis (adqspri.xpt).

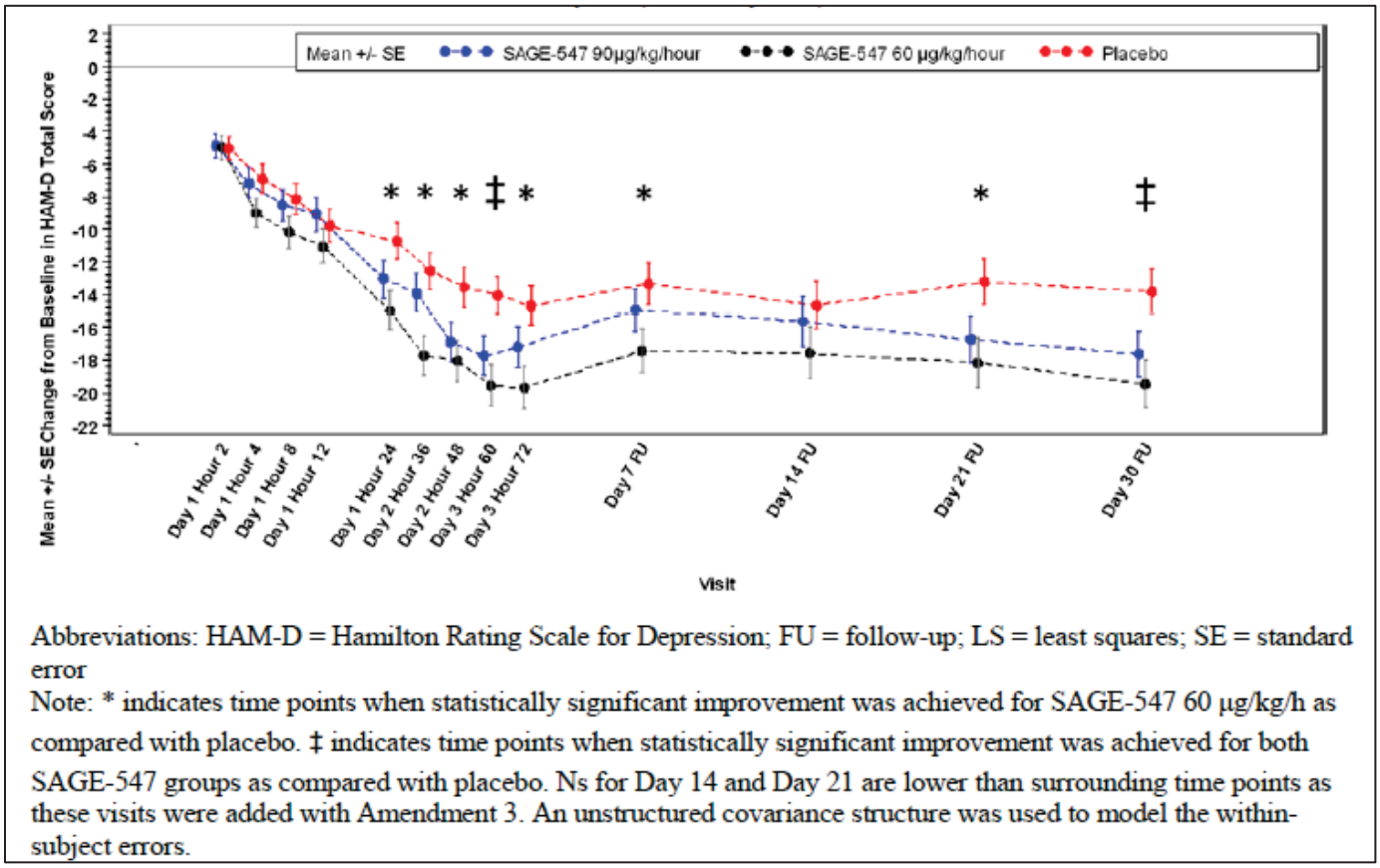
Figure 17. Histogram of the Magnitude of Improvement from Baseline in HAM-D Total at Day 30, Study 202B.



Source: Biostatistics Reviewer's Analysis (adqspri.xpt).

As displayed in Figure 18, all treatment groups showed a decrease in HAM-D total score over the first 72 hours, with numerically greater change from baseline for both brexanolone groups at all time points starting at Hour 24. As in Study 202A, the full drug-placebo difference was observed at 24 hours, when a dose of 60 µg/kg/h was used in both treatment groups.

Figure 18. Least Squared Mean (±SE) Change-from-Baseline over Time in HAM-D Total Score, Study 202B (Applicant Figure).

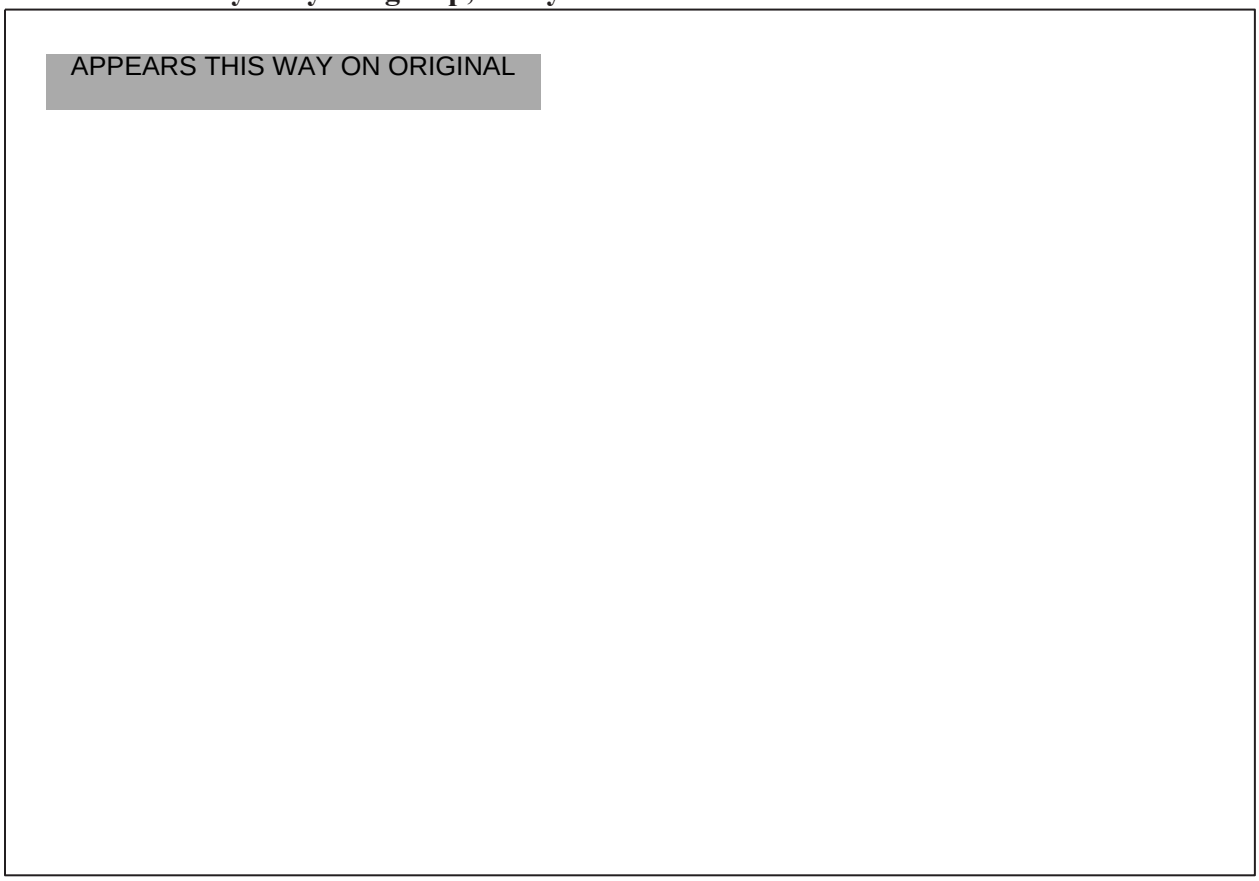


Abbreviations: HAM-D = Hamilton Rating Scale for Depression; FU = follow-up; LS = least squares; SE = standard error
Note: * indicates time points when statistically significant improvement was achieved for SAGE-547 60 µg/kg/h as compared with placebo. ‡ indicates time points when statistically significant improvement was achieved for both SAGE-547 groups as compared with placebo. Ns for Day 14 and Day 21 are lower than surrounding time points as these visits were added with Amendment 3. An unstructured covariance structure was used to model the within-subject errors.

Note: the significance at each timepoint was not adjusted for multiplicity.
Source: Clinical Study Report Figure 3.

Further exploratory subgroup analyses on the primary endpoint were assessed by age group (18 to 24 vs. 25 to 45), race, baseline antidepressant use, baseline BMI, onset of PPD, and family history of PPD. Results are shown in Figure 19.

Figure 19. Least Squared Mean Difference between Brexanolone and Placebo (with 95% Confidence Interval) for Change-from-Baseline at Hour 60 in HAM-D Total Score Based on MMRM Analysis by Subgroup, Study 202B.



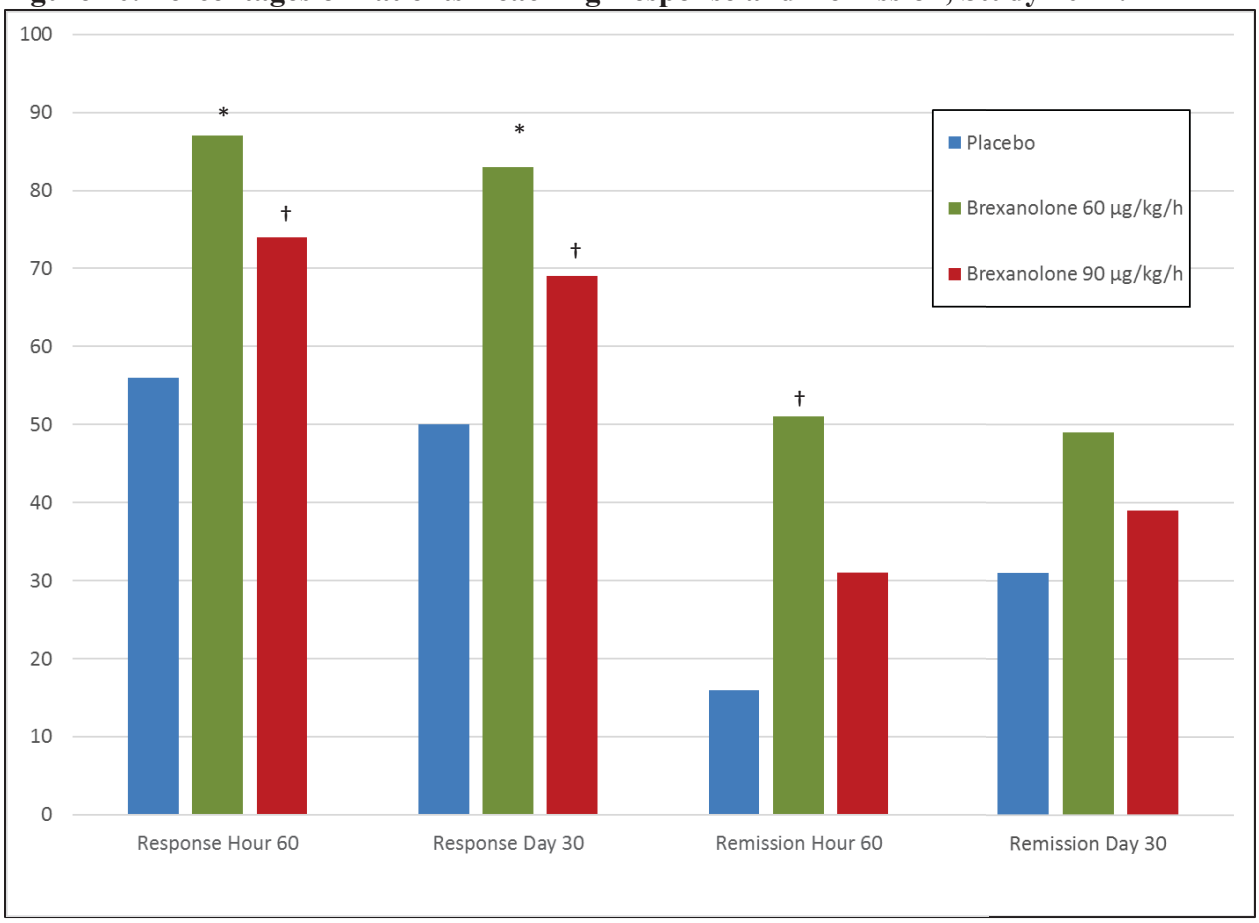
Source: Biometrics Reviewer's Analysis (adsl.xpt and adqspri .xpt).

Biostatistics Reviewer Comment: The estimates in the smaller subgroups are subject to large sampling variation, but no apparent subgroup differences were observed.

Exploratory Endpoints

The Applicant defined response as a reduction of HAM-D score of at least 50% compared with baseline. Remission was having a HAM-D score ≤ 7. At Hour 60 and Day 30, more patients on brexanolone (both the 90 and the 60 μg/kg/h arms) than placebo reached response (see Figure 20). At Hour 60, more patients receiving brexanolone 60 μg/kg/heached remission than patients receiving placebo.

Figure 20. Percentages of Patients Reaching Response and Remission, Study 202B.



*=p<0.01; †=p<0.05 when compared to placebo arm. P-values for these exploratory endpoints did not take into account multiplicity adjustment.
Source: Clinical reviewer's analysis.

Table 46 includes exploratory endpoints from Study 202B. These results represent a range of patient experience with the drug (in addition to the primary and pre-specified secondary endpoints). Of note, CGI-I response is defined as a score of 1 (very much improved) or 2 (much improved). There were no statistically significant differences in any of the eight sub-scores of the SF-36 or on the HCRU (data not shown).

Clinical Review Comment: Although few results were statistically significant, numerically results are consistent with the primary and prespecified endpoints.

Table 48. Exploratory Endpoints, Study 202B.

Timepoint	Scale	Parameter	Placebo (n=43)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=41)
Hour 60	CGI-I	Response, %	56	**84***	**82†**
	GAD-7	LS Mean Change from Baseline (SE)	-7.7 (7.2)	-8.1 (7.3)	-7.3 (6.4)
	EPDS	LS Mean Change from Baseline (SE)	-8.7 (1.0)	-10.3 (1.1)	-9.8 (1.1)
	PHQ-9	LS Mean Change from Baseline (SE)	-7.9 (1.1)	-8.8 (1.2)	-8.9 (1.2)
Day 30	CGI-I	Response, %	52	80†	72†
	GAD-7	LS Mean Change from Baseline (SE)	-8.5 (7.3)	-9.9 (6.8)	-8.9 (6.5)
	EPDS	LS Mean Change from Baseline (SE)	-9.2 (1.1)	**-12.8 (1.3)†**	-11.0 (1.3)
	PHQ-9	LS Mean Change from Baseline (SE)	-9.5 (1.1)	-12.0 (1.3)	-11.9 (1.2)
	BIMF	LS Mean Change from Baseline (SE)	20.4 (2.8)	**29.0 (3.0)†**	25.4 (3.0)

*=p<0.01; †=p<0.05 when compared to placebo arm. P-values for these exploratory endpoints did not take into account multiplicity adjustment.
Source: Applicant's analysis.

Dose and Dose Response

In Study 202B, the Sponsor explored a brexanolone target dose of 60 µg/kg/h. The 60 µg dose was not directly compared to the 90 µg dose, but it showed numerically greater HAM-D score reductions and significant separation from placebo earlier than the 90 µg dose. Exploratory endpoints of percentage of patients reaching response and remission also show a greater effect for the 60 µg arm.

Clinical Reviewer Comment: The 60 µg arm begins to separate from the 90 µg arm at Hour 24—when both groups are receiving 60 µg/kg/h. This indicates that patients in the 60 µg arm may be inherently different from those in the 90 µg arm, and that differences in the two groups may not be attributable to the brexanolone dose. Error bars in the HAM-D measurements from the 60 and 90 µg arms overlap at most time points (other than Hour 36). This seems to indicate that although the 60 µg arm appears better than the 90 µg arm, there is no meaningful difference between their HAM-D scores. It is clear, however, that there is no suggestion that the 90 µg dose has a greater effect.

Durability of Response with Continued Administration

Brexanolone is delivered as a one-time infusion. The Applicant did not investigate durability of response with continued administration.

Persistence of Effect

See Table 47.

8.3. 547-PPD-202C

8.3.1. Study Design

Overview and Objective

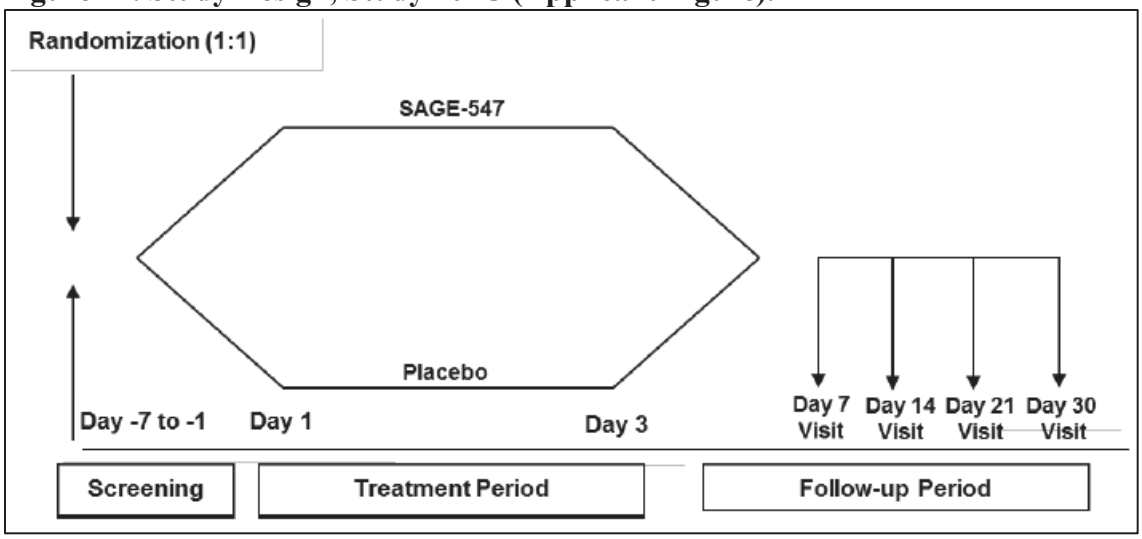
The primary objective was to determine if brexanolone infused intravenously at up to 90 µg/kg/h for 60 hours reduces depressive symptoms in subjects with moderate PPD compared to placebo injection as assessed by the change from baseline in HAM-D total score.

Trial Design

The trial design was very similar to the design of Trial 547-PPD-202A with the principal differences being the addition of randomization stratification by antidepressant use at baseline, the larger study size, and the severity of PPD in patients enrolled (moderate in 202C versus severe in 202A and B). Randomization was initially un-stratified. Starting in January 2017, following a recommendation from FDA, randomization was stratified by antidepressant use at baseline. Follow up visits at Day 14 and 21 were added in January 2017. Therefore, the sample sizes at these two visits are smaller than the sample sizes of the other visits. Eligible subjects were randomized in 1:1 ratio to receive 60 hours of IV treatment with either brexanolone injection or placebo.

The trial was conducted entirely in the United States. The study design is presented in Figure 21. HAM-D total score was scheduled to be assessed at baseline and Hours 1, 2, 4, 8, 12, 24, 36, 48, 60, 72 and Days 7 and 30 (see Table 38). Entry criteria were a diagnosis of PPD starting during the third trimester or within 4 weeks of delivery and a HAM-D score of 20 to 25.

Figure 21. Study Design, Study 202C (Applicant Figure).



Source: Clinical Study Report Figure 1.

Study Assessments and Endpoints

The change from baseline in HAM-D total score at the end of the treatment period (Hour 60) was the primary efficacy endpoint. The change from baseline in HAM-D total score at Day 30 was the prespecified secondary efficacy endpoint.

Statistical Analysis Plan

The SAP was evaluated in the statistical review dated 12/13/2016 in DARRTS under IND 122279. The Efficacy Set included all randomized subjects who started the infusion of study drug and who had a valid baseline HAM-D assessment and at least one postbaseline HAM-D assessment. This analysis population was used for all efficacy analyses. There was no interim analysis. Subgroup analyses by age group (18 to 24 vs. 25 to 45), race, baseline antidepressant use baseline BMI, onset of PPD, and family history of PPD were planned. A sensitivity analysis based on Missing Not at Random (MNAR) to handle missing data was pre-specified.

Change from baseline to each assessment time point in HAM-D total score was analyzed using MMRM. The model included the change from baseline at each visit time point as the dependent variable, center (pooled), treatment, baseline antidepressant use, baseline HAM-D total score, visit time point, and visit time point-by-treatment interaction terms as explanatory variables. All explanatory variables including pooled center were treated as a fixed effect in the primary analysis. An unstructured covariance structure was used to model the within-subject errors. If there was a convergence issue with the unstructured covariance model, Toeplitz, Autoregressive (1), then compound symmetry covariance structure was to be used, following this sequence until convergence was achieved. If convergence was not achieved, no results were reported.

Protocol Amendments

Studies 202B and 202C were conducted concurrently. See Section 8.2.1 for the list of protocol amendments.

8.3.2. Study Results

Patient Disposition

This trial was conducted in 32 centers in the United States. One hundred and eight subjects were randomized, 104 of whom received study treatment (51 brexanolone and 53 placebo). Of the subjects who received study treatment, four discontinued the study early (three brexanolone and one placebo). One subject from the brexanolone group withdrew from the study because of an AE. Of the 104 subjects, 101 (97.1%) had primary efficacy endpoint assessment (Hour 60) and 100 (96.1%) had the pre-specified secondary endpoint assessment (Day 30).

Protocol Deviations

There were no major protocol violations (i.e., a protocol deviation that reduces the quality or completeness of the data, makes the informed consent document inaccurate, or impacts a subject's safety, rights, or welfare).

There were 41 protocol deviations in the brexanolone arm and 45 deviations in the placebo arm. Most were considered minor (e.g., an assessment performed out of window). Three brexanolone patients and four placebo patients had major protocol deviations.

- Subject (b) (6); brexanolone: Patient's infusion rates were not calculated correctly and the patient received a lower than appropriate dose. Her coagulation parameters were not assessed.
- Subject (b) (6); brexanolone: Patient's baseline HAM-D score was 26, but she was randomized to 202C (202B was for patients with a HAM-D of ≥ 26; 202C was for patients with a HAM-D of 20 to 25).
- Subject (b) (6); brexanolone: Patient's antidepressant dose was not stable in the 14 days prior to screening (patient had discontinued escitalopram 9 days before screening).
- Subject (b) (6); placebo: Patient's antidepressant dose was not stable in the 14 days prior to screening (patient had discontinued sertraline and initiated paroxetine 13 days before screening).
- Subject (b) (6); placebo: Patient's Hour 60 HAM-D was collected 20 min prior to window because assessment was scheduled during sleeping period.
- Subject (b) (6); placebo: Patient randomized despite HAM-D score of 19 (202B was for patients with a HAM-D of ≥ 26; 202C was for patients with a HAM-D of 20 to 25).
- Subject (b) (6); placebo: Patient did not have Hour 60 HAM-D.

Clinical Reviewer Comment: It seems unlikely that these deviations would have affected the efficacy results.

Table of Demographic Characteristics

All subjects (100%) were female. The study was conducted entirely in the United States. A summary of the other demographic data is presented in Table 49.

Table 49. Demographic Characteristics, Study 202C.

Characteristic	Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
Age, years		
Mean (SD)	27.3 (5.9)	28.2 (6.1)
Median	27	27
Min, Max	18, 44	19, 42
Race, n (%)		
AA/Black	19 (36%)	22 (43%)
White	33 (62%)	29 (57%)
Other	1 (2%)	0
Ethnicity, n (%)		
Hispanic	14 (26%)	10 (20%)
Non-hispanic	39 (74%)	41 (80%)
Height, cm		
Mean (SD)	162.6 (8.4)	164.3 (6.2)
Median	161.3	164.0
Min, Max	142.0, 184.0	152.0, 181.0
Weight, kg		
Mean (SD)	86.6 (24.5)	87.3 (24.8)
Median	82.0	84.2
Min, Max	50.8, 159.7	44.9, 150.2
BMI, kg/m^2		
Mean (SD)	32.6 (8.2)	32.2 (8.5)
Median	32.5	30.6
Min, Max	21.5, 52.2	18.0, 52.0

Clinical Reviewer Comment: As with 202B, mean/median ages do not appear skewed.

Other Baseline Characteristics (e.g., disease characteristics, important concomitant drugs)

See Table 50 for baseline characteristics of interest in Study 202B. Table 51 includes medications with potential antidepressant action being taken at baseline.

Table 50. Baseline Patient Characteristics, Study 202C.

Characteristic	Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
Prior antidepressant treatment, n (%)	19%	24%
Onset of PPD, n (%)		
Third Trimester	12 (23%)	11 (22%)
Within 4 weeks of delivery	41 (77%)	40 (78%)
Previous episodes of depression, n (%)		
1	15 (28%)	13 (26%)
2	3 (6%)	4 (8%)
3	3 (6%)	0
>3	0	0
Previous episodes of PPD, n (%)		
Yes	21 (40%)	23 (45%)
Severity of Depression at Baseline		
HAM-D, Mean (SD)	22.7 (1.6)	22.6 (1.6)
EPDS, Mean (SD)	18.5 (4.0)	18.8 (3.9)

Clinical Reviewer Comment: The groups were well-matched at baseline. The mean HAM-D at baseline indicates that, on average, the patients' PPD was, indeed, moderate (defined by the Applicant as a HAM-D score of 20 to 25).

Table 51. Antidepressant Medication Use at Baseline, Study 202C.[a]

Class	Medication	Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
At least one antidepressant		15 (28%)	12 (24%)
SNRI	Duloxetine	0	1 (2%)
	Venlafaxine	1 (2%)	2 (4%)
SSRI	Citalopram	2 (4%)	0
	Escitalopram	2 (4%)	3 (6%)
	Fluoxetine	1 (2%)	0
	Paroxetine	1 (2%)	0
	Sertraline	5 (10%)	6 (12%)
Other Antidepressants	Bupropion	1 (2%)	1 (2%)
	Mirtazapine	1 (2%)	0
	Trazodone	1 (2%)	0
Other Drugs	Aripiprazole	0	1 (2%)
	Lamotrigine	1 (2%)	0
	Lithium	0	1 (2%)
	Quetiapine	2 (4%)	0

[a]Patients could be taking more than one of these drugs.

Clinical Reviewer Comment: One-quarter of the 202C patients were on an antidepressant at the start of the trial. As with 202B, the enrolled sample is representative of patients both already taking a psychotropic medication and those not already taking a psychotropic medication. However, patients were on a wide variety of medications and it is impossible to say how or whether brexanolone interacts with any individual drug or class.

Treatment Compliance and Rescue Medication Use

Because the study drug was administered as an infusion in a monitored setting, compliance was 100%.

Table 52 presents rescue antidepressant use in the study.

Table 52. Rescue Antidepressant Use, Study 202C.

Rescue Antidepressant	Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
Initiation of New Antidepressant or Increase in Antidepressant Dose, n (%)	7 (13%)	5 (10%)

Efficacy Results: Primary Endpoint and Prespecified Secondary Endpoints

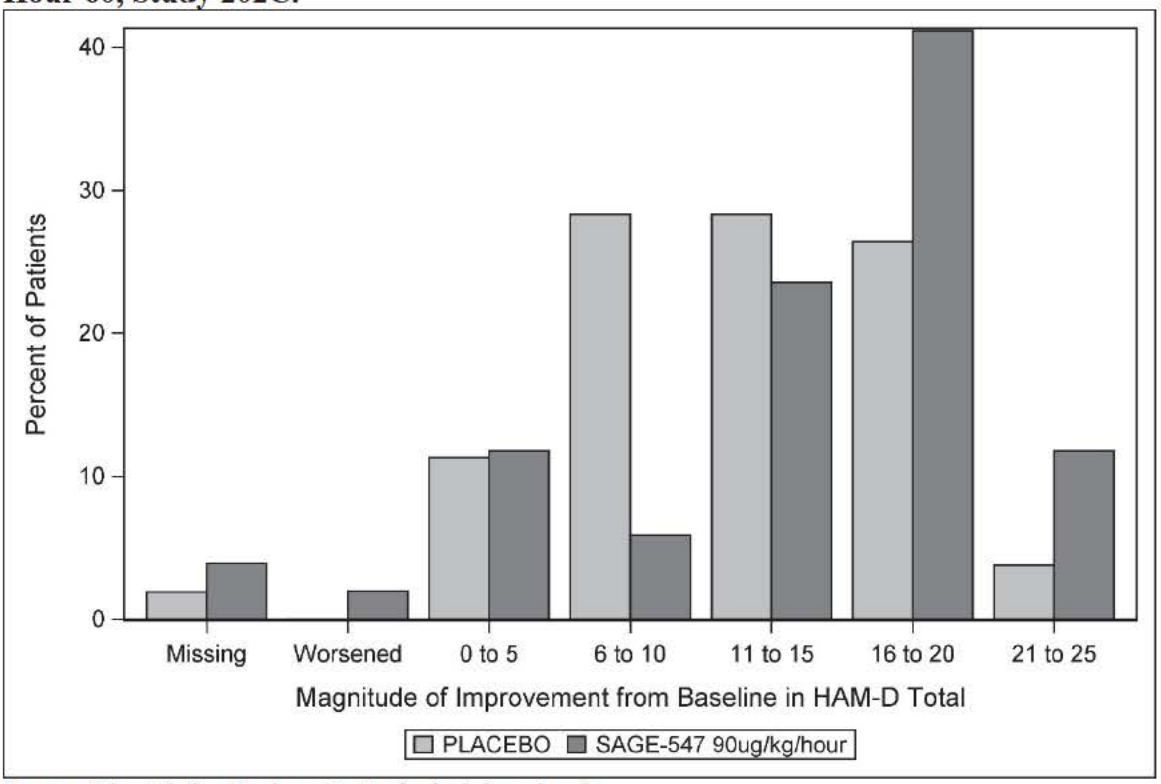
A summary of statistical significance for the primary and the prespecified secondary efficacy endpoints according to the hierarchical testing procedure is provided in Table 53. The primary efficacy endpoint was considered statistically significant. But the Day 30 data did not show an effect. No sensitivity analysis was performed because of the negligible amount of missing data. Figure 22 and Figure 23 display the histograms of the magnitude of improvement from baseline in HAM-D total at Hour 60 and Day 30, respectively.

Table 53. Primary Efficacy and Secondary Efficacy Results, Study 202C.

Timepoint		Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
Hour 60	Mean score at Baseline (SD)	22.7 (1.59)	22.6 (1.56)
	Mean Score at Hour 60 (SD)	10.7 (5.52)	8.5 (5.94)
	LS mean Change from Baseline (SE)	-12.1 (0.77)	-14.6 (0.78)
	Placebo -subtracted Difference (95% CI)		-2.5 (-4.5, -0.5)
	P-value (unadjusted)		0.0160
	Significance (MCP-adjusted)		Yes
Day 30	Mean score at Baseline (SD)	22.7 (1.59)	22.6 (1.56)
	Mean Score at Day 30 (SD)	7.6 (6.34)	8.4 (6.54)
	LS mean Change from Baseline (SE)	-15.2 (0.93)	-14.7 (0.96)
	Placebo -subtracted Difference (95% CI)		0.5 (-2.0, 3.1)
	P-value (unadjusted)		0.6710
	Significance (MCP-adjusted)		No

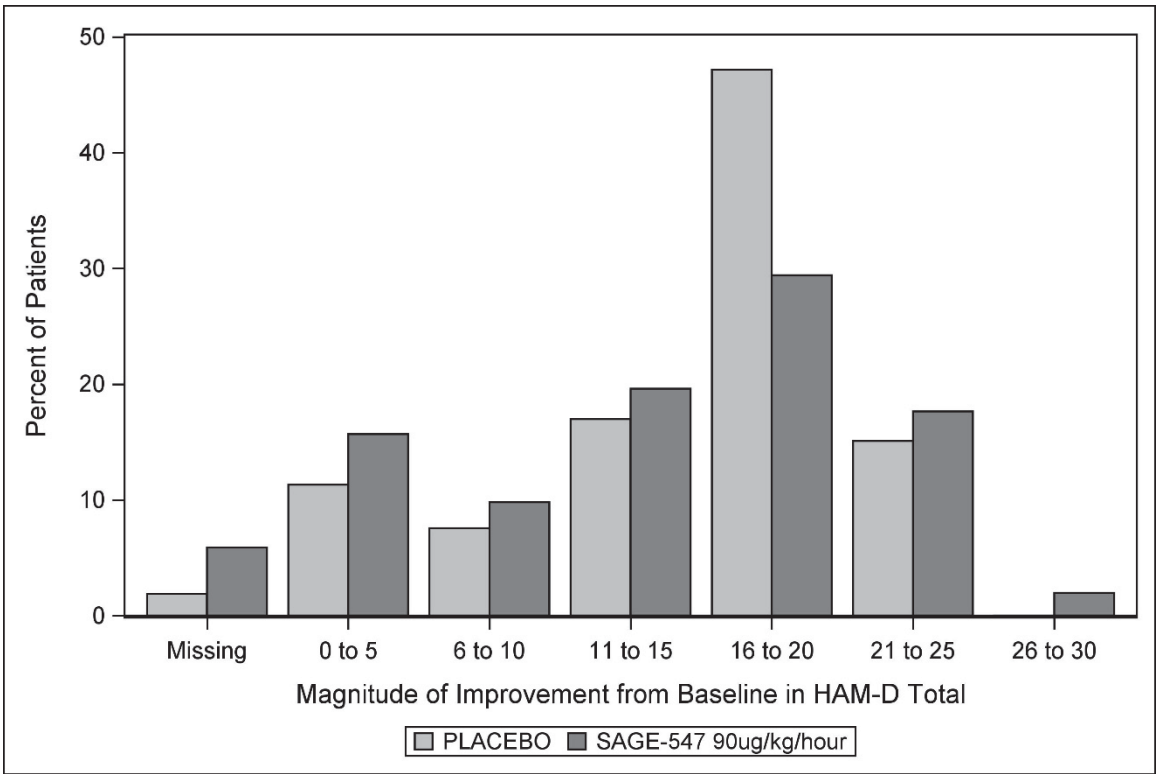
MCP=Multiple comparison procedures.
Source: Biostatistics Reviewer's Analysis (adqspri.xpt) based on Applicant's Clinical Study Reports.

Figure 22. Histogram of the Magnitude of Improvement from Baseline in HAM-D total at Hour 60, Study 202C.



Source: Biostatistics Reviewer's Analysis (adqspri.xpt).

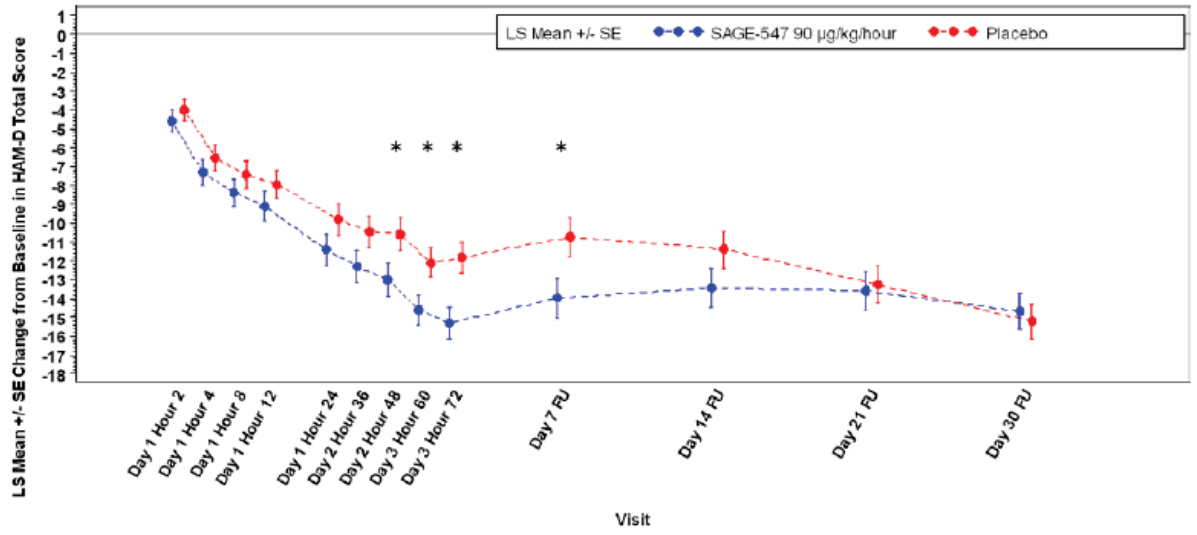
Figure 23. Histogram of the Magnitude of Improvement from Baseline in HAM-D total at Day 30, Study 202C.



Source: Biostatistics Reviewer's Analysis (adqspri.xpt).

As displayed in Figure 24, both treatment groups showed a decrease in HAM-D total score over the first 72 hours, with numerically greater change from baseline for the SAGE-547 group at all time points through Day 21.

Figure 24. Least Squared Mean (±SE) Change-from-Baseline over Time in HAM-D Total Score, Study 202C (Applicant Figure).



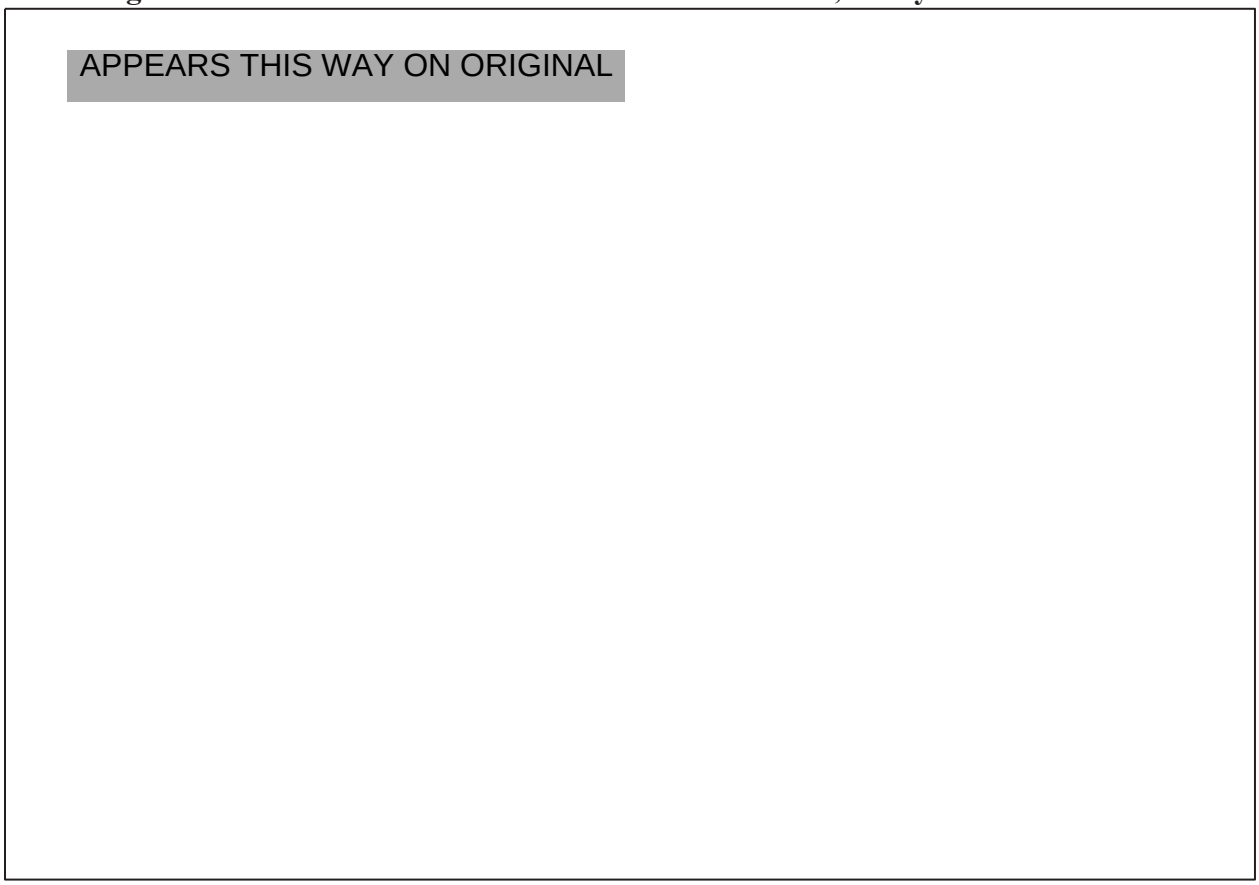
Abbreviations: HAM-D = Hamilton Rating Scale for Depression; FU = follow-up; LS = least squares; SE = standard error
Note: * indicates time points when statistically significant improvement was achieved for SAGE-547 as compared with placebo. Ns for Day 14 and Day 21 are lower than surrounding time points as these visits were added with Amendment 3.

Source: Clinical Study Report Figure 2.

Clinical Reviewer Comment: In the first 14 days, improvement in the brexanolone arm was similar to that observed in Study 202B. However, the placebo group in 202C had a much more robust response after 14 days and the brexanolone group was not significantly different from placebo at Day 30. Indeed, this might be a function of random effect in the small sample size, but could indicate that moderate PPD is more likely to respond to placebo or spontaneously resolve over time.

Further exploratory subgroup analyses on the primary endpoint were assessed by age group (18 to 24 vs 25 to 45), race, baseline antidepressant use, baseline BMI, onset of PPD, and family history of PPD. Results are shown in Figure 25. No apparent subgroup differences were observed.

Figure 25. Least Squared Mean Difference between Brexanolone and Placebo with 95% CI for Change-from-Baseline at Hour 60 in HAM-D Total Score, Study 202C.

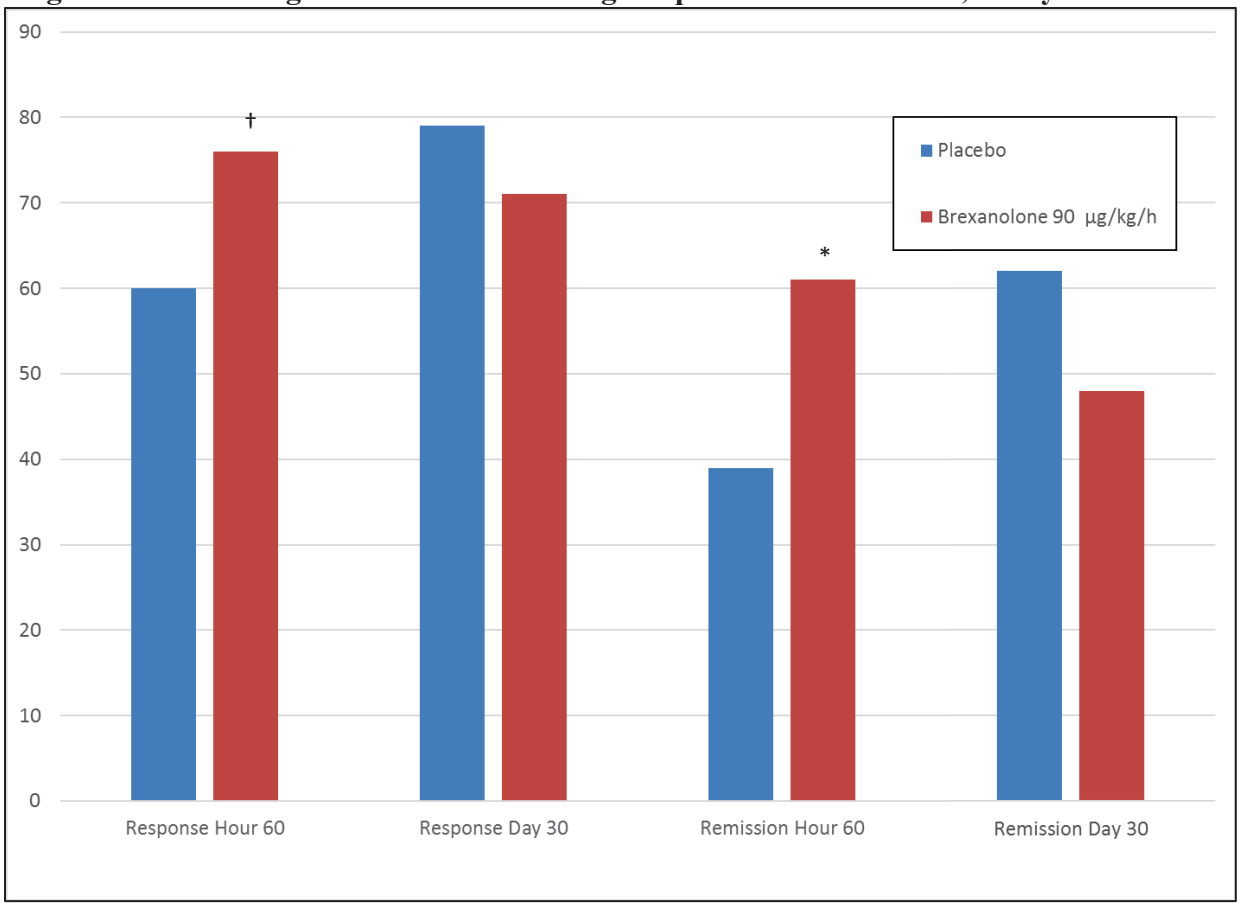


Source: Biometrics Reviewer's Analysis (adsl.xpt and adqspri.xpt).

Exploratory Endpoints

At Hour 60, more patients on brexanolone than placebo reached response (reduction of HAM-D score by at least 50% compared with baseline) and remission (HAM-D score ≤ 7; see Figure 26).

Figure 26. Percentages of Patients Reaching Response and Remission, Study 202C.



*=p<0.01; †=p<0.05 when compared to placebo arm. P-values for these exploratory endpoints did not take into account multiplicity adjustment.
Source: Clinical reviewer's analysis.

Table 54 includes exploratory endpoints from Study 202C. These results represent a range of patient experience with the drug (in addition to the primary and pre-specified secondary endpoints). Only the Hour 60 CGI-I showed a statistically significant effect. There were no statistically significant differences in any of the eight sub-scores of the SF-36 or on the HCRU (data not shown).

Table 54. Exploratory Endpoints, Study 202C.

Timepoint	Scale	Parameter	Placebo (n=53)	Brexanolone 90 µg/kg/h (n=51)
Hour 60	CGI-I	Response, %	56	**80***
	GAD-7	LS Mean Change from Baseline (SE)	-6.4 (0.8)	-7.6 (0.9)
	EPDS	LS Mean Change from Baseline (SE)	-7.0 (0.9)	-8.8 (1.0)
	PHQ-9	LS Mean Change from Baseline (SE)	-6.9 (1.0)	-8.1 (1.0)
Day 30	CGI-I	Response, %	79	81
	GAD-7	LS Mean Change from Baseline (SE)	-9.9 (0.9)	-10.2 (0.9)
	EPDS	LS Mean Change from Baseline (SE)	-11.2 (1.0)	-10.8 (1.0)
	PHQ-9	LS Mean Change from Baseline (SE)	-11.8 (0.9)	-12.3 (0.9)
	BIMF	LS Mean Change from Baseline (SE)	24.9 (2.3)	25.0 (2.4)

*=p<0.01 when compared to placebo arm. P-values for these exploratory endpoints did not take into account multiplicity adjustment.
Source: Applicant's analysis.

Dose and Dose Response

There was no exploration of dose response in this study.

Durability of Response with Continued Administration

Brexanolone is delivered as a one-time infusion. The Applicant did not investigate durability of response with continued administration.

Persistence of Effect

See Table 53.

9 Integrated Review of Effectiveness

9.1. Assessment of Efficacy Across Trials

9.1.1. Primary Endpoints

The efficacy of brexanolone in the treatment of PPD has been evaluated in three placebo-controlled studies (two in subjects with severe PPD, one in subjects with moderate PPD). All the

studies were conducted in the United States. The change from baseline in HAM-D total score at the end of the treatment period (at 60 hours) was the primary efficacy endpoint for all three studies. The primary efficacy results of the three positive studies are summarized in Table 55.

Table 55. Primary Efficacy Results (Change in HAM-D Total at Hour 60) for Positive Efficacy Studies.

Study		Placebo	Brexanolone 60 µg/kg/h	Brexanolone 90 µg/kg/h
202A 12/15/2015 – 06/22/2016 Severe PPD (HAM-D ≥ 26)	**n**	11	-	10
	Mean score at Baseline (SD)	28.8 (1.99)	-	28.1 (1.29)
	Mean Score at Hour 60 (SD)	19.7 (9.59)	-	7.5 (8.72)
	LS mean Change from Baseline (SE)	-8.8 (2.80)	-	-21.0 (2.94)
	Placebo -subtracted Difference (95% CI)		-	-12.2 (-20.8, -3.7)
	P-value (unadjusted)		-	0.008
	Significance (MCP-adjusted)		-	Yes
202B 8/1/2016 – 10/19/2017 Severe PPD (HAM-D ≥ 26)	**n**	43	38	41
	Mean score at Baseline (SD)	28.6 (2.54)	29.0 (2.70)	28.4 (2.47)
	Mean Score at Hour 60 (SD)	14.6 (7.55)	9.2 (7.01)	10.7 (5.78)
	LS mean Change from Baseline (SE)	-14.40 (1.15)	-19.5 (1.23)	-17.7 (1.19)
	Placebo -subtracted Difference (95% CI)		-5.5 (-8.8, -2.2)	-3.7 (-6.9, -0.5)
	P-value (unadjusted)		0.0013	0.0252
	Significance (MCP-adjusted)		Yes	Yes
202C 07/25/2016 – 10/11/2017 Moderate PPD (HAM-D 20-25)	**n**	53	-	51
	Mean score at Baseline (SD)	22.7 (1.59)	-	22.6 (1.56)
	Mean Score at Hour 60 (SD)	10.7 (5.52)	-	8.5 (5.94)
	LS mean Change from Baseline (SE)	-12.1 (0.77)	-	-14.6 (0.78)
	Placebo -subtracted Difference (95% CI)		-	-2.5 (-4.5, -0.5)
	P-value (unadjusted)		-	0.0160
	Significance (MCP-adjusted)		-	Yes

MCP=Multiple comparison procedures.
Source: Biostatistics Reviewer's Analysis (dqshamd.xpt, adqspri.xpt).

9.1.2. Secondary and Other Endpoints

The change from baseline in HAM-D total score at Day 30 was the prespecified secondary efficacy endpoint for 202B and 202C. There was no prespecified secondary efficacy endpoint in 202A. The secondary efficacy results are summarized in Table 56. The Applicant is seeking labeling claims based on time course of treatment response, remission rate, and CGI-I.

Biometrics and Clinical Reviewer Comments: The plot of brexanolone's treatment response time course should be presented in the product labeling without indications of statistical significance.

Table 56. Secondary Efficacy Results (Change in HAM-D Total at Day 30) for Positive Efficacy Studies.

Study		Placebo	Brexanolone 60 µg/kg/h	Brexanolone 90 µg/kg/h
202B 8/1/2016 – 10/19/2017 Severe PPD (HAM-D ≥ 26)	**n**	43	38	41
	Mean score at Baseline (SD)	28.6 (2.54)	29.0 (2.70)	28.4 (2.47)
	Mean Score at Day 30 (SD)	14.7 (9.46)	9.1 (7.97)	11.0 (8.34)
	LS mean Change from Baseline (SE)	-13.8 (1.32)	-19.5 (1.44)	-17.6 (1.40)
	Placebo -subtracted Difference (95% CI)		-5.6 (-9.5, -1.8)	-3.8 (-7.6, -0.0)
	P-value (unadjusted)		0.0044	0.0481
	Significance (MCP-adjusted)		Yes	Yes
202C 07/25/2016 – 10/11/2017 Moderate PPD (HAM-D 20-25)	**n**	53	-	51
	Mean score at Baseline (SD)	22.7 (1.59)	-	22.6 (1.56)
	Mean Score at Day 30 (SD)	7.6 (6.34)	-	8.4 (6.54)
	LS mean Change from Baseline (SE)	-15.2 (0.93)	-	-14.7 (0.96)
	Placebo -subtracted Difference (95% CI)		-	0.5 (-2.0, 3.1)
	P-value (unadjusted)		-	0.6710
	Significance (MCP-adjusted)		-	No

MCP=Multiple comparison procedures.
Source: Biostatistics Reviewer's Analysis (dqshamd.xpt, adqspri.xpt).

9.1.3. Subpopulations

The effect of brexanolone in population subgroups has been consistent across age group (18 to 24 vs 25 to 45), race, baseline antidepressant use, baseline BMI, onset of PPD, and family history of PPD in 202B and 202C. Subgroup analysis was not performed on 202A because of the small sample size (21 subjects in total).

9.2. Additional Efficacy Considerations

9.2.1. Considerations on Benefit in the Postmarket Setting

Based on the proposed postmarketing use of the product and the product's REMS (see separate Division of Risk Management review), it is expected that brexanolone will be used in the same manner it was studied.

9.2.2. **Other Relevant Benefit**

Not applicable for this application.

9.3. **Integrated Assessment of Effectiveness**

The three studies presented by the Applicant provide substantial evidence of effectiveness for brexanolone in the treatment of postpartum depression. The studies demonstrate a clinically meaningful effect because the improvement in depressive symptoms is both consistent with the effects of other antidepressants and occurs much more quickly (after 60 hours versus 4 weeks).

10 **Review of Safety**

10.1. **Safety Review Approach**

The safety data supporting this application are largely based on the Phase 2 study 202A and the Phase 3 studies 202B and 202C; all three were randomized, double-blind, placebo-controlled, multicenter studies of 60-hour brexanolone infusions in women with PPD. These studies are described in more detail in Section 7.1: *Clinical Effectiveness Studies*. The *Integrated Assessment of Safety* (Section 10.10) summarizes the review of safety and provides an overall safety assessment. The *Benefit-Risk Assessment* (Section 1.3) weighs the safety findings against clinical need and the possibility of effective treatment for this product. Table 57 contains all Applicant-submitted studies. The Applicant provided safety data from all trials at submission and no mid-review safety update was required.

The Agency did not issue clinical holds for this development program. The review team identified loss of consciousness as the primary safety concern related to this product (see Section 10.4.1).

Table 57. Studies Submitted for Safety Review.

Indication	Study	Phase	Description	N
PPD	547-PPD-201	2	Open-label, baseline HAM-D ≥20	4
	202A	2	PBO-controlled, baseline HAM-D ≥26	21
	202B	3	PBO-controlled, baseline HAM-D ≥26	138
	202C	3	PBO-controlled, baseline HAM-D 20 to 25	108
Clinical Pharmacology	547-CLP-101	1	Healthy males, radio-labelled brexanolone for metabolism and excretion	8
	102	1	Double-blind, human abuse study	138
	103	1	Hepatic impairment study	32
	104	1	Renal impairment study	17
	105	1	Drug-drug interaction study with phenytoin	29
	106	1	QT study	30
	107	1	Oral bioavailability/food-effect study	8
	108	1	Breast milk brexanolone concentrations	12
Essential Tremor	547-ETD-201	2	Cross-over followed by open-label	25

Note that the randomization for 202B included two brexanolone arms. Therefore, the randomization ratio was 2 brexanolone: 1 placebo for this study. Combining all brexanolone arms from 202B with the brexanolone arms from 202A and C (which had 1:1 randomization ratios) raises the possibility of Simpson's Paradox (a statistical phenomenon where trends in subgroups disappear or reverse when groups with different randomization ratios are combined). To counter this possibility, data are presented for 60 and 90 µg/kg/h doses separately as well as combined.

Clinical Reviewer Comment: Detailed reviews of the Phase 1 studies can be found in the Clinical Pharmacology Section (Section 6) and the consultant reviews from the Controlled Substance Staff and the QT Interdisciplinary Review Team (separate from this Unireview).

The Applicant's adverse event dataset contained flags for treatment emergent adverse events and whether the adverse event occurred during study drug infusion. For my primary AE analysis, I selected data from the PPD-202 studies with a "Y" for these flags.

10.2. Review of the Safety Database

10.2.1. Overall Exposure

The PPD studies 202A, 202B, and 202C all included a similar dosing regimen (see Section 1.1 *Product Introduction* and Table 58.). These studies used the same titration schedule to target a brexanolone dose of 90 µg/kg/h. Study 202B also included an arm with a target brexanolone dose of 60 µg/kg/h. All patients in these studies received a brexanolone or placebo infusion for 60 hours.

Table 58. Safety Population, Size, and Denominators.

Clinical Trials	Placebo (n= 107)	Brexanolone Target Dose 60µg/kg/h (n= 38)	Brexanolone Target Dose 90µg/kg/h (n= 102)
PPD-202A	11	-	10
PPD-202B	43	38	41
PPD-202C	53	-	51

Studies CLP-102, 106, and ETD-201 included dosing greater than 90 µg/kg/h (see Table 59.)

Table 59. Brexanolone Studies with Doses >90µg/kg/h.

Study	Dose (µg/kg)	Duration of Exposure	n
CLP-102 Dose Selection Phase	120	Single dose	6
	150	Single dose	6
	180	Single dose	6
	210	Single dose	6
	240	Single dose	6
	270	Single dose	6
CLP-102 Treatment Phase	180	Single dose	40[a]
	270	Single dose	
CLP-106	120	1 h	27[a]
	150	1 h	
	180	1 h	
EDT-201	120	5 h	16[a]
	150	8 h	

[a]Same subjects received all listed doses for this protocol.

10.2.2. **Relevant characteristics of the safety population:**

For a discussion of the patients' demographic characteristics, see Sections 8.1.2 (202A), 8.2.2 (202B), and 8.3.2 (202C).

10.2.3. **Adequacy of the safety database:**

Brexanolone is intended for use as a single, 60-hour infusion and not for chronic or chronic intermittent use. The Agency agreed that the exposures in the PPD development program were sufficient to support submission of the NDA.

10.3. Adequacy of Applicant's Clinical Safety Assessments

10.3.1. Issues Regarding Data Integrity and Submission Quality

The data quality was acceptable for review. Datasets, study reports, and patient narratives were consistent. Initial adverse event datasets included treatment group, but not the specific dose patients were receiving at the time of the AE. An information request yielded a new ISS AE dataset with this information. Additional information requests:

- Requested information on the infusion setting (location, protocols, available staff, etc.)
- Requested additional details on loss of consciousness/syncope cases
- Requested time since delivery before infusion for 202B and C patients
- Requested method of delivery (vaginal versus Cesarean section) for 202B and C patients (the Applicant did not collect this data)

The Applicant responded in a timely and complete manner to these requests.

10.3.2. Categorization of Adverse Events (AEs)

The Applicant used the Medical Dictionary for Regulatory Activities (MedDRA) Version 19.1 for their ISS submission. Studies that relied on earlier MedDRA versions were recoded based on Lower Level Terms. AEs, treatment-emergent AEs (TEAEs), and serious AEs (SAEs) were appropriately defined. During infusions, AEs were collected as per Table 38. For the Phase 3 studies, AEs were collected through the 30-day follow-up. The severity of AEs was classified based on the following:

- Mild (discomfort noticed, but no disruption to daily activity)
- Moderate (discomfort sufficient to reduce or affect normal daily activity, but was not hazardous to health; prescription drug therapy may have been employed to treat the AE)
- Severe (inability to work or perform normal daily activity and represented a definite hazard to health; prescription drug therapy and/or hospitalization may have been employed to treat the AE)

Clinical Reviewer Comment: The Applicant's AE monitoring and severity determinations are reasonable. I also examined the Applicant's mapping of verbatim-to-preferred terms for the Phase 3 studies. The mapping was acceptable. Sedation and somnolence were split based on the verbatim terms, but combined in my analysis.

Note that the PPD-202 studies were conducted in a monitored healthcare setting. Therefore, adverse events that may have triggered a hospital visit (by definition a serious AE) in an outpatient clinical setting may not have in this situation (i.e., investigators may have had a higher threshold for referring to a higher level of care).

10.3.3. Routine Clinical Tests

Clinical laboratory assessments were collected at screening, 72 hours after the start of infusion

(12 hours after the end of the infusion), and at Day 7. Laboratory studies included hematology, coagulation parameters, serum chemistries (including liver function tests), and thyroid stimulating hormone. Patients received a serum pregnancy test at screening and a urine pregnancy test on Day 1 and Day 30.

Vital signs and EKGs were collected as per Table 38. Vital signs included temperature, respiratory rate, heart rate, and blood pressure supine and standing. Vital signs were waived between the hours of 2300 and 0600 if the patient was sleeping.

Clinical Reviewer Comment: The laboratory tests and collection schedule for labs, vital signs, and EKGs were reasonable. Although the Applicant did not collect clinical labs during the infusion, the collection at 72 hours balanced the need for clinical labs at a time near the infusion with the burden of blood collection for the patients (who were also getting blood draws for PK measurements during the infusion).

The Applicant collected several exploratory labs (estrogen, progesterone, progesterone metabolites, oxytocin, tryptophan, kynurenine), but these were not included in the submitted analysis dataset.

10.4. **Safety Results**

There were no deaths in this development program.

10.4.1. **Serious Adverse Events**

There were two SAEs in this development program.

- Subject (b) (6) Study 202B, 60 µg/kg/h arm; 25-year-old white female: 2 days after completing the infusion, she reported suicidal ideation and intentional overdose on Percocet, Norco, and Flexaril. The patient informed her boyfriend of the overdose. Acetaminophen levels in the emergency department were inconsistent with the reported overdose amount (estimated fewer than five pills consumed). The emergency department noted the patient had a complex social situation (was married, but also had a boyfriend) and believed she was "attention seeking." The patient was not admitted.

- Subject (b) (6) Study 202C, 90 µg/kg/h arm; 25-year-old white female: syncope/altered state of consciousness (see Section 10.4.1: *Loss of Consciousness* for more details).

Clinical Reviewer Comment: The presentation of Subject (b) (6) appears consistent with borderline personality disorder. Although having borderline personality disorder does not preclude a concurrent diagnosis of PPD, I believe the suicidal ideation and intentional overdose are much more likely the result of a personality disorder than either the PPD or a drug effect. See Section 10.4.2: Suicidal Ideation and Behavior *for a discussion of the C-SSRS results.*

10.4.2. **Dropouts and/or Discontinuations Due to Adverse Effects**

Four patients discontinued study drug because of adverse effects:

- Subject (b) (6) Study 202B, placebo arm; discontinued study drug after 59 hours of infusion due to infusion site extravasation.
- Subject (b) (6), Study 202B, brexanolone 60 µg/kg/h arm; discontinued study drug after 57 hours of infusion due to infusion site pain.
- Subject (b) (6), Study 202C, brexanolone 90 µg/kg/h arm; discontinued study drug after 8 hours of infusion due to SAEs of syncope and altered state of consciousness.
- Subject (b) (6) Study 202C, brexanolone 90 µg/kg/h arm; discontinued study drug after 37 hours of infusion due to vertigo and presyncope.

Section 10.4.1: *Loss of Consciousness*, contains more details on Subjects (b) (6)

Clinical Reviewer Comment: Aside from complications from the IV procedures (which affected the placebo arm as well)—and the loss-of-consciousness issue—brexanolone appears well-tolerated.

10.4.3. **Significant Adverse Events**

Dose reduction and/or interruption was required in 10 brexanolone patients and three placebo patients as per Table 60. For patients whose dose was interrupted, only one had recurrence upon rechallenge. Subject (b) (6) (Study 202A) was randomized to the brexanolone 90 µg/kg/h arm of study 202A. She developed somnolence at the 30 µg/kg/h dose that continued through the escalation to 60 µg/kg/h at Hour 4. The somnolence resolved at Hour 12 after the dose was decreased to 30 µg/kg/h. At Hour 24, her dose was increased again to 60 µg/kg/h. However, she again experienced somnolence and, after approximately 6 hours, the dose was lowered to 30 µg/kg/h for the remainder of the 60 hours (she never received the 90 µg/kg/h dose).

Table 60. Dose Reductions and/or Interruptions in Studies 202A, B, and C.

Treatment	Adverse Event	n	Reduction or interruption
Placebo	Extremity pain/edema	1	Interrupted
	Infusion site pain	1	Interrupted
	Dizziness	1	Reduced
Brexanolone	Somnolence	2	Interrupted (1) Reduced (1)
	Syncope	3	Interrupted
	Infusion site pain/edema/itching	2	Interrupted
	Infusion site extravasation	1	Interrupted
	Fatigue	1	Reduced
	Hypotension	1	Reduced

Clinical Reviewer Comment: As with the AEs leading to premature discontinuation, the pattern of AEs leading to dose reductions or interruptions show most were related to complications from the IV procedures or sedation/loss of consciousness.

10.4.4. **Treatment Emergent Adverse Events and Adverse Reactions**

Brexanolone adverse events greater than two percent and at least twice the rate of placebo are presented in Table 61. There were only six AEs rated as severe in five patients (see Table 62).

Table 61. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group in Studies 202A, B, and C; n (%).

Adverse Event	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Sedation, somnolence	6 (6%)	21 (15%)	8 (21%)	13 (13%)
Dizziness, lightheadedness, presyncope, vertigo	7 (7%)	17 (12%)	5 (13%)	12 (12%)
Dry mouth, thirst	1 (1%)	7 (5%)	4 (11%)	3 (3%)
LOC, syncope	-	5 (4%)	2 (5%)	3 (3%)
Flushing, hot flush	-	4 (3%)	2 (5%)	2 (2%)
Diarrhea	1 (1%)	3 (2%)	1 (3%)	2 (2%)
Oropharyngeal pain	-	3 (2%)	1 (3%)	2 (2%)
Tachycardia	-	3 (2%)	-	3 (3%)
Dyspepsia, indigestion	-	2 (1%)	-	2 (2%)

At 72 h, one placebo patient and two brexanolone 90 µg/kg/h patients had sedation and two brexanolone 90 µg/kg/h patients had dizziness/lightheadedness.

Table 62. Severe AEs in Studies 202A, B, and C.

Study	Treatment	n	Preferred Term
202A	Placebo	1	insomnia
202B	brexanolone 60 µg/kg/h	1	loss of consciousness, somnolence
202C	brexanolone 90 µg/kg/h	1	presyncope
		1	fatigue
	Placebo	1	headache

Clinical Reviewer Comment: The AE dataset initially provided by the Applicant categorized AEs by assigned brexanolone treatment group. I requested that the Applicant also categorize the AE

dataset by what treatment the patient was receiving at the time of the AE. I present this information in Table 63.

For the calculations in Table 63, I have assumed all brexanolone patients (n=140) received 30 and 60 µg/kg/h doses (during titration). Note also that patients who experienced the same AE at multiple doses are counted for each dose. Therefore, the row totals from this table may not match total brexanolone numbers in Table 61.

Table 63. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment at the Time of the AE in Studies 202A, B, and C; n (%).

Adverse Event	Brexanolone Dose		
	30 µg/kg/h (n=140)	60 µg/kg/h (n=140)	90 µg/kg/h (n=102)
Sedation, somnolence	16 (14%)	7 (5%)	-
Dizziness, lightheadedness, presyncope, vertigo	7 (5%)	9 (6%)	4 (4%)
Dry mouth, thirst	1 (1%)	5 (4%)	1 (1%)
LOC, syncope	1 (1%)	3 (2%)	1 (1%)
Flushing, hot flash	1 (1%)	3 (2%)	-
Diarrhea	1 (1%)	2 (1%)	-
Oropharyngeal pain	1 (1%)	1 (1%)	1 (1%)
Tachycardia	1 (1%)	1 (1%)	2 (2%)
Dyspepsia, indigestion	-	1 (1%)	1 (1%)

In examining the distribution of AEs based on brexanolone dose at the time of the AE, there is no obvious dose effect. Indeed, sedation AEs are more common during the 30 µg/kg/h dose as patients start the infusion and none occurred at the highest dose. This lack of a dose-response for AEs suggests that lowering the dose of the infusion would not necessarily lead to better tolerability. Although it is possible that AEs diminish with exposure time, this finding informs my decision on PMCs (i.e., it would not seem useful to patients to find a lower efficacious dose if it would not improve tolerability).

Because there are sedation and dizziness AEs at 72 hours, the label should contain warning language regarding driving, etc. at discharge.

10.4.5. **Laboratory Findings**

Numbers of patients with potentially clinically significant hematology and coagulation values are presented in Table 64.

Table 64. Number of Patients with Potentially Clinically-Relevant Hematology and Coagulation Values in Studies 202A, B, and C; n (%).

Lab Test/ Day	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Hematocrit < 37 %				
Screening	15 (14%)	14 (10%)	2 (5%)	12 (12%)
72 h	11 (10%)	12 (9%)	4 (11%)	8 (8%)
Day 7	12 (11%)	20 (14%)	4 (11%)	16 (16%)
Hemoglobin < 12.0 g/dL				
Screening	28 (26%)	29 (21%)	11 (29%)	18 (18%)
72 h	26 (24%)	31 (22%)	12 (32%)	19 (19%)
Day 7	25 (23%)	38 (27%)	13 (34%)	25 (25%)
WBC < 4.0 x10^9/L				
Screening	2 (2%)	5 (4%)	1 (3%)	4 (4%)
72 h	5 (5%)	5 (4%)	1 (3%)	4 (4%)
Day 7	4 (4%)	3 (2%)	-	3 (3%)
Platelets < 150 x10^9/L				
Screening	1 (1%)	-	-	-
72 h	-	-	-	-
Day 7	-	-	-	-
INR >1.3				
Screening	3 (3%)	6 (4%)	1 (3%)	5 (5%)
72 h	2 (2%)	5 (4%)	2 (5%)	3 (3%)
Day 7	6 (6%)	4 (3%)	1 (3%)	3 (3%)
aPTT >40 sec				
Screening	5 (5%)	8 (6%)	2 (5%)	6 (6%)
72 h	7 (7%)	5 (4%)	2 (5%)	3 (3%)
Day 7	11 (10%)	6 (4%)	1 (3%)	5 (5%)

One brexanolone subject (90 Mg/kg/h) had a serum sodium of 127 mmol/L at Day 7 (139 mmol/L on Day 3). There were no cases of hypo- or hyperkalemia. There were five cases of low serum glucose (less than 4.0 mmol/L) at Day 3; two in the placebo group, one with brexanolone 60 µg/kg/h dose, and one with brexanolone 90 µg/kg/h.

Table 65. presents the numbers of patients with elevated TSH.

Table 65. Number of Patients with TSH >4.5 mIU/L in Studies 202A, B, and C; n (%).

Day	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Screening	1 (1%)	1 (1%)	-	1 (1%)
72 h	3 (3%)	6 (4%)	1 (3%)	5 (5%)
Day 7	2 (2%)	1 (1%)	-	1 (1%)

There was one patient (Subject (b) (6) Study 202B) in the brexanolone 90 µg/kg/h group who developed ALT and AST elevations approximately seven times the upper limit of normal. The subject's baseline ALT and AST were clinically unremarkable (58 and 43 U/L, respectively). However, on Day 3, the patient's ALT was 373 and AST was 234 U/L. By Day 7 the AST had decreased to 51 U/L, but the ALT remained elevated at 192 U/L. At Day 30, the ALT had decreased to 29. Throughout this time, the patient's bilirubin remained 8.6 to 14.3 µmol/L (within the normal range of 1.7 to 20.5 µmol/L). The patient's alkaline phosphatase ranged from 89 to 117 U/L (within the normal range of 30 to 140 U/L). This patient had no relevant medical history. In addition to brexanolone, she was taking sertraline. There were no other patients with transaminase elevations.

Clinical Reviewer Comment: There is no pattern consistent with a drug effect in the numbers of patients with potentially clinically-relevant hematology, coagulation, serum electrolyte, or TSH results.

Although there was one subject in the brexanolone 90 µg/kg/h group with significant ALT and AST elevations, the case did not meet criteria for Hy's Law (no elevation of bilirubin). The patient was also taking sertraline, which has been associated with rare instances of marked elevations in liver enzymes 2 to 24 weeks after starting the drug (National Institutes of Health, 2018). Although there is no clear cause for this subject's transaminase elevations, I believe the sertraline is a more likely culprit than the brexanolone, which is an analogue of an endogenous hormone.

10.4.6. **Vital Signs**

Numbers of patients with potentially clinically significant heart rate or blood pressure are presented in Table 66, Table 67, and Table 68. Figure 27 and Figure 28 show the minimum and maximum respiratory rates by drug in Studies 202B and 202C, respectively. Initially, protocols required pulse oximetry monitoring every 2 hours. This was amended to continuous pulse oximetry in December 2015. In February 2017, the protocol was further amended to completely remove the requirement for pulse oximetry. No pulse oximetry data were submitted; case report forms did not include a space for reporting pulse oximetry.

Table 66. Number of Patients with Potentially Clinically Relevant Heart Rate in Studies 202A, B, and C; n (%).

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Heart Rate > 110 bpm				
Screening	-	-	-	-
Baseline	-	1 (1%)	-	1 (1%)
2 h	-	1 (1%)	-	1 (1%)
4 h	-	1 (1%)	-	1 (1%)
8 h	1 (1%)	-	-	-
12 h	-	-	-	-
18 h	-	-	-	-
24 h	-	-	-	-
30 h	-	1 (1%)	1 (3%)	-
36 h	-	-	-	-
42 h	-	1 (1%)	-	1 (1%)
48 h	-	1 (1%)	-	1 (1%)
54 h	-	-	-	-
60 h	-	1 (1%)	-	1 (1%)
66 h	-	1 (1%)	-	1 (1%)
72 h	1 (1%)	2 (1%)	1 (3%)	1 (1%)
Day 7	1 (1%)	1 (1%)	-	1 (1%)
Day 30	-	1 (1%)	-	1 (1%)

Table 66 continued.

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Heart Rate < 50 bpm				
Screening	1 (1%)	3 (2%)	1 (3%)	2 (2%)
Baseline	1 (1%)	1 (1%)	1 (3%)	-
2 h	3 (3%)	2 (1%)	2 (5%)	-
4 h	-	-	-	-
8 h	1 (1%)	-	-	-
12 h	1 (1%)	-	-	-
18 h	-	1 (1%)	-	1 (1%)
24 h	2 (2%)	-	-	-
30 h	-	-	-	-
36 h	-	-	-	-
42 h	-	-	-	-
48 h	-	1 (1%)	1 (3%)	-
54 h	-	-	-	-
60 h	-	1 (1%)	-	1 (1%)
66 h	-	-	-	-
72 h	1 (1%)	1 (1%)	-	1 (1%)
Day 7	2 (2%)	-	-	-
Day 30	-	-	-	-

Table 67. Number of Patients with Potentially Clinically-Relevant Supine Systolic Blood Pressure in Studies 202A, B, and C; n (%).

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Systolic Blood Pressure > 150 mmHg				
Screening	2 (2%)	3 (2%)	1 (3%)	2 (2%)
Baseline	3 (3%)	2 (1%)	1 (3%)	1 (1%)
2 h	1 (1%)	1 (1%)	-	1 (1%)
4 h	1 (1%)	2 (1%)	-	2 (2%)
8 h	2 (2%)	-	-	-
12 h	2 (2%)	2 (1%)	-	2 (2%)
18 h	-	-	-	-
24 h	1 (1%)	1 (1%)	-	1 (1%)
30 h	1 (1%)	1 (1%)	1 (3%)	-
36 h	1 (1%)	2 (1%)	-	2 (2%)
42 h	1 (1%)	-	-	-
48 h	1 (1%)	2 (1%)	-	2 (2%)
54 h	-	2 (1%)	1 (3%)	1 (1%)
60 h	-	4 (3%)	2 (5%)	2 (2%)
66 h	-	1 (1%)	1 (3%)	-
72 h	-	1 (1%)	1 (3%)	-
Day 7	3 (3%)	1 (1%)	1 (3%)	-
Day 30	2 (2%)	3 (2%)	1 (3%)	2 (2%)

Table 67 continued.

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Systolic Blood Pressure < 90 mmHg				
Screening	-	-	-	-
Baseline	1 (1%)	1 (1%)	-	1 (1%)
2 h	1 (1%)	1 (1%)	1 (3%)	-
4 h	-	2 (1%)	-	2 (2%)
8 h	-	1 (1%)	-	1 (1%)
12 h	-	1 (1%)	-	1 (1%)
18 h	-	1 (1%)	-	1 (1%)
24 h	2 (2%)	3 (2%)	1 (3%)	2 (2%)
30 h	-	4 (3%)	-	4 (4%)
36 h	-	1 (1%)	-	1 (1%)
42 h	-	-	-	-
48 h	1 (1%)	4 (3%)	2 (5%)	2 (2%)
54 h	-	-	-	-
60 h	-	1 (1%)	-	1 (1%)
66 h	-	-	-	-
72 h	-	2 (1%)	-	2 (2%)
Day 7	1 (1%)	3 (2%)	-	3 (3%)
Day 30	-	-	-	-

Table 68. Number of Patients with Potentially Clinically-Relevant Supine Diastolic Blood Pressure in Studies 202A, B, and C; n (%).

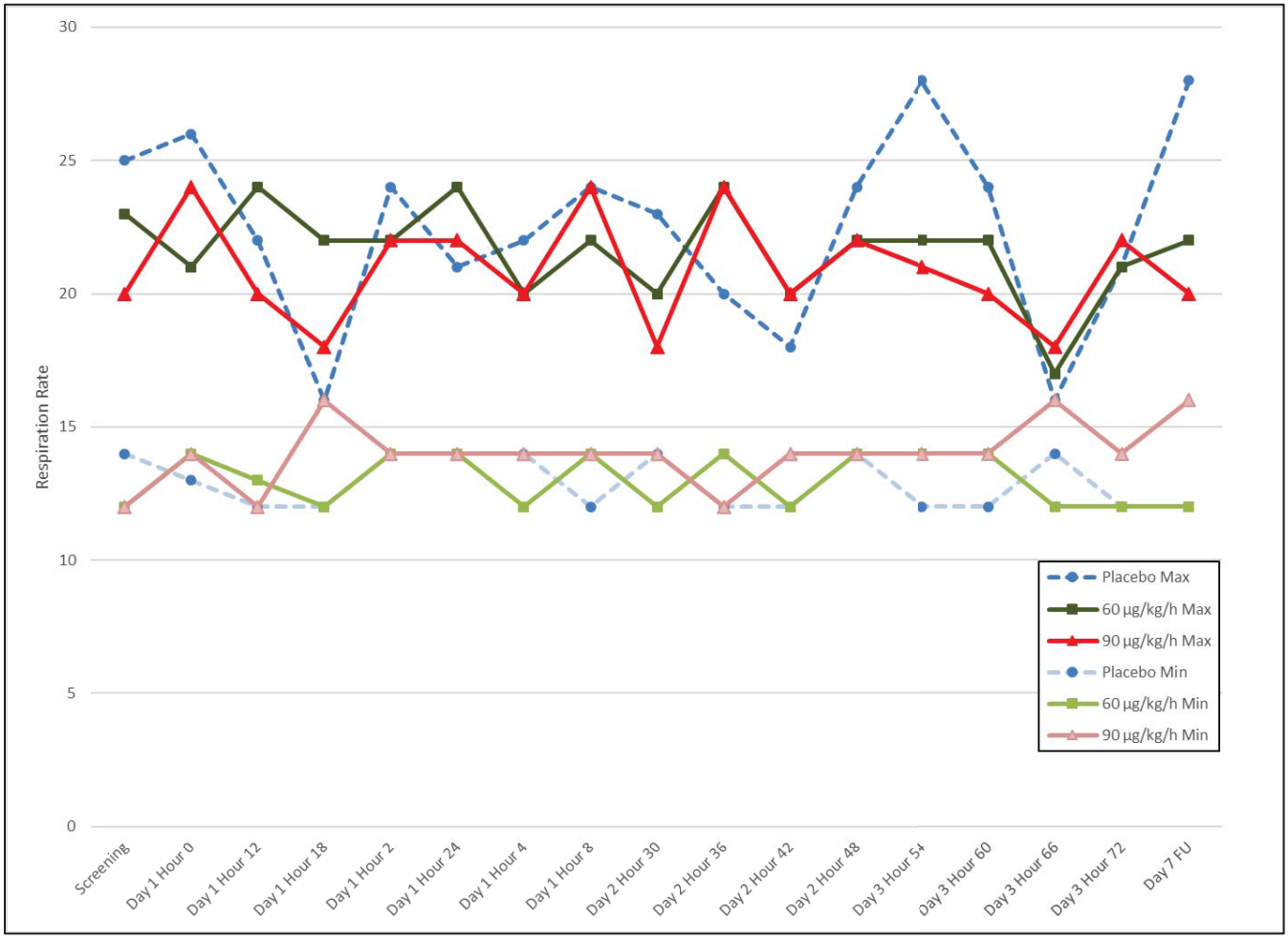
Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Diastolic Blood Pressure > 100 mmHg				
Screening	1 (1%)	2 (1%)	1 (3%)	1 (1%)
Baseline	1 (1%)	1 (1%)	-	1 (1%)
2 h	1 (1%)	-	-	-
4 h	1 (1%)	1 (1%)	-	1 (1%)
8 h	-	1 (1%)	-	1 (1%)
12 h	-	-	-	-
18 h	-	-	-	-
24 h	-	1 (1%)	-	1 (1%)
30 h	-	-	-	-
36 h	1 (1%)	1 (1%)	-	1 (1%)
42 h	-	-	-	-
48 h	-	-	-	-
54 h	-	-	-	-
60 h	-	-	-	-
66 h	-	-	-	-
72 h	-	-	-	-
Day 7	2 (2%)	-	-	-
Day 30	-	1 (1%)	1 (3%)	-

Table 68 continued.

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Diastolic Blood Pressure < 60 mmHg				
Screening	2 (2%)	5 (4%)	2 (5%)	3 (3%)
Baseline	8 (7%)	7 (5%)	2 (5%)	5 (5%)
2 h	10 (9%)	10 (7%)	3 (8%)	7 (7%)
4 h	7 (7%)	18 (13%)	3 (8%)	15 (15%)
8 h	5 (5%)	10 (7%)	3 (8%)	7 (7%)
12 h	5 (5%)	7 (5%)	3 (8%)	4 (4%)
18 h	3 (3%)	4 (3%)	-	4 (4%)
24 h	12 (11%)	7 (5%)	2 (5%)	5 (5%)
30 h	7 (7%)	12 (9%)	4 (11%)	8 (8%)
36 h	4 (4%)	11 (8%)	3 (8%)	8 (8%)
42 h	1 (1%)	-	-	-
48 h	5 (5%)	13 (9%)	4 (11%)	9 (9%)
54 h	8 (7%)	9 (6%)	1 (3%)	8 (8%)
60 h	6 (6%)	6 (4%)	3 (8%)	3 (3%)
66 h	2 (2%)	3 (2%)	1 (3%)	2 (2%)
72 h	9 (8%)	7 (5%)	2 (5%)	5 (5%)
Day 7	7 (7%)	6 (4%)	-	6 (6%)
Day 30	1 (1%)	-	-	-

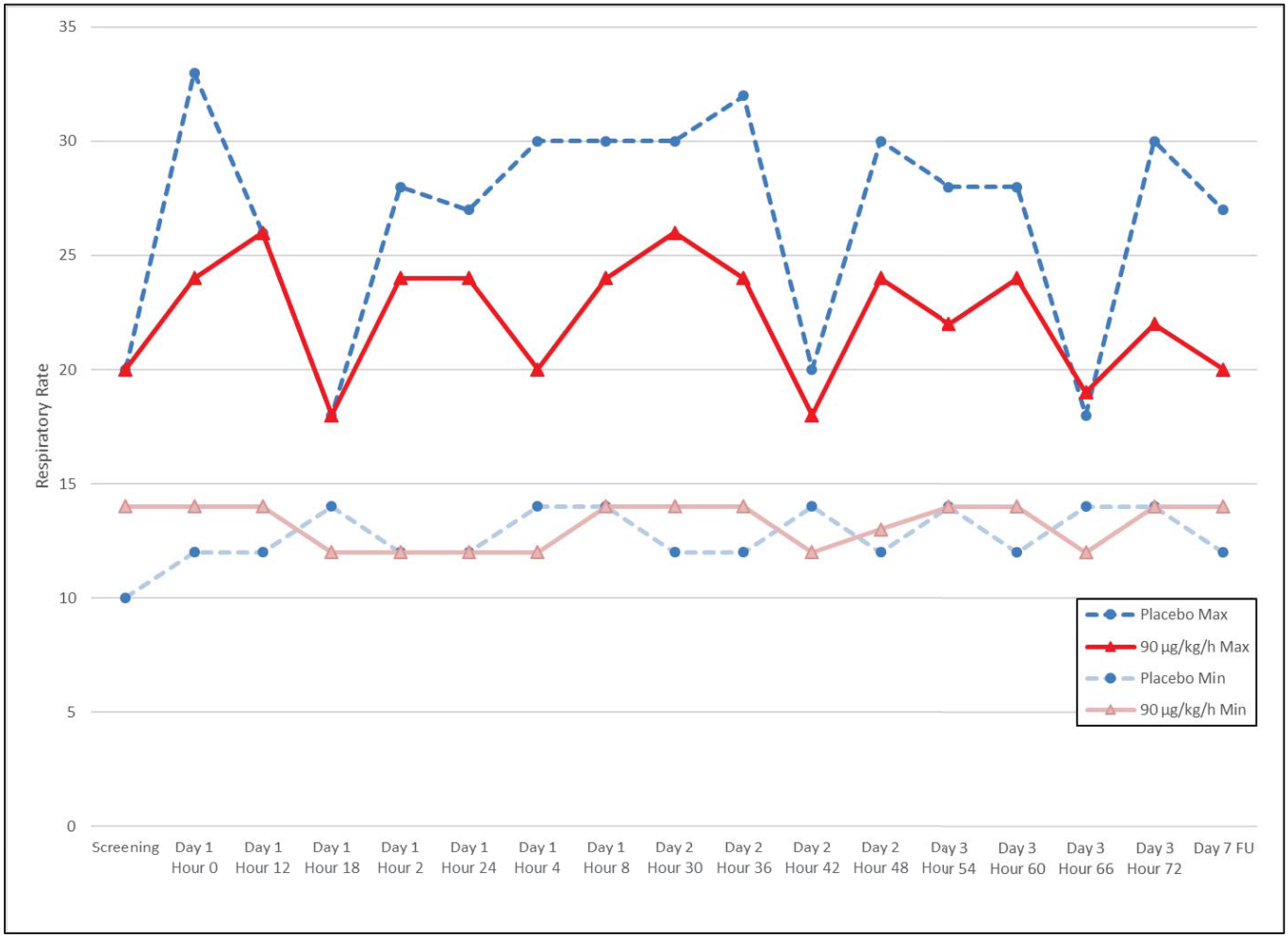
There were fewer patients with blood pressure measurements while standing (values not presented), but the results were similar to supine blood pressure. Only one patient (Study 202C, brexanolone 90 µg/kg/h arm) was orthostatic by blood pressure criteria alone (standing heart rate was not measured). Other patients with postural dizziness were not orthostatic.

Figure 27. Minimum and Maximum Respirations per Minute by Drug Assignment in Study 202B.



Source: Clinical Reviewer generated.

Figure 28. Minimum and Maximum Respirations per Minute by Drug Assignment in Study 202C.



Source: Clinical Reviewer generated.

Clinical Reviewer Comment: For all vital sign measurements, if the patient was asleep at night, the vitals were waived. Therefore, because the patients started the infusion in the morning, there are few values at 18 and 42 hours after the start of the infusion. Although sustained measurements of blood pressure greater than 140/90 define hypertension, I chose a slightly higher threshold for determining clinically significant elevated blood pressure in order to account for minor elevations associated with experimental procedures. I did the same for heart rate.

There was a male subject who experienced apnea during the thorough QT Study (see Section 10.4.1: Loss of Consciousness*). Because of this, and a respiratory signal in some nonclinical studies (see Section 5.5.1:* General Toxicology*), I chose to graphically represent the minimum and maximum respiratory rates for patients by drug assignment. As shown in Figures Figure* 27 *and Figure* 28*, brexanolone was not associated with a pattern of respiratory distress (more respirations per minute than placebo) nor with respiratory depression (fewer respirations per minute than placebo). It is unfortunate that the Applicant discontinued pulse oximetry*

monitoring. Based on the case of apnea and the possibility that brexanolone could act like a barbiturate at the $GABA_A$ receptor, I recommend continuous pulse oximetry monitoring during infusions. The consequences of allowing the infusions to continue after loss of consciousness is unclear. Including continuous pulse oximetry monitoring will allow for intervention in the event the patient "loses consciousness" while sleeping. This should be included in the label.

I do not feel there is a pattern of vital sign values consistent with a drug effect. Although there are some time points where more patients on brexanolone appeared to have low diastolic blood pressure (36 h), there were other infusion time points when more patients on placebo had low diastolic blood pressure (24 h). These fluctuations are most likely an artifact of the sample sizes.

10.4.7. **Electrocardiograms (ECGs)**

Table 69 presents potentially clinically relevant PR and QTc intervals. The QT interval was corrected using Fridericia's formula. The QT Interdisciplinary Review Team evaluated the Applicant's thorough QT study (CLP-106) in a separate document. Their findings are summarized in Section 10.3.13: *QT*.

Table 69. Number of Patients with Potentially Clinically-Relevant ECG Findings, in Studies 202A, B, and C; n (%).

Day/Time	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
PR Interval > 200 msec				
Screening	1 (1%)	4 (3%)	1 (3%)	3 (3%)
48h	1 (1%)	3 (2%)	1 (3%)	2 (2%)
Day 7	2 (2%)	3 (2%)	2 (5%)	1 (1%)
QTc Interval > 460 msec				
Screening	2 (2%)	3 (2%)	1 (3%)	2 (2%)
48h	2 (2%)	1 (1%)	-	1 (1%)
Day 7	1 (1%)	2 (1%)	-	2 (2%)
QTc Interval < 350 msec				
Screening	-	-	-	1 (1%)
48h	1 (1%)	2 (1%)	1 (3%)	1 (1%)
Day 7	-	-	-	-

Clinical Reviewer Comments: I do not feel there is a pattern of ECG interval values consistent with a drug effect.

10.4.8. **QT**

As per the QT Interdisciplinary Review Team's (IRT) conclusions:

> No significant QTc prolongation effect of brexanolone (SAGE-547) treatment (a 5-hour intravenous infusion starting at a rate of 60 µg/kg/h and increasing each hour to 90, 120, 150, and 180 µg/kg/h) was detected in TQT study 547-CLP-106. The largest upper bound of the

2-sided 90% CI for the mean difference between brexanolone treatment and placebo was below 10 ms, the threshold for regulatory concern as described in ICH E14 guidelines. The largest lower bound of the two-sided 90% CI for the ΔΔQTcF for moxifloxacin was greater than 5 ms, and the moxifloxacin profile over time is adequately demonstrated…indicating that assay sensitivity was established (p. 1; QT IRT Review archived by Moh Jee Ng on July 26, 2018).

The IRT recommends the following language be included in the Phamacodynamics section of labeling:

The effect of brexanolone on the QTc interval was evaluated in a Phase 1 randomized, placebo and positive controlled, double-blind, three-period crossover thorough QTc study in 30 healthy adult subjects. At 1.9-fold of the therapeutic exposures for highest recommended clinical dose, brexanolone did not prolong the QTc interval to any clinically relevant extent (p. 2).

10.4.9. **Immunogenicity**

Not applicable to this application.

10.5. **Analysis of Submission-Specific Safety Issues**

10.5.1. **Loss of Consciousness**

There were six patients with loss of consciousness, syncope, or presyncope in Studies 202A, B, and C (see Table 70). Patient (b) (6) appeared to have a vasovagal reaction to a blood draw. Patient (b) (6) reported dizziness and vertigo that improved when she sat down (she never lost consciousness). The remaining four patients seemed to experience an abrupt onset of deep sleep.

Table 70. Cases of Loss of Consciousness, Syncope, Presyncope in Studies 202A, B, and C.

Subject ID (Study)	Demographics	Description of Event	Dose at time of LOC Event (µg/kg/h)	Timeline: Nearest PK to Event (ng/mL)
(b) (6) (202B)	31 yo, AA BMI 28.1 kg/m^2 78 days after delivery h/o MDD Medication -medroxyprogesterone	-Vasovagal syncope during venipuncture for PK sampling (reported fear of needles)	60	(b) (6) 0800: Infusion start (b) (6) 0759: 64.6 0800: Syncope 1400: 64.0
(b) (6) (202B)	25 yo, W BMI 40 kg/m^2 40 days after delivery h/o anxiety Medication -labetalol -lansoprazole -promethazine -acetaminophen	-Infusion pump malfunction, dose unclear -BP lability before and during the event (71/48 to 140/101 mmHg) -LOC occurred 14 h after starting 90µg/kg/h (actual dose unclear) -LOC x 30 sec, "as if in deep, sound sleep" -Infusion stopped; felt well after 10 min	90[a]	(b) (6) 0815: Infusion start (b) (6) 2005: 79.3 2238: LOC (b) (6) 0805: 102
(b) (6)	28 yo, W	-Infusion pump malfunction, dose	30[a]	(b) (6)

(202B)	BMI 35 kg/m^2 82 days after delivery Medication -none	unclear -Asked if the drug made one sleepy, then fell forward "abruptly"; snoring -No change in vitals -Infusion stopped; recovered after 14 min		0845: Infusion start 1016: LOC 1235: 29.5
(b) (6) (202B)	24 yo, W BMI 29 kg/m^2 185 days after delivery h/o anxiety, MDD Medication -ASA/acetaminophen/ caffeine	-Reported dizziness 20 h after starting 60 µg/kg/h -10 h later was extremely somnolent and unaware of surroundings -Infusion stopped; improved after 15 min, resolved after 45 min	60	(b) (6) 1110: Infusion start (b) (6) 1102: 152 2200: LOC 2319: 103
(b) (6) (202C)	25 yo, W BMI 30 kg/m^2 189 days after delivery h/o anxiety, MDD Medication -sertraline (since 2016) -single dose ondansetron	-Reported dizziness 5 h after starting 60 µg/kg/h -Was eating Jell-O when abruptly dropped spoon and became unresponsive -Opened eyes to verbal stimuli after 10 min, but not responsive for 1 h -Sent to emergency department -No memory for event	60	(b) (6) 0937: Infusion start 1741: 51.6 1815: Syncope

Table 70 continued.

Subject ID (Study)	Demographics	Description of Event	Dose at time of LOC Event (µg/kg/h)	Timeline: Nearest PK to Event (ng/mL)
(b) (6) (202C)	36 yo, AA BMI 51 kg/m^2 115 days after delivery h/o HTN Medication -medroxyprogesterone -methadone (since 2012) -metoprolol -naproxen -lisinopril/HCTZ	-Reported dizziness and somnolence at 30 and 60 µg/kg/h -Presyncope/vertigo 13 h after starting 90 µg/kg/h -Sat down and presyncope resolved after 10 min, vertigo after 2 h	90	(b) (6) 0800: Infusion start (b) (6) 0750: 82.1 1115: Presyncope 1400: 138

AA=African-American, ASA=aspirin, BMI=body mass index, BP=blood pressure, HCTZ=hydrochlorothiazide, HTN=hypertension, h/o=history of, LOC=loss of consciousness, MDD=major depressive disorder, W=white, yo=year old.
[a]Because of IV pump malfunction, actual dose unclear. PK samples from these patients do not indicate abnormally high doses, but the Applicant reports a biphasic elimination and that the drug is rapidly cleared.

Subject (b) (6) in Study CLP-106 (cardiac repolarization study) also lost consciousness. This subject was a 55-year-old man with no reported past medical history. He developed somnolence, confusion, dizziness, and less than 1 minute of apnea while receiving brexanolone 150 µg/kg/h.

His blood level was 144 ng/mL. He was not obese. This was the only subject in this or the other development programs for brexanolone with apnea.

Clinical Reviewer Comments: Most of the LOC events resolved when the infusion was interrupted with no lasting effects. However, Patient (b) (6) *was not fully responsive for an hour and had amnesia for the LOC events. The subject with apnea is also concerning. None of the women in the PPD studies experienced apnea. It is possible that the apneic event is a result of the subject falling into a deep sleep, but the subject had no history of sleep apnea and was not obese.*

There is no discernable pattern to the LOC events. Dosing, time elapsed since start of dose, blood levels, BMI, past medical history, and medication all varied. I considered the possibility that LOC might be related to women who had Cesarean deliveries (i.e., surgical patients). However, although the Applicant did not collect method of delivery, time since delivery indicates this is an unlikely explanation. Removing the case of presyncope and the vasovagal reaction, we are left with white females in their 20s. However, most study patients were in their 20s and most patients were white—and there is not a plausible mechanism that would result in racial differences in the absence of a PK effect. It is possible that the cases with an infusion pump malfunction experienced increased dosing, but brexanolone blood levels do not support this.

Because the events are sudden, unpredictable, and require intervention (i.e., stopping the infusion), delivery of the infusion will require constant monitoring for the safety of the patient and her infant. Patients will all require dose titration and taper. But, in accordance with the treatment protocol used, may also require dose adjustment based on tolerability. Coupled with the possibility of apnea, supervision must be from a medical professional and peripheral oxygen saturation must be monitored (pulse oximetry).

10.5.2. **Suicidal Ideation and Behavior**

The Applicant used the Columbia Suicide Severity Rating Scale (C-SSRS) to establish lifetime suicidal ideation and behavior at baseline as well as to monitor for these AEs during the study. Results are presented in Table 71.

Table 71. C-SSRS Results during Studies 202A, B, and C; n (%).

Day	Event	Placebo (n=107)	Any Brexanolone (n=140)	Brexanolone 60 µg/kg/h (n=38)	Brexanolone 90 µg/kg/h (n=102)
Baseline[a]	None	72 (67%)	83 (59%)	18 (47%)	65 (64%)
	SI	28 (26%)	42 (30%)	15 (39%)	27 (26%)
	SB	7 (7%)	15 (11%)	5 (13%)	10 (10%)
Day 2	None	103 (96%)	135 (96%)	37 (97%)	98 (96%)
	SI	3 (3%)	3 (2%)	-	3 (3%)
	SB	-	-	-	-
Day 3	None	104 (97%)	132 (94%)	37 (97%)	95 (93%)
	SI	3 (3%)	3 (2%)	-	3 (3%)
	SB	-	-	-	-
Day 4	None	104 (97%)	135 (96%)	37 (97%)	98 (96%)
	SI	3 (3%)	1 (1%)	-	1 (1%)
	SB	-	-	-	-
Day 7	None	101 (94%)	123 (88%)	35 (92%)	88 (86%)
	SI	5 (5%)	9 (6%)	1 (3%)	8 (8%)
	SB	-	2 (1%)	1 (3%)	1 (1%)
Day 14[b]	None	62 (98%) n=63	75 (91%) n=82	23 (100%) n=23	52 (88%) n=59
	SI	1 (2%)	4 (5%)	-	4 (7%)
	SB	-	1 (1%)	-	1 (2%)
Day 21[b]	None	58 (92%) n=63	79 (96%) n=82	21 (91%) n=23	58 (98%) n=59
	SI	4 (6%)	2 (2%)	1 (4%)	1 (2%)
	SB	-	-	-	-
Day 30	None	100 (93%)	124 (89%)	35 (92%)	89 (87%)
	SI	5 (5%)	5 (4%)	-	5 (5%)
	SB	-	-	-	-

SB=suicidal behavior (including non-suicidal self-injurious behavior); SI=suicidal ideation.
[a]Baseline values are lifetime incidence.
[b]Visits 14 and 21 were added to Studies 202B and C via amendment and include less patients.

Clinical Reviewer Comment: Unfortunately, the baseline C-SSRS values represent lifetime suicidal ideation and behavior rather than a baseline for the current depressive episode. Therefore, it is difficult to put the first few data points into context.

Visits on Day 7 and 14 appear to capture a few individuals with suicidal behaviors—all in the brexanolone groups. However, one individual on Day 7 is Subject (b) (6) *(discussed earlier in Section 10.3.6:* Serious Adverse Events*) and one individual (Subject* (b) (6) *reported non-suicidal self-injurious behavior on both Day 7 and Day 14.*

Considering that the behaviors captured on Days 7 and 14 either do not represent suicidal intent or are better accounted for by an underlying personality disorder, there does not appear to be a drug-related suicide signal for brexanolone in the submitted data. However, suicide events are so rare it would be impossible to conclude there is no underlying signal with the number of subjects exposed thus far. Previous suicide signals in antidepressants required pooling of studies and thousands of patients for detection.

10.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

Study 202A included the Stanford Sleepiness Scale (SSS): a patient-reported, Likert-type scale assessing sleepiness from 1 ("feeling active, vital, alert, or wide awake") to 7 ("no longer fighting sleep, sleep onset soon; having dream-like thoughts"). The SSS was administered as per Table 38 unless the patient was asleep. Results are presented in Table 72. Most patients were sleeping at the 18-, 42-, and 66-hour time points and no data from those times are presented in the table.

Table 72. Study 202A SSS Results; Mean (SD).

Time	Parameter	Placebo (n=11)	Brexanolone 90 µg/kg/h (n=10)
Baseline	Mean (SD) Min, Max	2.6 (2) 1, 6	2.7 (1) 1, 4
2 h	Mean (SD) Min, Max	3.0 (1) 1, 6	2.8 (1) 1, 5
4 h	Mean (SD) Min, Max	2.3 (1) 1, 4	3.0 (2) 1, 7
8 h	Mean (SD) Min, Max	2.6 (2) 1, 5	2.2 (2) 1, 6
12 h	Mean (SD) Min, Max	2.5 (1) 1, 4	3.6 (2) 1, 5
24 h	Mean (SD) Min, Max	2.6 (1) 1, 5	1.9 (1) 1, 3
30 h	Mean (SD) Min, Max	1.4 (1) 1, 2	2.0 (1) 1, 4
36 h	Mean (SD) Min, Max	2.1 (1) 1, 3	2.0 (2) 1, 6
48 h	Mean (SD) Min, Max	1.8 (1) 1, 3	1.5 (1) 1, 3
54 h	Mean (SD) Min, Max	1.5 (1) 1, 3	1.3 (1) 1, 3
60 h	Mean (SD) Min, Max	2.0 (1) 1, 4	1.4 (1) 1, 4
72 h	Mean (SD) Min, Max	1.7 (1) 1, 3	1.4 (1) 1, 3

Clinical Reviewer Comment: Considering the most common AEs with brexanolone are sedation-related, it is quite surprising that drug and placebo arms in 202A did not consistently differ in their self-report of somnolence (either mean or maximum), especially because three 202A brexanolone patients actually had AEs of mild to moderate sedation/somnolence. This may reflect poor scale validity or a chance finding from extremely small sample sizes.

10.7. Safety Analyses by Subgroups

Table 73 describes the incidence of AEs by age. Although there are several points at which a break could be made, this analysis uses the median age of 27 as a cut-point.

Table 74 describes the incidence of AEs by race. Patients who were not either African-American/black or white were too few to include in the racial analysis (one American Indian/Alaskan Native, one Native Hawaiian/Pacific Islander, one Asian, and four "other").

Table 74 describes the incidence of AEs by weight. Brexanolone is dosed by weight. Heavier women received a larger dose. Dividing patients at 85 kg produced similar sample size numbers for the placebo and brexanolone groups. To account for body type, AEs are also presented by BMI (Table 75).

Table 76 describes the incidence of AEs by baseline antidepressant and benzodiazepine use. There were no AEs in the single patient receiving a benzodiazepine, but not receiving an antidepressant. Table 77 describes the incidence of sedation/somnolence and dizziness/lightheadedness/presyncope/vertigo in Study 202C patients based on concomitant opioids. There were no concomitant opioids in Study 202A. In Study 202B, one 90 μg/kg/h patient, two 60 μg/kg/h patients, and three placebo patients received opioids. None of these patients reported a sedation/somnolence AE; one 60 μg/kg/h patient reported dizziness.

A caveat to all subgroup analyses is the resulting extremely small sample sizes. Therefore, group differences must be quite large to be potentially clinically meaningful.

Table 73. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group and by Age in Studies 202A, B, and C; n(%).

Adverse Event	Placebo n (%)		Any Brexanolone		Brexanolone 60 mcg/kg/h		Brexanolone 90 mcg/kg/h	
	<27 years (n=47)	≥27 years (n=60)	<27 years (n=64)	≥27 years (n=76)	<27 years (n=17)	≥27 years (n=21)	<27 years (n=47)	≥27 years (n=55)
Sedation, somnolence	4 (9%)	5 (8%)	8 (13%)	13 (17%)	3 (18%)	5 (24%)	5 (11%)	8 (15%)
Dizziness, lightheadedness, presyncope, vertigo	2 (4%)	5 (8%)	9 (14%)	8 (11%)	1 (6%)	4 (19%)	8 (17%)	4 (7%)
Dry mouth, thirst	0	1 (2%)	9 (14%)	3 (4%)	2 (12%)	2 (10%)	2 (4%)	1 (2%)
LOC, syncope	0	0	3 (5%)	2 (3%)	1 (6%)	1 (5%)	2 (4%)	1 (2%)
Flushing, hot flush	0	0	3 (5%)	1 (1%)	1 (6%)	1 (5%)	2 (4%)	0
Diarrhea	0	1 (2%)	0	3 (4%)	0	1 (5%)	0	2 (4%)
Oropharyngeal pain	0	0	1 (2%)	2 (3%)	0	1 (5%)	1 (2%)	1 (2%)
Tachycardia	0	0	3 (5%)	0	0	0	3 (6%)	0
Dyspepsia, indigestion	0	0	1 (2%)	1 (1%)	0	0	1 (2%)	1 (2%)

Clinical Reviewer Comment: Flushing/hot flush and tachycardia appeared more common on the younger age group while diarrhea was more common in the older age group. Given the small numbers of patients with events and the largely arbitrary cut-point, I do not expect that these differences are clinically meaningful.

Table 74. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group and by Race in Studies 202A, B, and C; n (%).

Adverse Event	Placebo		Any Brexanolone		Brexanolone 60 µg/kg/h		Brexanolone 90 µg/kg/h	
	AA (n=40)	White (n=65)	AA (n=49)	White (n=86)	AA (n=12)	White (n=25)	AA (n=37)	White (n=61)
Sedation, somnolence	2 (5%)	4 (6%)	9 (18%)	12 (14%)	4 (33%)	4 (16%)	5 (14%)	8 (13%)
Dizziness, lightheadedness, presyncope, vertigo	1 (3%)	6 (9%)	3 (6%)	14 (16%)	0	5 (20%)	3 (8%)	9 (15%)
Dry mouth, thirst	0	1 (2%)	3 (6%)	4 (5%)	1 (8%)	3 (12%)	2 (5%)	1 (2%)
LOC, syncope	0	0	1 (2%)	4 (5%)	0	2 (8%)	1 (3%)	2 (3%)
Flushing, hot flush	0	0	2 (4%)	2 (2%)	0	2 (8%)	2 (5%)	0
Diarrhea	0	1 (2%)	1 (2%)	2 (2%)	0	1 (4%)	1 (3%)	1 (2%)
Oropharyngeal pain	0	0	2 (4%)	1 (1%)	1 (8%)	0	1 (3%)	1 (2%)
Tachycardia	0	0	1 (2%)	2 (2%)	0	0	1 (3%)	2 (3%)
Dyspepsia, indigestion	0	0	0	1 (1%)	0	0	0	1 (2%)

AA=African-American/black.

Clinical Reviewer Comment: There appears to be a consistent pattern of increased dizziness/vertigo in white patients; however, this pattern is also true in the placebo group. Therefore, this observation is not consistent with a drug effect.

Table 75. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group and by Weight in Studies 202A, B, and C; n (%).

Adverse Event	Placebo		Any Brexanolone		Brexanolone 60 µg/kg/h		Brexanolone 90 µg/kg/h	
	≤85 kg (n=62)	>85 kg (n=45)	≤85 kg (n=76)	>85 kg (n=64)	≤85 kg (n=18)	>85 kg (n=20)	≤85 kg (n=58)	>85 kg (n=44)
Sedation, somnolence	4 (6%)	2 (4%)	10 (13%)	11 (17%)	2 (11%)	6 (30%)	8 (14%)	5 (11%)
Dizziness, lightheadedness, presyncope, vertigo	4 (6%)	3 (7%)	11 (14%)	6 (9%)	4 (22%)	1 (5%)	7 (12%)	5 (11%)
Dry mouth, thirst	1 (2%)	0	4 (5%)	3 (5%)	2 (11%)	2 (10%)	2 (3%)	1 (2%)
LOC, syncope	0	0	3 (4%)	2 (3%)	1 (6%)	1 (5%)	2 (3%)	1 (2%)
Flushing, hot flash	0	0	1 (1%)	3 (5%)	0	2 (10%)	1 (2%)	1 (2%)
Diarrhea	0	1 (2%)			1 (6%)	0	0	2 (5%)
Oropharyngeal pain	0	0	0	3 (5%)	0	1 (5%)	0	2 (5%)
Tachycardia	0	0	3 (4%)	0	0	0	3 (5%)	0
Dyspepsia, indigestion	0	0	2 (3%)	0	0	0	2 (3%)	0

Table 76. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group and by BMI in Studies 202A, B, and C; n (%).

Adverse Event	Placebo				Any Brexanolone			
BMI (kg/m^2):	<25 (n=28)	25-<30 (n=26)	30-<40 (n=37)	≥40 (n=16)	<25 (n=33)	25-<30 (n=32)	30-<40 (n=49)	≥40 (n=26)
Sedation, somnolence	2 (7%)	1 (4%)	3 (8%)	0	4 (12%)	4 (13%)	7 (14%)	6 (23%)
Dizziness, lightheadedness, presyncope, vertigo	2 (7%)	1 (4%)	1 (3%)	3 (19%)	4 (12%)	7 (22%)	3 (6%)	3 (12%)
Dry mouth, thirst	1 (4%)	0	0	0	3 (9%)	1 (3%)	2 (4%)	1 (4%)
LOC, syncope	0	0	0	0	0	3 (9%)	1 (2%)	1 (4%)
Flushing, hot flash	0	0	0	0	1 (3%)	0	2 (4%)	1 (4%)
Diarrhea	0	0	0	1 (6%)	0	1 (3%)	1 (2%)	1 (4%)
Oropharyngeal pain	0	0	0	0	0	0	1 (2%)	2 (8%)
Tachycardia	0	0	0	0	2 (6%)	0	1 (2%)	0
Dyspepsia, indigestion	0	0	0	0	1 (3%)	0	1 (2%)	0

Clinical Reviewer Comment: Because the brexanolone dose is weight-based, heavier women received a larger brexanolone dose. Based on the data in Table 74, there is no consistent pattern of AEs in either weight group. For example, more of the heavier women in the brexanolone 60 µg/kg/h group experienced sedation (30% versus 11% in the lighter group)—however, this pattern was not true in the 90 µg/kg/h group. Likewise, when grouped by BMI, there is no consistent pattern of AEs.

Table 77. Adverse Events ≥ 2% and Twice the Rate of Placebo by Treatment Group and Baseline Antidepressant and Benzodiazepine Medication in Studies 202A, B, and C; n (%).

Adverse Event	Placebo			Any Brexanolone		
	AD Only (n=21)	AD+ Benzo (n=5)	Neither (n=80)	AD Only (n=23)	AD+ Benzo (n=11)	Neither (n=106)
Sedation, somnolence	1 (5%)	0	5 (6%)	4 (17%)	5 (45%)	12 (11%)
Dizziness, lightheadedness, presyncope, vertigo	2 (10%)	2 (40%)	3 (4%)	4 (17%)	3 (27%)	10 (9%)
Dry mouth, thirst	0	0	1 (1%)	1 (4%)	1 (9%)	5 (5%)
LOC, syncope	0	0	0	1 (4%)	0	4 (4%)
Flushing, hot flash	0	0	0	2 (9%)	0	2 (2%)
Diarrhea	1 (5%)	0	0	3 (13%)	0	0
Oropharyngeal pain	0	0	0	0	1 (9%)	2 (2%)
Tachycardia	0	0	0	0	0	3 (3%)
Dyspepsia, indigestion	0	0	0	0	1 (9%)	1 (1%)

AD=antidepressant, Benzo=benzodiazepine

Clinical Reviewer Comment: A greater percentage of patients on antidepressants and brexanolone (26%) reported sedation AEs compared with patients on brexanolone alone (11%), placebo and antidepressants (4%), or placebo alone (6%). Patients on benzodiazepines as well as antidepressants had an even greater percentage reporting sedation and dizziness AEs. However, the sample sizes are small and there are similar absolute numbers of patients reporting these AEs in both the medication groups. Nevertheless, it is consistent with physiology that benzodiazepines (which act at a GABA receptor site distinct from that of brexanolone) and antidepressants (because sedation is largely related to non-GABAergic mechanisms such as histamine blockade) would produce additive sedation and dizziness-type AEs in patients taking brexanolone. Indeed, a similar pattern of dizziness AEs is observed in the placebo patients (those on an antidepressant and a benzodiazepine had a higher percentage reporting these AEs than those on an antidepressant and those on an antidepressant had a higher percentage reporting these AEs than those on neither drug).

Labeling should reflect this possible additive risk for sedation and dizziness.

Table 78. Sedation and Dizziness AEs by Concomitant Opioid Use in Study 202C; n (%).

Adverse Event	No Opioids		Concomitant Opioids	
	Placebo (n=48)	Brexanolone 90 µg/kg/h (n=46)	Placebo (n=5)	Brexanolone 90 µg/kg/h (n=5)
Sedation, somnolence	2 (4%)	6 (13%)	0	2 (40%)
Dizziness, lightheadedness, presyncope, vertigo	3 (6%)	4 (9%)	0	1 (20%)

Clinical Reviewer Comment: A greater percentage of patients in Study 202C who received an opioid during the study reported sedation and dizziness AEs compared with patients who did not receive an opioid. This analysis includes as-needed opioids as well as chronic opioids. Although the sample sizes are extremely small—and only Study 202C included enough patients on an opioid reporting the AEs of interest—additive sedation is consistent with known physiology and drug pharmacodynamics. Therefore, I recommend labeling reflect the possible additive risk for sedation and dizziness in patients receiving brexanolone and a concomitant opioid.

10.8. Specific Safety Studies/Clinical Trials

Not applicable to this application.

10.9. Additional Safety Explorations

10.9.1. Human Carcinogenicity or Tumor Development

Not applicable to this application.

10.9.2. Human Reproduction and Pregnancy

Not applicable to this application.

10.9.3. Pediatrics and Assessment of Effects on Growth

Not applicable to this application.

10.9.4. Overdose, Drug Abuse Potential, Withdrawal, and Rebound

As per the Controlled Substance Staff's review, preclinical and clinical findings indicate that brexanolone has abuse potential similar to that of benzodiazepines.

The preclinical evaluation of the abuse potential of brexanolone includes receptor binding studies, functional studies, and animal behavioral studies, which demonstrate the following:

- Receptor binding studies indicate that brexanolone has significant affinity for GABA-chloride channels, androgen, progesterone, and GABA-benzodiazepine receptors.
- Functional studies indicate that brexanolone acts as an agonist at GABA receptor sites.
- In general animal behavioral studies, brexanolone produces dose-dependent depressant effects such as sedation and muscle relaxation in rats and dogs and decreased locomotion in mice.
- In a drug-discrimination study in rats, brexanolone produces full generalization to the benzodiazepine, midazolam (>99%). This suggests that brexanolone produces effects that are similar to a sedative with known abuse potential.
- A physical dependence study conducted in rats was not conclusive, as the positive control, midazolam, did not produce a strong withdrawal signal upon abrupt discontinuation.

Clinical studies with brexanolone further support that brexanolone produces subjective effects comparable to benzodiazepines, based on the following:

- A human abuse potential study produced dose-dependent subjective effects indicative of abuse potential. At the high dose tested (270 µg/kg/IV/1-hour infusion) brexanolone produced Drug Liking scores similar to those of alprazolam 3 mg.
- In phase2/3 double-blind studies, no events of euphoria were reported; however, sedation was reported in 4-30% (mean 5.7%) of subjects on brexanolone and 0-2% (mean 0.9%) of subjects on placebo. Somnolence, which may not necessarily be an abuse related adverse event (AE), was reported as an AE separate from sedation and occurred at higher rates in the active drug group compared to placebo.

10.10. Safety in the Postmarket Setting

10.10.2. Safety Concerns Identified Through Postmarket Experience.

Not applicable to this application.

10.10.3. Expectations on Safety in the Postmarket Setting

Brexanolone will be the subject of a REMS (see separate Division of Risk Mitigation review). The healthcare setting will monitor safety and will report infusion-related adverse events to the REMS.

10.11. Integrated Assessment of Safety

The Applicant submitted sufficient information to adequately assess brexanolone's safety profile. The Agency's major safety concern is the possibility of LOC during the infusion (6 of 140 women exposed to brexanolone). After examining dose, timing of dose, blood level, concurrent medications, available medical history, and patient characteristics (e.g., age, body mass index) we found no relationships between these factors and the LOC events. Because LOC can be abrupt, and there is no way to predict the event, the Agency did not feel the risk could be mitigated solely through labeling. Therefore, this product will be approved with a REMS to mitigate the risk of adverse events associated with sedation and sudden LOC caused by

brexanolone. Aside from the risk of sedation and LOC, brexanolone appeared reasonably well-tolerated.

11 SUMMARY AND CONCLUSIONS

Conclusions and Recommendations

Evidence of brexanolone' s effectiveness as a 60-hour infusion in treating PPD was assessed in three controlled studies: 547-PPD-202A, 202B, and 202C. The primary efficacy endpoint in these studies was change from baseline on the Hamilton Depression Scale at 60 hours after start of the brexanolone infusion. All three studies showed a statistically significant reduction in depressive symptoms with brexanolone infusion. Dosages of 90 [redacted] and 60 [redacted] were studied, the larger dosage in both of the phase 3 studies, the smaller dosage in only one. The higher dosage did not show a greater effect, but did not appear to cause more adverse reactions. It will be the recommended dosage.

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The Applicant submitted sufficient information to adequately assess brexanolone's safety profile. The Agency's major safety concern is the possibility of LOC during the infusion (6 of 140 women exposed to brexanolone). After examining dose, timing of dose, blood level, concurrent medications, available medical history, and patient characteristics (e.g., age, body mass index) we found no relationships between these factors and the LOC events. Because LOC can be abrupt, and there is no way to predict the event, the Agency did not feel the risk could be mitigated solely through labeling. Brexanolone will be approved with a REMS mitigate the risk of adverse events associated with sedation and LOC. Aside from the risks of sedation and LOC, brexanolone appeared reasonably well-tolerated.

Considering the seriousness of PPD, the lack of identified effective treatments, and the risks and benefits of brexanolone, the review team recommends approval. We do not believe additional studies are needed prior to marketing to further characterize the LOC risk. However, we recommend additional efficacy studies to determine whether the infusion can be given in an interrupted manner (only during the daytime) or shortened—potentially broadening available administration settings.

X	X	X
Jinglin Zhong, PhD Primary Statistical Reviewer	Peiling Yang, PhD Statistical Team Leader	Hsien Ming (James) Hung, PhD Biometrics Division Director

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Bernard Fischer, MD
Primary Clinical Reviewer & Team Leader

12 Advisory Committee Meeting and Other External Consultations

The Agency convened a joint meeting of the Psychopharmacologic Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee on November 2, 2018. Three voting questions and three discussion questions were presented to the Committees. The complete discussion is available in the public record via the transcript of the meeting.

1. **VOTE:** Has substantial evidence been presented by the Applicant to support a claim of effectiveness for brexanolone for the treatment of postpartum depression?

   Result: *Yes: 18* *No: 0* *Abstain: 0*

2. **VOTE:** Has the Applicant adequately characterized the safety profile of brexanolone for the treatment of postpartum depression? Do you believe the loss of consciousness events have been characterized sufficiently to enable safe use of brexanolone?

   Result: *Yes: 16* *No: 2* *Abstain: 0*

3. **VOTE:** Given the efficacy as presented, and when used in a certified facility by qualified staff and as outlined in the FDA's proposed REMS, do the benefits outweigh the risks of brexanolone for the treatment of postpartum depression?

   Result: *Yes: 17* *No: 1* *Abstain: 0*

4. **DISCUSSION:** There is evidence that both a 60 µg/kg/h and a 90 µg/kg/h dose (after 24 hours) are effective. Please discuss, if approved, which dose should be the recommended dose.

- Start at 90 µg/kg/h with the option to decrease the dose to 60 µg/kg/h based on tolerability
- Start at 60 µg/kg/h with the option to increase the dose to 90 µg/kg/h based on response

The Committees agreed with the Agency that the starting dose could not be determined based on the Applicant's data. They declined to recommend a starting dose, deferring to the Agency.

5. **DISCUSSION**: Discuss whether the FDA's proposed REMS would ensure safe use of brexanolone. If no, please discuss what additional safeguards would be needed.

Individual Committee Members' Recommendations:	***Actions to Address Committees' Concerns:***
• *Simplify the dosing regimen.*	• *The approved dosing regimen will be based on the way the drug was studied. However, postmarketing studies will examine whether the duration of the infusion can be shortened.*

• *Use a sedation scale at predetermined intervals during the infusion.*	• *The use of a particular scale may complicate delivery (based on determining which scale or scales should be used, whether training is necessary, etc.). The REMS will require checks for excessive sedation at regular intervals.*
• *Develop a standardized order set to be used nationally.*	• *The Applicant has discussed their intent to develop such an order set.*
• *Consider using capnography rather than pulse oximetry for monitoring.*	• *The Agency believes pulse oximetry is adequate and the limited availability of capnography could present an access issue for patients in need of treatment.*
• *Limit the infusion setting to inpatient facilities only.*	• *The Agency weighted the risks and benefits of this limitation. We believe it would limit access to patients while not offering a proportional increase in safety.*
• [redacted] (b) (4)	

6. **DISCUSSION:** If approved, what additional data will be needed to support safe use of brexanolone at home and address outstanding issues?

The Committees recommended the Applicant determine the utility of brexanolone for a wider population (e.g., bipolar depression, suicidal ideation, patients with psychosis), whether the infusion could be given in interrupted pulses (e.g., during waking hours for 3 days), and find the optimal dosage.

13 Pediatrics

The Applicant and FDA agreed to a randomized, double-blind, placebo-controlled study in adolescents 15 to less than 18 years old with PPD. The Applicant [redacted] (b) (4)

14 Labeling Recommendations

14.1. Prescription Drug Labeling

The table below summarizes significant changes to the proposed prescribing information made by FDA. This labeling was under negotiation at the time of this review. The remainder of this section will only focus on high-level issues.

Summary of Significant Labeling Changes (High level changes and not direct quotations)		
Section	**Proposed Labeling**	**Approved Labeling**
Highlights		
Boxed Warning	*See comments below in FPI Black Box Warning for more information.*	
Indications and Usage	*See comments below in FPI Indications and Usage for more information.*	
Dosage and Administration	*See comments below in FPI Dosage and Administration for more information.*	
Warnings and Precautions	*See comments below in FPI Warnings and Precautions for more information.*	
Adverse Reactions	*See comments below in FPI Adverse Reactions for more information.*	
Use in Specific Populations	*See comments below in FPI Use in Specific Population for more information.*	
Full Prescribing Information		
Boxed Warning	(b) (4) warning was added.	(b) (4) was removed from the boxed warning. (b) (4)
1. Indications and Usage	ZULRESSO is indicated for the treatment of postpartum depression (PPD).	Retained.
2. Dosage and Administration	Dosing, preparation and administration language was provided.	This language was edited to simplify instructions.
4. Contraindications	ZULRESSO is (b) (4)	(b) (4)
5. Warnings and Precautions	Clinical Worsening and	Excessive sedation was

	Suicide Risk, Sedation, Potential Interactions with CNS Depressants and Impaired Alertness sections were provided.	added. Suicidal thoughts and behaviors was retained, but modified for applicability to brexanolone.
6. Adverse Reactions	(b) (4) [redacted]	The table of adverse reactions was limited to those observed during infusion and at a higher rate than in placebo. Warnings and precautions were edited.
7. Drug Interactions	This language was provided.	Language for increased risk of sedation with concomitant use of CNS depressants was maintained. The increased risk of sedation with antidepressants was added.
8. Use in Specific Populations	Pregnancy, lactation, pediatric use, hepatic and renal impairment were discussed.	Section 8.1: Data obtained from published literature on apoptotic neurodegeneration with drugs that enhance GABAergic inhibition was added to this section.
9. Drug Abuse and Dependence	9.1 (b) (4) [redacted] 9.2 Abuse (b) (4) [redacted], 3% of subjects administered ZULRESSO 90 mcg/kg reported euphoric mood compared to none administered placebo. 9.3 Dependence	FDA is recommending Schedule IV.

	[redacted]	
10. Overdosage	[redacted] [redacted] Management of Overdose In case of overdose, stop the infusion immediately and initiate supportive measures as necessary. [redacted]	The cases of IV pump malfunction resulting in overdosage were added to this section.
11. Description	[redacted]	Revised for clarity. [redacted] betadex sulfobutyl ether sodium. Added a second established pharmacological class "neuroactive steroid."
12. Clinical Pharmacology	12.1 Mechanism of Action [redacted] 12.3 Pharmacokinetics [redacted]	12.1 Mechanism of Action: Language was edited. 12.2 Pharmacodynamics: Section was added. 12.3 Pharmacokinetics: This language was edited for clarity. [redacted] was removed. Betadex Sulfobutyl Ether Sodium Pharmacokinetics was added.
13. Nonclinical Toxicology	[redacted] have not been	Retained.

	performed. Brexanolone was not genotoxic when tested in an in vitro microbial mutagenicity (Ames) assay, an in vitro micronucleus assay in human peripheral blood lymphocytes, and an in vivo rat bone marrow micronucleus assay. (b) (4) brexanolone was associated with decreased mating and fertility indices.	
14. Clinical Studies	The overview of the clinical development program is provided. Primary endpoint results are displayed in a chart, and the Change from Baseline in HAM-D Total Score Over Time (Days) in (b) (4) is displayed in a graph.	This section was edited to (b) (4) with results from 202B and 202C separately. (b) (4)
16. How Supplied/ Storage and Handling	(b) (4) How Supplied ZULRESSO is supplied as 100 mg brexanolone in 20 mL single-use vials (5 mg/mL). (b) (4) Storage and Handling Store ZULRESSO at 2°C to 8°C (36°F to 46°F). Do not freeze. Store protected from light.	This section was edited when the Applicant provided additional data on length of storage of the diluted product.
17. Patient Counseling	Patients are advised to read the FDA-approved patient labeling (Medication Guide). Counseling points were provided for Suicide Thoughts and Behaviors, (b) (4), Concomitant Medications, (b) (4) Pregnancy (b) (4)	Counseling points on the ZULRESSO Risk Evaluation and Mitigation Strategy (REMS) was added.

15 Risk Evaluation and Mitigation Strategies (REMS)

Refer to the separate REMS review and Applicant-submitted documents for more details. The REMS goal is to mitigate the risk of serious harm resulting from excessive sedation and loss of consciousness during the ZULRESSO infusion by:

i. Ensuring that ZULRESSO is administered only to patients in a medically supervised setting that provides monitoring while ZULRESSO is administered.
ii. Ensuring pharmacies and healthcare settings that dispense ZULRESSO are certified.
iii. Ensuring that each patient is informed of the adverse events of excessive sedation and loss of consciousness and the need for monitoring while ZULRESSO is administered.
iv. Enrollment of all patients in a registry to characterize the risks and support safe use.

Healthcare settings and pharmacies that dispense ZULRESSO will be certified to dispense and/or administer ZULRESSO. Healthcare settings will enroll patients in the ZULRESSO REMS registry. The Applicant will provide training materials for healthcare settings and pharmacies. They will also develop patient education material, a healthcare provider REMS letter, and establish and maintain a REMS program website.

16 Postmarketing Requirements and Commitments

16.1. Postmarketing Requirements (PMRs)

16.1.1. Pediatric Research Equity Act (PREA) PMR 3535-1

Given that adolescents also experience PPD, the Applicant has agreed to conduct a randomized, double-blind, placebo-controlled, parallel-group study evaluating the efficacy and safety of brexanolone in adolescent females ages 15 to less-than-18 years, diagnosed with PPD.

16.1.2. PMR 3535-2

Based on data from published animal studies that reported that administration of drugs that enhance GABAergic inhibition to neonatal rats caused widespread apoptotic neurodegeneration in the developing brain, the Applicant has agreed to conduct an animal neurotoxicity study to determine if these effects will be observed with brexanolone.

16.2. Postmarketing Commitments (PMCs)

The Applicant studied target dosages of 60 and 90 µg/kg/h for their infusions. The review team felt that, although both dosages appeared effective (with a favorable benefit:risk ratio), the 90 µg/kg/h target had more supportive evidence. However, we noted that most (over 65%) of the observed effect occurred within the first 24 hours (when all dosage schedules were receiving 60 µg/kg/h). It is unclear whether the dosage needs to reach 90 µg/kg/h to observe a clinical effect and/or whether the infusion needs to continue for 60 hours. We are requesting the Applicant to

study this by conducting two [redacted] (b) (4) to evaluate the efficacy and safety of alternate dosing regimens.

16.2.1. **PMC 3535-3**

For this PMC, the Applicant will study [redacted] (b) (4)

16.2.2. **PMC 3535-4**

For this PMC, the Applicant will study the effect of interrupted infusions [redacted] (b) (4) If brexanolone appears safe and effective using this regimen, it may significantly increase the number of settings where patients could receive the drug.

17 Division Director

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Tiffany R. Farchione, MD
Acting Division Director

18 **Division Director (OCP)**

X

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Mehul Mehta, PhD
Director

19 Division Director (OB)

X

APPEARS THIS WAY ON ORIGINAL

Hsien Ming (James) Hung, PhD
Director

20 Division Director (Clinical)

X

APPEARS THIS WAY ON ORIGINAL

Tiffany R. Farchione, MD
Acting Division Director

21 Office Director (or designated signatory authority)

X

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Robert Temple, MD
Deputy Director of Clinical Science

22 Appendices

22.1. References

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22.2. Financial Disclosure

The Applicant could not obtain financial disclosure forms from two sub-investigators at (b) (6) However, the site did not enroll patients.

(b) (6) Study PPD-201. (b) (6) was a principal investigator for the PPD-202 studies (b) (6) but did not participate in collecting the outcome measures.

(b) (6) received stock options for service on the (b) (6). He was a sub-investigator for the PPD-202 studies (b) (6) but did not participate in collecting the outcome measures.

Covered Clinical Study (Name and/or Number): 547-PPD-202B, -202C

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 334		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 2		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 1 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator: 1 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 2		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

22.3. Nonclinical Pharmacology/Toxicology

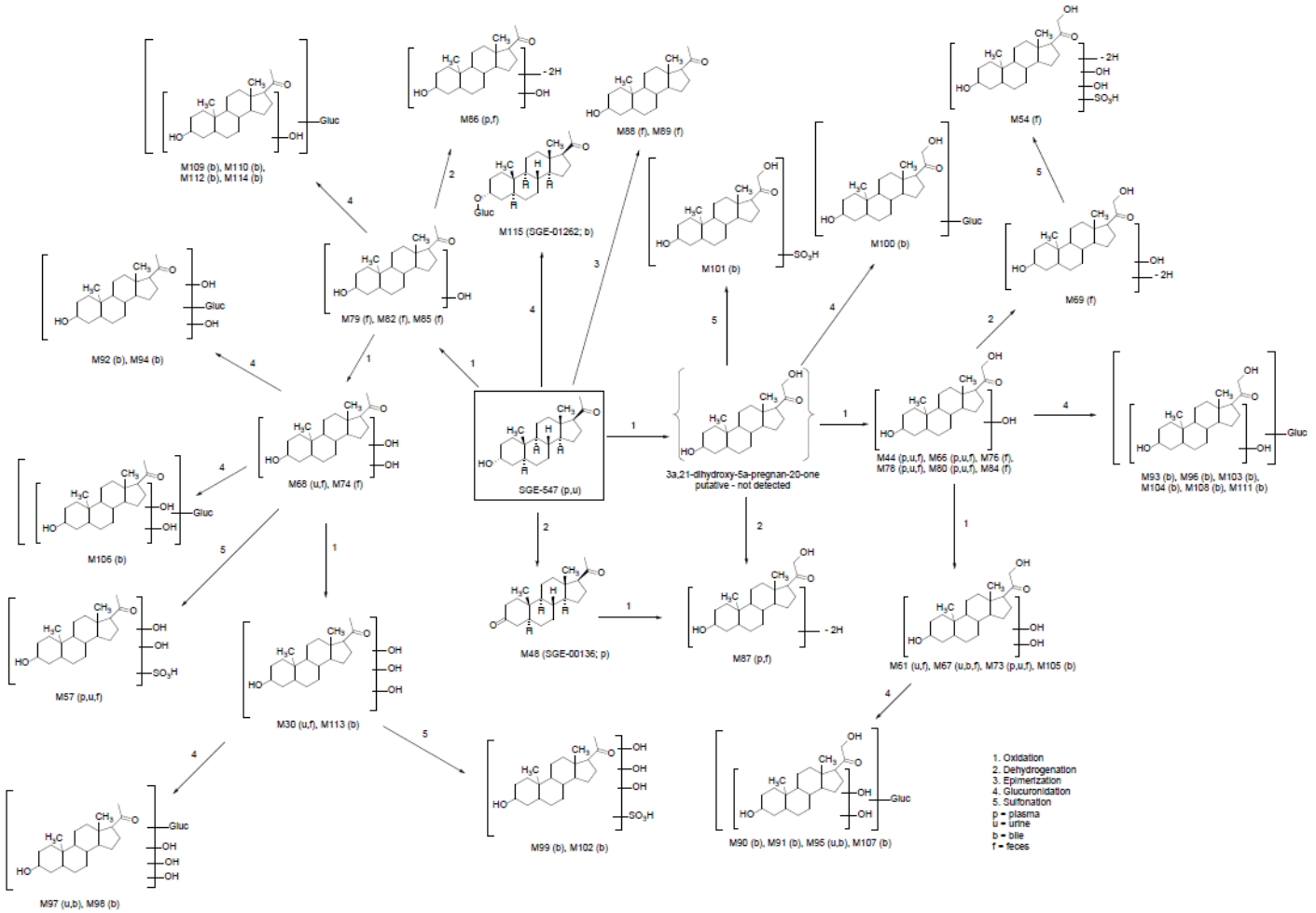
ADME/PK

Table 79: PK of brexanolone in mice and rats following single oral administration of 20 mg/kg brexanolone in 30% SBECD

Parameter	Mice	Rats
AUC_{last} (ng.h/mL)	9.16	59.3
C_{max} (ng/mL)	13	12.1
$T_{1/2}$ (h)	NA	2.46
T_{max} (h)	0.5	0.5
F (%)	0.563	2.32

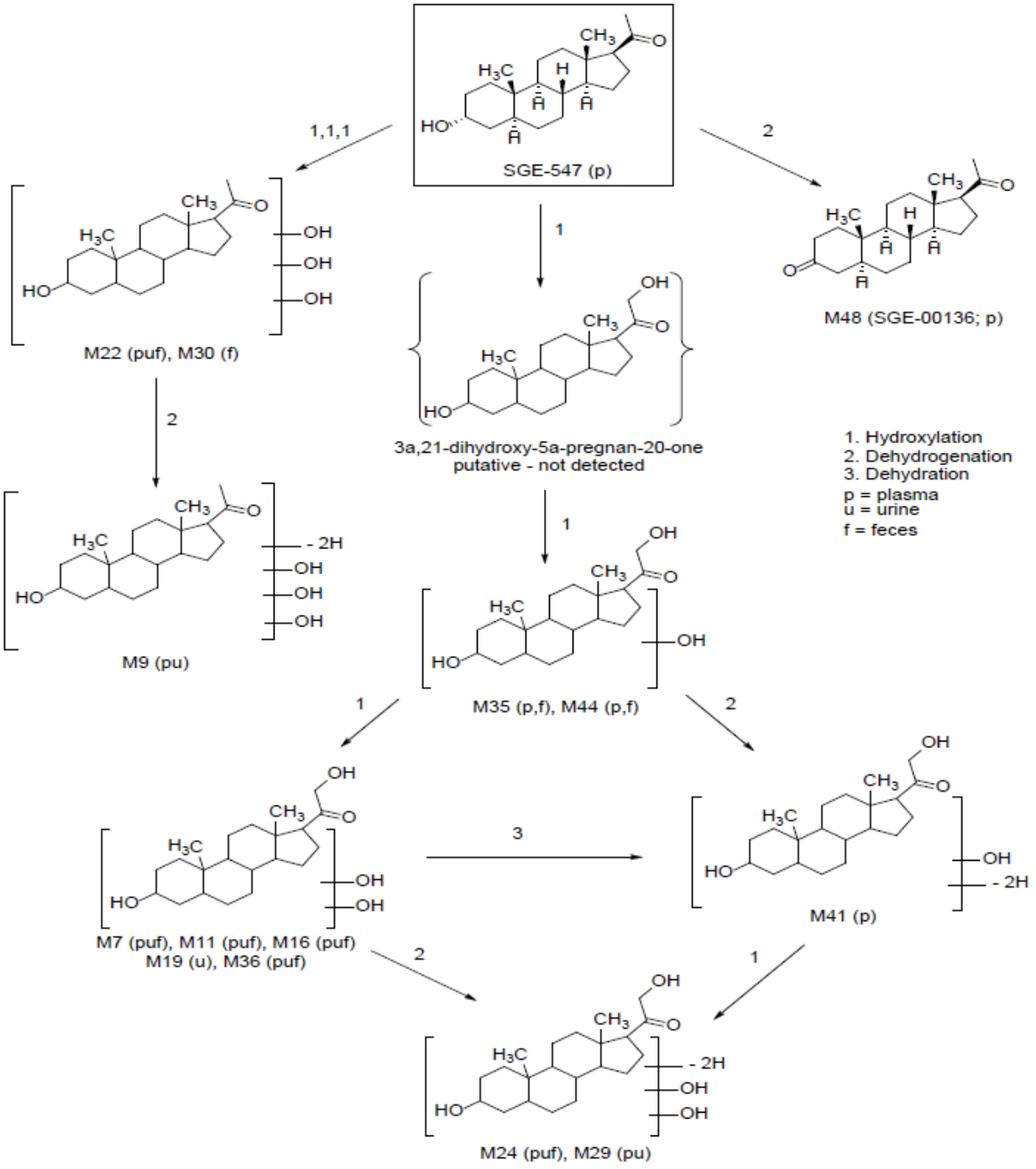
AUC_{last}: area under the curve from zero to the time of the last quantifiable concentration; C_{max}: maximum plasma concentration; $T_{1/2}$: terminal elimination half-life; T_{max}: time to reach maximum plasma concentration; F: bioavailability; NA: not available

Figure 29: Proposed metabolic pathways for brexanolone in rats



Source: Applicant's Table, Pharmacokinetics Written Summary, p.53.

Figure 30: Proposed metabolic pathways for brexanolone in dogs



Source: Applicant's Table, Pharmacokinetics Written Summary, p.55.

Table 80: Summary of transporter inhibition for brexanolone metabolites M133 (SGE-03211), M136 (SGE-03212), and M137 (SGE-03227)

Transporter and **Assay Type**	**Maximum Inhibition (% of Control)**		
	SGE-03211	**SGE-03212**	**SGE-03227**
BCRP VT	24[a]	NIO[a]	NIO[a]
BSEP VT	88	89	89
MDR1 VT	46	42	NIO
MRP3 VT	NIO	78	99
MRP4 VT	71	81	32
MATE1 UPT	NIO	NIO	NIO
MATE2-K UPT	NIO	NIO	NIO
OAT1 UPT	NIO	NIO	27
OAT3 UPT	44	61	NIO
OATP1B1 UPT	90	90	53
OATP1B3 UPT	98	95	54
OCT1 UPT	35	NIO	23
OCT2 UPT	NIO	NIO	NIO

Abbreviations: BCRP = breast cancer resistance protein; BSEP = bile salt export pump; MATE = multidrug and toxin extrusion; MDR = multi-drug resistance; MRP = multidrug resistance-associated protein; NIO = no interaction; OAT = organic anion transporter; OATP = organic anion-transporting polypeptide; OCT = organic cation transporter; UPT = uptake assay; VT = vesicular transport assay

[a] Stimulated BCRP-mediated E3S transport

Source: Applicant's Table, Pharmacokinetics Written Summary, p.69.

Table 81: Inhibition of CYP and UGT isoforms by brexanolone

Enzyme	Substrate	IC_{50} (µM)	Maximum Inhibition (%)
CYP1A2	Phenacetin	>30	21
CYP2C19	S-Mephenytoin	29 ± 6	47
CYP2B6	Efavirenz	>30	30
CYP2C8	Amodiaquine	23 ± 7	49
CYP2C9	Diclofenac	0.41	95
CYP2D6	Dextromethorphan	>30	9.9
CYP3A4/5	Midazolam	>30	34
CYP3A4/5	Testosterone	>30	38
UGT1A1	17β-Estradiol	>30	18
UGT1A3	Chenodeoxycholic acid	21 ± 5	52
UGT1A4	Trifluoperazine	26 ± 8	47
UGT1A6	1-Naphthol	>30	7.5
UGT1A9	Propofol	>30	23
UGT2B7	Morphine	23 ± 5	50
UGT2B15	Oxazepam	6.3 ± 1.3	67
UGT2B17	Testosterone	1.7 ± 0.2	86

Abbreviations: CYP = cytochrome P450 enzyme; IC_{50} = median inhibitory concentration; UGT = uridine 5'-diphospho-glucuronosyltransferase
Source: Applicant's Table, Pharmacokinetics Written Summary, p.56.

Table 82: Inhibition of CYP Isoforms by brexanolone metabolites M133 (SGE-03211), M136 (SGe-03212), M137 (SGE-02080)

Enzyme	Substrate	SGE-03211		SGE-03212		SGE-03277	
		IC_{50} ± SE	Max Inh. (%)	IC_{50} ± SE	Max Inh. (%)	IC_{50} ± SE	Max Inh. (%)
CYP1A2	Phenacetin	>100	51	84 ± 6	56	>100	6.9
CYP2B6	Efavirenz	49 ± 4	80	42 ± 3	81	>100	26
CYP2C8	Amodiaquine	17 ± 1	100	12 ± 1	100	>100	23
CYP2C9	Diclofenac	40 ± 5	80	85 ± 19	56	>100	6.0
CYP2C19	S-Mephenytoin	>100	39	>100	25	>100	13
CYP2D6	Dextromethorphan	>100	32	>100	30	>100	9.7
CYP3A4/5	Midazolam	91 ± 10	55	13 ± 3	89	>100	22
CYP3A4/5	Testosterone	>100	36	57 ± 6	64	>100	21

Abbreviations: CYP = cytochrome P450 enzyme; IC_{50} = median inhibitory concentration; Inh. = inhibition; SE = standard error
Source: Applicant's Table, Pharmacokinetics Written Summary, p.58.

A 28-Day Intravenous Infusion Toxicity Study of SAGE-547 in the Albino Rat Followed by a 28-Day Recovery Period/Study No. SSN-01272

Observations and Results

Mortality

APPEARS THIS WAY ON ORIGINAL

Table 83: Preterminal mortalities in the 28-day repeat dose rat toxicity study

SAGE-547 Dose Level (mg/kg/day)	Animal Number	Study Day	Status [b]	Noteworthy Findings
0[a]	Female No.2505	7	FD	The day prior to death, the animal was observed to have decreased activity level, signs of dehydration, blue discoloration of the abdominal and generalized dorsal skin, and increased respiratory rate. The death of this animal was attributed to hepatocellular necrosis.
10	Female No. 3502	6	FD	There were no SAGE-547 or vehicle (Captisol)-related clinical observations preceding death, which was attributed to thrombosis with bacteria noted at the infusion site.
30	Male No. 4004	24	FD[c]	The animal was observed to have decreased activity on the day preceding its accidental death. Inflammation with bacteria at the infusion site was noted.
30	Male No. 4016	17	FD	The day prior to death, the animal was observed to have decreased activity level, lying on side, skin pallor, ungroomed fur and both eyes partially closed. The death of this animal was attributed to the inflammation with bacteria at the infusion site.
30	Female No. 4502	23	FD	The day prior to death, the animal was observed to have decreased activity level. The death of this animal was attributed to the inflammation with bacteria at the infusion site.
60	Male No. 5002	14	UE	The animal condition quickly deteriorated and it was observed to have decreased activity level, weakness, thin, signs of dehydration and increased respiratory rate, leading to euthanasia. Low platelets and reticulocyte counts were noted for this animal on the day of death. There were no other abnormal clinical observations noted on the days preceding death, which was attributed to the inflammation with bacteria at the infusion site.
60	Male No. 5005	22	FD	The animal showed signs of weakness and decreased activity level between Day 14 to 21. Its general condition appeared to have stabilized over this time period following the addition of wet pellets. The death of this animal was attributed to the inflammation with bacteria at the infusion site.
60	Male No. 5012	26	FD	Prior to its death, the animal showed signs of decreased activity level between Day 22 to 25. The death of this animal was attributed to the inflammation with bacteria at the infusion site.
60	Male No. 5104	25	UE	The animal started showing signs of decreased activity level on Day 14, followed by ungroomed fur on Day 17. Its general health condition deteriorated rapidly on Day 25 where it was also noted to be cold to touch with generalized skin pallor and increased respiratory rate, leading to euthanasia. The death of this animal was attributed to the inflammation with bacteria at the infusion site.

SAGE-547 Dose Level (mg/kg/day)	Animal Number	Study Day	Status [b]	Noteworthy Findings
60	Female No. 5602	10	UE	The animal condition quickly deteriorated and it was observed to have decreased activity level, weakness, hunched posture, skin pallor, erected fur, brown fur staining (periorbital and muzzle), and eyes partially closed, leading to euthanasia. Low platelet count was noted for this animal on the day of death. There were no other abnormal clinical observations noted on the days preceding death, which was attributed to the thrombosis with bacteria at the infusion site.
60	Female No. 5516	29	UE	The animal condition quickly deteriorated and it was observed to have decreased activity level, weakness, hunched posture, signs of dehydration, thin, warm to touch, red fur staining (periorbital and muzzle), eyes partially closed and labored breathing, leading to euthanasia. Elevated white blood cells, low platelets and reticulocyte counts were noted for this animal on the day of death. The death of this animal was attributed to the inflammation with bacteria at the infusion site.
60	Female No. 5520*	9	UE	The animal condition quickly deteriorated and it was observed to have decreased activity level, weakness, lying on side, skin pallor, erected fur, brown fur staining (muzzle) and eyes partially closed, leading to euthanasia. The cause of death of this animal is undetermined.

[a] Captisol dose of 3000 mg/kg/day
[b] UE = Unscheduled euthanasia; FD = Found dead
[c] Accidental death
* Toxicokinetic study (gross examination only)

Source: Applicant's Table, SSN-01272, p.33.

Clinical Signs

Table 84: Clinical signs in the 28-day repeat dose rat toxicity study

Dose SAGE-547 (mg/kg)		0	0	10	30	60
Amount SBECD (mg/kg)		0	3000	500	1500	3000
Parameter	Sex	Observations/No. of Rats				
Decreased Activity	M	12/3	90/15	35/5	81/14	180/16
	F	8/4	17/7	19/5	122/13	106/15
Limited Usage of hindlimbs	M	0	0	2/1	12/1	9/3
	F	0	0	0	59/4	0
Hunched Posture	M	0	4/4	0	0	5/1
	F	0	2/2	0	2/2	2/2

Limited usage of hindlimbs and/or hunched posture noted for vehicle-dosed rats may be associated with the presence of masses and/or other procedural-related lesions (inflammation and/or bacterial sepsis) observed at the infusion site.

Gross Pathology

Pale discoloration of the kidney was noted in most rats that received the vehicle and enlargement of kidneys was noted in most VCM and 1 -2 HDM, VCF, MDF, and HDF. The findings in the kidney were not observed at the end of the recovery period.

Table 85: Vehicle-related gross pathology findings in the 28-day rat repeat dose study

	Male					Female				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**10**	**30**	**60**	**0**	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**0**	**3000**	**500**	**1500**	**3000**	**0**	**3000**	**500**	**1500**	**3000**
No. Animals Examined	**10**	**10**	**10**	**9**	**6**	**10**	**9**	**9**	**9**	**8**
Kidney (No. Examined)	10	10	10	9	6	10	9	9	9	8
Discoloration pale	1	9	5	7	6	0	7	4	8	8
Enlargement	1	7	0	0	2	0	1	0	2	2

Source: Applicant's Table, SSN-01272, p.40.

The masses noted at the infusion site of some rats were located at the tip or entry of the catheter into the iliac vein and up to the catheter tip, the kidney, or occasionally the liver. The masses were pale, firm, and varied in size (10X10X7 mm to 55X15X15 mm) and a pale thick material was generally noted at the cut surface. These masses and the swelling at the infusion site correlated with microscopic findings of moderate to severe neutrophilic inflammation that often contained bacteria. Other macroscopic changes were noted in miscellaneous tissues and were thought to be secondary to the bacteria-related neutrophilic inflammation at the infusion site.

Table 86: Infusion site gross pathology findings in the 28-day rat repeat dose study

	Male					Female				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**10**	**30**	**60**	**0**	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**0**	**3000**	**500**	**1500**	**3000**	**0**	**3000**	**500**	**1500**	**3000**
No. Animals Examined	**10**	**10**	**10**	**9**	**6**	**10**	**9**	**9**	**9**	**8**
Infusion site (No. Examined)	10	10	10	9	6	10	9	9	9	8
Mass	3	2	1	4	2	1	3	0	4	0
Swelling	0	2	1	3	0	0	1	1	0	1
Thick	0	0	0	0	0	3	0	2	0	1
Firm	1	0	0	1	0	1	0	0	0	0

Source: Applicant's Table, SSN-01272, p.40.

Organ Weights

There were no microscopic findings observed in the ovaries to correlate with the decreased ovary weights. The increased kidney weights correlated with microscopic findings of tubular vacuolation in VC and HD groups.

Table 87: Organ weight findings in the in the 28-day rat repeat dose study

	Male				Female			
Group	**2**	**3**	**4**	**5**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**10**	**30**	**60**	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**3000**	**500**	**1500**	**3000**	**3000**	**500**	**1500**	**3000**
No. Animals per Group	**10**	**10**	**9**	**6**	**9**	**9**	**9**	**8**
Kidney[a]								
Absolute value (1)	**46.41**	3.33	14.19	**31.55**	**45.91**	7.58	20.78	**47.11**
Absolute value (2)	-	**-29.42**	-22.01	-10.15	-	**-26.27**	**-17.22**	0.82
% of body weight (1)	**60.65**	**9.42**	**23.49**	**38.74**	**45.70**	4.33	**14.38**	**32.75**
% of body weight (2)	-	**-31.89**	**-23.13**	-13.64	-	**-28.39**	**-21.49**	-8.89
% of brain weight (1)	**49.97**	6.03	**18.35**	**35.23**	**47.79**	5.49	**20.33**	**45.86**
% of brain weight(2)	-	**-29.30**	-21.08	-9.83	-	**-28.62**	**-18.58**	-1.30
Ovary[a]								
Absolute value (1)					12.08	10.26	-13.78	-17.21
Absolute value (2)					-	-1.63	**-23.08**	**-26.14**
% of body weight (1)					11.11	7.39	-17.44	**-25.20**
% of body weight (2)					-	-3.35	**-25.70**	**-32.68**
% of brain weight (1)					13.07	7.81	-14.07	-18.33
% of brain weight (2)					-	-4.65	**-24.00**	**-27.77**

[a] All values expressed as percent difference of saline control (1) or vehicle control (2) group means. Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Source: Applicant's Table, SSN-01272, p.42.

Table 88: Organ weight findings after the recovery period in the 28-day rat repeat dose study

	Males			Females		
Group	**2**	**4**	**5**	**2**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**30**	**60**	**0**	**30**	**60**
Captisol Dose (mg/kg/day)	**3000**	**1500**	**3000**	**3000**	**1500**	**3000**
No. Animals per Group	**6**	**5**	**6**	**6**	**6**	**6**
Kidney[a]						
Absolute value (1)	7.99	12.31	12.59	**19.34**	7.00	**26.54**
Absolute value (2)	-	4.00	4.26	-	-10.34	6.04
% of body weight (1)	**15.03**	9.75	**15.98**	**28.89**	10.00	**24.62**
% of body weight (2)	-	-4.59	0.83	-	**-14.66**	-3.32
% of brain weight (1)	6.28	8.42	13.17	16.21	7.07	**23.06**
% of brain weight (2)	-	2.01	6.48	-	-7.86	5.89

[a] All values expressed as percent difference of saline control (1) or vehicle control (2) group means. Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Source: Applicant's Table, SSN-01272, p.43.

Histopathology
Peer Review: Yes

Histological Findings:

Table 89: SBECD-related histopathologic findings in the 28-day rat repeat dose study

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**10**	**30**	**60**	**0**	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**0**	**3000**	**500**	**1500**	**3000**	**0**	**3000**	**500**	**1500**	**3000**
No. Animals Examined[b]	**10**	**10**	**6**	**7**	**6**	**10**	**9**	**4**	**8**	**8**
Kidney (No. Examined)	10	10	6	7	6	10	9	4	8	8
Vacuolation, tubular	(0)[a]	(10)	(6)	(7)	(6)	(0)	(9)	(4)	(8)	(8)
Minimal	0	0	2	0	0	0	0	3	0	0
Mild	0	0	4	7	0	0	0	1	8	0
Moderate	0	9	0	0	6	0	9	0	0	8
Marked	0	1	0	0	0	0	0	0	0	0
Urinary Bladder (No. Examined)	10	10	0	2	6	10	9	2	1	8
Vacuolation; urothelial	(0)	(7)	0	(1)	(5)	(0)	(9)	(0)	(1)	(8)
Minimal	0	7	0	1	4	0	9	0	1	8
Mild	0	0	0	0	1	0	0	0	0	0
Infusion Site (No. Examined)	10	10	1	5	6	10	9	2	4	8
Vacuolation; macrophage	(0)	(5)	(1)	(3)	(5)	(0)	(7)	(1)	(4)	(5)
Minimal	0	2	0	2	2	0	3	0	1	3
Mild	0	3	1	1	3	0	4	1	3	2
Bone Femur (No. Examined)	10	10	0	0	6	10	9	0	0	8
Vacuolation; synovial	(0)	(10)	0	0	(6)	(0)	(9)	0	0	(8)
Minimal	0	5	0	0	3	0	9	0	0	8
Mild	0	5	0	0	3	0	0	0	0	0
Lymph node, Mandibular (No. Examined)	10	10	2	0	6	10	9	0	2	8
Vacuolation; macrophage	(0)	(10)	0	0	(6)	(0)	(7)	(0)	0	(8)
Minimal	0	4	0	0	2	0	4	0	0	3
Mild	0	6	0	0	4	0	3	0	0	5
Lymph node, Mesenteric (No. Examined)	10	10	0	0	6	10	9	0	0	8
Vacuolation; macrophage	(0)	(6)	0	0	(5)	(0)	(5)	(0)	0	(6)
Minimal	0	4	0	0	4	0	5	0	0	6
Mild	0	2	0	0	1	0	0	0	0	0
Uterus (No. Examined)						10	9	0	0	8
Vacuolation; interstitial						(0)	(7)	0	0	(7)
Minimal						0	7	0	0	7
Heart (No. Examined)	10	10	0	0	6	10	9	0	0	8
Vacuolation; interstitial	(0)	(6)	0	0	(2)	(0)	(2)	0	0	(2)
Minimal	0	6	0	0	1	0	2	0	0	2
Mild	0	0	0	0	1	0	0	0	0	0

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Numbers of animals/tissues examined in the low and mid dose group represent the number of animals with gross abnormalities.

Source: Applicant's Table, SSN-01272, p.45.

Table 90: SBECD-related histopathologic findings after the recovery period in the 28-day rat repeat dose study

Group	1	2	4	5	1	2	4	5
SAGE-547 Dose (mg/kg/day)	0	0	30	60	0	0	30	60
Captisol Dose (mg/kg/day)	0	3000	1500	3000	0	3000	1500	3000
No. Animals Examined[b]	6	6	1	6	6	6	2	6
Kidney (No. Examined)	6	6	1	6	6	6	0	6
Vacuolation, tubular	(0)[a]	(6)	(1)	(6)	(0)	(6)	(0)	(6)
Mild	0	6	1	6	0	6	0	6
Urinary Bladder (No. Examined)	6	6	0	6	6	6	0	6
Vacuolation; urothelial	(0)	(5)	(0)	(6)	(0)	(6)	(0)	(5)
Minimal	0	5	0	6	0	6	0	5
Infusion Site (No. Examined)	6	6	0	6	6	6	2	6
Vacuolation; macrophage	(0)	(3)	(0)	(2)	(0)	(1)	(1)	(4)
Minimal	0	2	0	0	0	0	1	3
Mild	0	1	0	2	0	1	0	1
Bone Femur (No. Examined)	6	6	0	6	6	6	0	6
Vacuolation; synovial	(0)	(4)	(0)	(2)	(0)	(3)	(0)	(2)
Minimal	0	4	0	2	0	3	0	2
Lymph node, Mandibular (No. Examined)	6	6	0	6	6	6	0	6
Vacuolation; macrophage	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Minimal	0	0	0	1	0	0	0	0
Lymph node, Mesenteric (No. Examined)	6	6	0	6	6	6	0	6
Vacuolation; macrophage	(0)	(2)	(0)	(2)	(0)	(0)	(0)	(0)
Minimal	0	2	0	2	0	0	0	0
Uterus (No. Examined)					6	6	0	6
Vacuolation; interstitial					(0)	(0)	(0)	(3)
Minimal					0	0	0	3

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Numbers of animals/tissues examined in mid dose group represent the number of animals with gross abnormalities.

Source: Applicant's Table, SSN-01272, pp. 48-49.

Other findings noted across all groups, including saline control, were related to the continuous infusion and included neutrophilic inflammation, bacteria, thrombosis, and inflammation (Table 90). The septic phlebitis also resulted in secondary changes observed throughout the rat. The findings related to the continuous infusion are not a concern clinically due to the much shorter duration of the clinical infusion (2.5 days vs. 28 days). After the recovery period, the incidence and severity were decreased for the infusion site changes (Table 91).

Table 91: Infusion site histopathologic findings in the 28-day rat repeat dose study

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**10**	**30**	**60**	**0**	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**0**	**3000**	**500**	**1500**	**3000**	**0**	**3000**	**500**	**1500**	**3000**
No. Animals Examined[b]	**10**	**10**	**1**	**5**	**6**	**10**	**9**	**2**	**4**	**8**
Infusion site (No. Examined)	10	10	1	5	6	10	9	2	4	8
Inflammation; neutrophilic	(3)[a]	(3)	(1)	(3)	(2)	(1)	(3)	(2)	(4)	(3)
Minimal	0	0	0	0	0	0	0	0	0	1
Mild	0	0	0	0	0	0	0	0	0	1
Moderate	0	1	0	0	0	0	0	2	0	0
Marked	2	0	1	3	0	1	2	0	2	0
Severe	1	2	0	0	2	0	1	0	2	1
Bacteria	(2)	(3)	(1)	(2)	(1)	(0)	(3)	(0)	(3)	(1)
Minimal	1	0	0	0	0	0	0	0	1	0
Mild	1	0	1	2	0	0	2	0	1	1
Moderate	0	2	0	0	1	0	1	0	1	0
Marked	0	1	0	0	0	0	0	0	0	0
Thrombosis acute	(0)	(0)	(0)	(0)	(1)	(3)	(2)	(0)	(0)	(3)
Minimal	0	0	0	0	1	1	0	0	0	2
Mild	0	0	0	0	0	1	1	0	0	1
Moderate	0	0	0	0	0	1	1	0	0	0
Thrombosis chronic	(3)	(6)	(0)	(1)	(2)	(6)	(3)	(0)	(0)	(1)
Minimal	1	4	0	1	1	3	1	0	0	0
Mild	2	2	0	0	1	2	2	0	0	1
Moderate	0	0	0	0	0	1	0	0	0	0
Inflammation, perivascular	(0)	(0)	(0)	(0)	(0)	(3)	(1)	(0)	(0)	(1)
Minimal	0	0	0	0	0	2	1	0	0	1
Mild	0	0	0	0	0	1	0	0	0	0

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Numbers of animals/tissues examined in the low and mid dose group represent the number of animals with gross abnormalities.

Source: Applicant's Table, SSN-01272, p.46.

Table 92: Infusion site histopathologic findings after the recovery period in the 28-day rat repeat dose study

Group	**1**	**2**	**4**	**5**	**1**	**2**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**30**	**60**	**0**	**0**	**30**	**60**
Captisol Dose (mg/kg/day)	**0**	**3000**	**1500**	**3000**	**0**	**3000**	**1500**	**3000**
No. Animals Examined	**6**	**6**	**5**	**6**	**6**	**6**	**6**	**6**
Infusion site (No. Examined)	6	6	0	6	6	6	2	6
Inflammation; neutrophilic	(1)[a]	(1)	(0)	(1)	(1)	(2)	(1)	(2)
Minimal	0	0	0	0	1	0	0	0
Mild	0	0	0	0	0	0	0	0
Moderate	1	0	0	0	0	0	1	1
Marked	0	1	0	1	0	2	1	0
Severe	0	0	0	0	0	0	0	1
Bacteria	(1)	(0)	(0)	(1)	(0)	(1)	(0)	(1)
Minimal	1	0	0	1	0	1	0	1
Thrombosis chronic	(1)	(2)	(0)	(1)	(2)	(2)	(0)	(1)
Minimal	1	2	0	1	1	0	0	0
Mild	0	0	0	0	1	2	0	1

[a] Numbers in parentheses represent the number of animals with the finding.

Source: Applicant's Table, SSN-01272, p.49.

A 28-Day Study of SAGE-547 by Intravenous Infusion in Beagle Dogs with a 28-Day Recovery Period/Study No. SSN-01273

Methods

Dosing administration was interrupted for five dogs during the study for the following reasons:

- Elevated body temperature that did not respond to non-steroidal anti-inflammatories (dogs were maintained on saline infusion until dosing resumed).
  - On Day 11 for No. 2506 (VCF). Dosing resumed on Day 12.
  - On Day 17 for No. 2002 (VCM). Dosing resumed on Day 19.
- Surgical repairs on the infusion catheters (dosing resumed the day following surgery).
  -

Animal No.	Study Day	Reason for Repair
3502	5	Infusion catheter repaired due to a suspected kinked catheter.
	11	Infusion catheter repaired
	24	Surgery was required to change the infusion catheter to left femoral vein.
5504	14	Surgery was required to change the infusion catheter to left femoral vein.
	22	Additional tacking sutures added to catheter placement.
4006	17	Surgery was required to change the infusion catheter to left femoral vein.

  - The dosing was extended for Nos. 4006 (MDM) and 3502 (LDF)so they could receive 28 days of dose administration. The dose administration for No. 5504 (HDF) was not extended due to the clinical condition of the dog.

Observations and Results

Mortality

A VCF (No. 2506) was euthanized on Day 17 due to poor/deteriorating condition. A HDF (No. 5505) was euthanized on Day 26 due to a severely swollen hind limb that precluded continuation of dosing. The cause of the poor clinical condition for No. 2506 and swollen hind limb for No. 5505 was attributed to an inflammatory reaction (associated with bacteria for No. 2506) at the infusion site and considered unrelated to drug or vehicle administration. The infusion site was characterized as having marked mixed cells to predominantly neutrophilic inflammation. The bacteriological culture of the infusion site swab yielded *Pseudomonas aeruginosa*. Hematology and clinical chemistry parameters that had changes prior to termination compared to predose values for Animal Nos. 2506 and 5505 are shown in Table 92. Microscopic findings noted in multiple organs (kidney, heart, lung, skeletal muscle, subcutaneous tissue, bone marrow, and spleen) of one or both dogs were considered secondary to the inflammatory reaction at the infusion site.

Table 93: Changed hematology and clinical chemistry parameters for Animal Nos. 2506 and 5505 prior to termination compared to predosing values in 28-day dog repeat dose study

Parameter	No. 2506	No. 5505
Red blood cells	↓16%	↓21%

Parameter	No. 2506	No. 5505
Hematocrit	↓19%	↓27%
Hemoglobin	↓22%	↓28%
Platelet counts	↓81%	↓74%
Fibrinogen	↑41%	↑171%
Leukocytes		↑119%
Neutrophils		↑149%
Monocytes		↑270%
Large unstained cells		↑550%
Alkaline phosphatase	↑696%	↑740%
Total bilirubin	↑71%	↑167%
Triglycerides		↑844%
Globulin		↑56%
Cholesterol		↑57%
Creatine kinase		↓44%
Glucose	↓32%	↓26%
Albumin	↓41%	↓51%
A/G ratio	↓60%	↓68%
Calcium	↓19%	↓26%
Phosphorus	↓25%	↓14%
Potassium	↓4%	↓25%

Clinical Signs
The incidence of clinical signs, except increased body temperature, is listed in Table 93. Test article-related clinical signs were limited to a MDM (No. 4006) which had a non-sustained convulsion 4 days after the end of dose administration on Day 35. Because of a dosing holiday, this dog received an additional two days of dose administration to complete 28 days of dosing and the last taper dose ended on Day 31. The Applicant could not determine the toxicological significance of the finding because it was "…noted in a single mid dose animal during the recovery period and well after the termination of dose administration."

Vehicle-related clinical signs included abnormal gait, decreased activity, lying on side, pale skin, weak, tremors, hunched posture, increased body temperature (noted in animals being followed by the veterinary group), reduced appetite, and limited usage of hindlimb/forelimb which generally occurred dose-dependently. These signs, except increased body temperature, generally occurred during the first week of dosing, were transient, and were not observed by the end of the 28-day recovery period. Increased body temperature that required Carprofen® treatment was observed from one to three times in 5 VCMs (Nos. 2001, 2002, 2003, 2005, 2006), 1 MDM (No. 4006), 2 HDM (Nos. 5001 and 5004), 2 VCFs (Nos. 2503 and 2506), and 1 HDF (No. 5504).

Table 94: Incidence of clinical signs in 28-day dog repeat dose study

Clinical Observation	Group 1 (Saline Control) M/F	Group 2 (0 mg/kg/day SAGE-547; 3600 mg/kg/day Captisol) M/F	Group 3 (12 mg/kg/day SAGE-547; 600 mg/kg/day Captisol) M/F	Group 4 (36 mg/kg/day SAGE-547; 1800 mg/kg/day Captisol) M/F	Group 5 (72 mg/kg/day SAGE-547; 3600 mg/kg/day Captisol) M/F
Abnormal Gait	0/0	2/0	1/0	2/0	2/1
Decreased Activity	0/0	5/3	1/3	3/0	4/3
Convulsion Non-Sustained	0/0	0/0	0/0	1/0	0/0
Tremors	0/0	1/0	0/0	0/0	1/0
Weak	0/0	0/0	0/0	0/0	1/0
Pale Skin	0/0	1/0	0/0	1/0	1/1
Hunched Posture	0/0	1/1	0/0	0/0	2/0
Limited Usage of limb(s) (hindlimb/forelimb)	0/0	4/1	1/1	1/0	2/0
Lying on Side	0/0	3/0	0/0	1/0	0/0
Reduced Appetite	2/1	3/3	1/2	2/3	3/4

Incidence= number of animals.
Source: Applicant's Table, SSN-01273, p.36.

Hematology

Increases in white blood cells, neutrophils, and monocytes are consistent with inflammation. Increases in fibrinogen were observed in VC (54% M, 80% F), LD (41% M, 37% F), MD (79% M, 38% F), and HD (93% M, 118% F) vs SC.

Table 95: SBECD-related hematology changes in 28-day dog repeat dose study

Hematology Parameters	Group 1 (Saline Control)		Group 2 (0 mg/kg/day SAGE-547;3600 mg/kg/day Captisol)		Group 3 (12 mg/kg/day SAGE-547; 600 mg/kg/day Captisol)		Group 4 (36 mg/kg/day SAGE-547; 1800 mg/kg/day Captisol)		Group 5 (72 mg/kg/day SAGE-547; 3600 mg/kg/day Captisol)		Historical Range	
	M	F	M	F	M	F	M	F	M	F	M	F
Red blood cell count (10^3/µL)	-	-	5.47 ↓14.60%	5.44 ↓12.11%	-	-	5.63 ↓12.05%	5.85 ↓5.50%	5.60 ↓12.62%	5.26 ↓14.89%	5.53-7.86	5.33-7.78
Platelet Count (10^3/µL)	-	-	96 ↓56.0%	111 ↓55.0%	-	-	98 ↓55.0%	120 ↓52.0%	54 ↓75.0%	144 ↓42.0%	181-427	220-484
Hemoglobin (g/dL)	-	-	12.2 ↓18.3%	12.5 ↓11.2%	-	-	13 ↓12.7%	13.4 ↓5.1%	12.5 ↓16.3%	11.8 ↓16.6%	12.8-18.1	12.3-17.6
Hematocrit %	-	-	36.6 ↓17.0%	36.8 ↓12.4%	-	-	38.3 ↓12.4%	39.0 ↓7.1%	36.6 ↓16.2%	34.3 ↓18.3%	35.8-52.4	34.5-52.3
White blood cell count (10^3/µL)	-	-	15.03 ↑57.40%	12.95 ↑30.76%	13.04 ↑36.59%	12.17 ↑22.85%	13.8 ↑44.57%	12.17 ↑22.80%	12.69 ↑32.94%	14.84 ↑49.84%	6.01-14.08	6.12-14.36
Neutrophil Count (10^3/µL)	-	-	10.68 ↑86.85%	8.89 ↑41.51%	9.40 ↑64.48%	8.70 ↑38.39%	10.30 ↑80.09%	8.40 ↑33.70%	9.19 ↑60.76%	11.10 ↑76.64%	3.18-10.14	3.43-9.9
Monocyte count (10^3/µL)	-	-	1.09 ↑128.42%	0.81 ↑42.52%	-	-	-	-	-	0.81 ↑41.82%	0.23-0.9	0.21-0.82

Bold = statistically significant. F=Female; M=Male; -= no effects.
% = % change from control value.
Source: Applicant's Table, SSN-01273, p.38.

Clinical Chemistry

Table 96: SBECD-related clinical chemistry changes in 28-day dog repeat dose study

Clinical Chemistry Parameters (mean values)	Group 1 (Saline Control)		Group 2 (0 mg/kg/day SAGE-547; 3600 mg/kg/day Captisol)		Group 3 (12 mg/kg/day SAGE-547; 600 mg/kg/day Captisol)		Group 4 (36 mg/kg/day SAGE-547; 1800 mg/kg/day Captisol)		Group 5 (72 mg/kg/day SAGE-547; 3600 mg/kg/day Captisol)		Historical Range	
SEX	M	F	M	F	M	F	M	F	M	F	M	F
Alkaline Phosphatase U/L	-	-	111 ↑42%	114 ↑15%	-	-	-	-	194 ↑148%	-	24-105	23-103
Creatine Kinase U/L	-	-	124 ↓36%	-	-	-	127 ↓35%	133 ↓24%	120 ↓38%	120 ↓32%	99-296	99-352
Albumin g/dL	-	-	2.7 ↓21.8%	2.9 ↓15.6%	-	-	-	-	2.5 ↓26.7%	2.7 ↓21.9%	3.1-3.9	3.1-4
A/G ratio	-	-	1.0 ↓31.9%	1.3 ↓25.9%	-	1.2 (-29.4%)	-	-	0.9 ↓39.6%	1.1 ↓36.5%	1.17-2	1.24-2.18
Calcium mg/dL	-	-	10.1 ↓7.3%	10.3 ↓5.2%	10.3 ↓5.5%	10.4 ↓4.3%	10.2 ↓6.6%	10.5 ↓3.2%	9.9 ↓8.9%	10.2 ↓6.1%	10.1-11.8	10-11.7

Bold = statistically significant. F = female; M = male. - = no effects
% = % change from control value.
Source: Applicant's Table, SSN-01273, p.39.

Gross Pathology

Pale discoloration of the kidney was noted in dogs from all vehicle dosed groups and enlargement of kidneys was noted in most VCM and 1 HDM, VCF, and LDF and correlated microscopically with tubular vacuolation. The findings in the kidney were not observed at the end of the recovery period. Enlargement of the iliac and/or mediastinal lymph nodes was noted in all vehicle dose groups and correlated with macrophage vacuolation observed microscopically. Enlargement of the lymph nodes was still observed after the recovery period.

Table 97: SBECD-related gross pathology findings in the 28-day dog repeat dose study

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE 547- Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Kidney (No. Examined)	4	4	4	4	4	4	3	4	4	3
Discoloration pale	0	2	1	0	2	0	3	1	2	0
Enlargement	0	3	0	0	1	0	1	1	0	0
Lymph node (No. Examined)	3	3	3	3	4	2	2	3	3	3
Enlargement[a]	0	2	1	1	4	0	1	1	0	2

[a] Vehicle (Captisol)-related gross finding with microscopic correlation of macrophage vacuolation.
Source: Applicant's Table, SSN-01273, p.41.

Table 98: SBECD-related gross pathology findings after the recovery period in the 28-day dog repeat dose study

	Males				Females			
Group	**1**	**2**	**4**	**5**	**1**	**2**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**36**	**72**	**0**	**0**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**1800**	**3600**	**0**	**3600**	**1800**	**3600**
No. Animals Examined	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Lymph node (No. Examined)	2	2	2	2	2	1	2	2
Enlargement[a]	0	2	1	1	0	1	0	1

[a] Vehicle (Captisol)-related gross finding with microscopic correlation of macrophage vacuolation.

Source: Applicant's Table, SSN-01273, p.42.

Findings at the infusion site were observed regardless of vehicle or dose group and correlated microscopically with inflammation and/or thrombosis at the infusion/surgical site. Procedure related findings at the infusion site partially recovered following the recovery period.

Table 99: Infusion site gross pathology findings in the 28-day dog repeat dose study

	Males					Females				
Group	**1**	**2**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Site infusion (No. Examined)	4	4	4	4	4	4	3	4	4	3
Thick	1	4	1	1	2	0	1	0	1	0
Mass	0	0	1	0	0	0	0	1	0	0
Abnormal consistency; firm	0	0	0	1	0	0	0	0	0	0
Swelling	1	1	1	0	0	0	1	0	0	0

Source: Applicant's Table, SSN-01273, p.42.

Table 100: Infusion site gross pathology findings after the recovery period in the 28-day dog repeat dose study

	Males				Females			
Group	**1**	**2**	**4**	**5**	**1**	**2**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**36**	**72**	**0**	**0**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**1800**	**3600**	**0**	**3600**	**1800**	**3600**
No. Animals Examined	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Site infusion (No. Examined)	2	2	2	2	2	2	2	2
Thick	0	0	1	2	0	1	0	0
Mass	1	0	0	0	0	0	0	0
Abnormal consistency; firm	0	0	0	0	1	0	0	2
Swelling	1	0	1	0	0	0	0	0

Source: Applicant's Table, SSN-01273, p.43.

Organ Weights

The increased kidney weights correlated with microscopic findings of tubular vacuolation in vehicle dosed dogs. The increased liver and spleen weights may also correlate with findings of vacuolation in these organs. The increased liver weight for HD males and females compared to VC is most likely unrelated to test article but associated with individual variability and the small

number of dogs. For example, HDF No. 5004 had an increased liver weight due to a moderate chronic passive congestion that was not test article related and VC Nos. 2501 and 2503 had lower individual liver weights.

Table 101: Organ weight findings in the in the 28-day dog repeat dose study

	Males				Females			
Group	**2[b]**	**3**	**4**	**5**	**2[b]**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**12**	**36**	**72**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**3600**	**600**	**1800**	**3600**	**3600**	**600**	**1800**	**3600**
No. Animals per Group	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Kidney (No. Weighed)[a]	4	4	4	4	3	4	4	3
Absolute value (1)	**31.457**	-6.907	3.713	**19.176**	30.742	13.305	17.109	44.416
Absolute value (2)	-	**-29.184**	**-21.105**	-9.342	-	-13.337	-10.428	10.459
% of body weight (1)	**29.999**	-8.741	6.081	15.908	31.157	15.459	10.083	41.677
% of body weight (2)	-	**-29.800**	**-18.399**	-10.839	-	-11.969	-16.067	8.021
% of brain weight (1)	**26.851**	-14.791	-3.449	9.817	31.969	11.689	20.866	44.895
% of brain weight (2)	-	**-32.827**	**-23.886**	13.429	-	-15.368	-8.413	9.795
Liver (No. Weighed)[a]	4	4	4	4	3	4	4	3
Absolute value (1)	26.496	13.579	13.481	64.605	17.210	-5.247	8.061	**43.950**
Absolute value (2)	-	-10.212	-10.289	30.126	-	-19.159	-7.806	22.814
% of body weight (1)	25.029	11.764	16.203	**60.240**	18.916	-2.929	2.045	**43.044**
% of body weight (2)	-	-10.609	-7.059	28.163	-	-18.370	-14.187	20.290
% of brain weight (1)	22.317	3.894	5.313	**52.456**	18.050	-7.313	11.616	**44.646**
% of brain weight (2)	-	-15.061	-13.901	24.640	-	-21.485	-5.451	22.529
Spleen (No. Weighed)[a]	4	4	4	4	3	4	4	3
Absolute value (1)	91.872	36.357	71.664	88.787	**95.330**	22.228	**91.484**	**109.325**
Absolute value (2)	-	-28.933	-10.532	-1.608	-	-37.425	-1.969	7.165
% of body weight (1)	90.954	34.990	76.453	84.593	**98.693**	25.878	**81.678**	**108.487**
% of body weight (2)	-	-29.308	-7.594	-3.331	-	-36.647	-8.563	4.929
% of brain weight (1)	83.716	25.070	57.014	74.704	92.009	17.589	96.132	**107.545**
% of brain weight (2)	-	-31.922	-14.535	-4.905	-	-38.759	2.147	8.091

[a] All values expressed as percent difference of saline (1) or Captisol vehicle (2) control group means.
[b] Group 2 is vehicle (Captisol) control group.
Based upon statistical analysis of group means, values highlighted in bold are significantly different from control group – $P \leq 0.05$; refer to data tables for actual significance levels and tests used.

Source: Applicant's Table, SSN-01273, p.44.

Histopathology
Peer Review: Yes

Histological Findings: Vehicle-related vacuolation was observed multiple organs.

Table 102: SBECD-related histopathologic findings in 28-day dog repeat dose study

	Males					Females				
Group	**1**	**2[b]**	**3**	**4**	**5**	**1**	**2[b]**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Kidney (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; tubular	(0)[a]	(4)	(2)	(4)	(4)	(0)	(3)	(2)	(4)	(3)
Minimal	0	0	1	1	0	0	1	1	1	0
Mild	0	4	1	1	0	0	2	1	3	0
Moderate	0	0	0	2	4	0	0	0	0	3
Vacuolation; urothelial; pelvis	(0)	(4)	(1)	(4)	(4)	(0)	(3)	(2)	(4)	(3)
Minimal	0	1	1	2	0	0	3	2	2	1
Mild	0	3	0	2	4	0	0	0	2	2
Urinary bladder (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; urothelial	(0)	(4)	(4)	(4)	(4)	(0)	(3)	(4)	(4)	(3)
Minimal	0	3	4	4	3	0	1	4	4	3
Mild	0	1	0	0	1	0	2	0	0	0
Vacuolation, macrophage; submucosal	(0)	(4)	(0)	(3)	(4)	(0)	(3)	(0)	(1)	(3)
Minimal	0	4	0	3	4	0	3	0	1	3
Bone, Femur (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; synovial	(0)	(4)	(2)	(4)	(4)	(0)	(3)	(0)	(4)	(3)
Minimal	0	0	2	4	0	0	0	0	4	0
Mild	0	3	0	0	4	0	2	0	0	3
Moderate	0	1	0	0	0	0	1	0	0	0
Vacuolation, macrophage; synovial	(0)	(4)	(0)	(3)	(4)	(0)	(3)	(0)	(1)	(3)
Minimal	0	1	0	3	0	0	0	0	1	0
Mild	0	2	0	0	3	0	2	0	0	3
Moderate	0	1	0	0	1	0	1	0	0	0
Brain (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage; interstitial; plexus choroid	(0)	(4)	(4)	(4)	(4)	(0)	(3)	(2)	(4)	(2)
Minimal	0	4	4	4	3	0	3	2	4	2
Mild	0	0	0	0	1	0	0	0	0	0
Ovary (No. Examined)	-	-	-	-	-	4	3	4	4	3
Vacuolation, macrophage; interstitial	-	-	-	-	-	(0)	(3)	(0)	(1)	(3)
Minimal	-	-	-	-	-	0	3	0	1	3
Cervix (No. Examined)	-	-	-	-	-	4	3	4	4	3
Vacuolation, macrophage; interstitial	-	-	-	-	-	(0)	(3)	(0)	(2)	(3)
Minimal	-	-	-	-	-	0	3	0	2	3
Uterus (No. Examined)	-	-	-	-	-	4	3	4	4	3
Vacuolation, macrophage; interstitial	-	-	-	-	-	(0)	(3)	(0)	(2)	(3)
Minimal	-	-	-	-	-	0	3	0	2	3
Vagina (No. Examined)	-	-	-	-	-	4	3	4	4	3
Vacuolation, macrophage; interstitial	-	-	-	-	-	(0)	(2)	(0)	(1)	(2)
Minimal	-	-	-	-	-	0	2	0	1	2
Epididymis (No. Examined)	4	4	4	4	4	-	-	-	-	-
Vacuolation; tubular	(0)	(3)	(4)	(4)	(4)	-	-	-	-	-
Minimal	0	0	1	0	0	-	-	-	-	-
Mild	0	3	3	4	3	-	-	-	-	-
Moderate	0	0	0	0	1	-	-	-	-	-

	Males					Females				
Group	**1**	**2[b]**	**3**	**4**	**5**	**1**	**2[b]**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
GALT (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage	(0)	(4)	(0)	(2)	(4)	(0)	(3)	(1)	(4)	(3)
Minimal	0	4	0	2	2	0	3	1	4	3
Mild	0	0	0	0	2	0	0	0	0	0
Gland, adrenal (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; cortical	(0)	(3)	(0)	(0)	(3)	(1)	(1)	(0)	(0)	(3)
Minimal	0	3	0	0	3	1	1	0	0	3
Gland, pituitary (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage; interstitial	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)	(1)
Minimal	0	0	0	0	0	0	1	0	0	1
Heart (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; interstitial	(0)	(2)	(0)	(1)	(1)	(0)	(1)	(0)	(1)	(1)
Minimal	0	2	0	1	1	0	1	0	1	1
Liver (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; Kupffer cell	(0)	(4)	(0)	(2)	(3)	(0)	(3)	(0)	(1)	(2)
Minimal	0	0	0	2	0	0	0	0	1	0
Mild	0	1	0	0	3	0	3	0	0	2
Moderate	0	3	0	0	0	0	0	0	0	0
Lung (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage; alveolar	(0)	(4)	(1)	(1)	(3)	(0)	(1)	(0)	(2)	(1)
Minimal	0	3	1	1	2	0	1	0	2	0
Mild	0	1	0	0	1	0	0	0	0	1
Spleen (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage; alveolar	(0)	(4)	(0)	(4)	(4)	(0)	(3)	(0)	(3)	(3)
Minimal	0	4	0	4	3	0	3	0	3	3
Mild	0	0	0	0	1	0	0	0	0	0
Stomach (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation, macrophage; submucosal	(0)	(4)	(0)	(1)	(4)	(0)	(3)	(0)	(3)	(3)
Minimal	0	3	0	1	2	0	3	0	3	3
Mild	0	1	0	0	2	0	0	0	0	0
Site, infusion (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; macrophage	(0)	(4)	(1)	(1)	(3)	(0)	(2)	(1)	(1)	(2)
Minimal	0	1	0	0	1	0	1	0	0	1
Mild	0	2	1	1	0	0	1	1	1	1
Moderate	0	1	0	0	2	0	0	0	0	0
Site, surgical (No. Examined)	2	1	1	0	2	0	0	1	0	0
Vacuolation; macrophage	(0)	(1)	(0)	(0)	(2)	(0)	(0)	(1)	(0)	(0)
Mild	0	0	0	0	1	0	0	1	0	0
Moderate	0	1	0	0	1	0	0	0	0	0
Lymph node, hepatic (No. Examined)	0	0	0	0	1	0	1	0	0	0
Vacuolation; macrophage	(0)	(0)	(0)	(0)	(1)	(0)	(1)	(0)	(0)	(0)
Mild	0	0	0	0	0	0	1	0	0	0
Moderate	0	0	0	0	1	0	0	0	0	0
Lymph node, iliac (No. Examined)	2	3	2	1	4	2	2	3	1	2
Vacuolation; macrophage	(0)	(3)	(1)	(1)	(4)	(0)	(2)	(1)	(1)	(2)
Minimal	0	0	0	0	0	0	0	1	0	0
Mild	0	3	1	0	0	0	0	0	0	0
Moderate	0	0	0	1	4	0	2	0	1	2

	Males					Females				
Group	**1**	**2[b]**	**3**	**4**	**5**	**1**	**2[b]**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Lymph node, mandibular (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; macrophage	(0)	(4)	(1)	(3)	(4)	(0)	(3)	(1)	(3)	(3)
Minimal	0	0	1	1	0	0	0	0	2	0
Mild	0	2	0	2	1	0	0	1	1	1
Moderate	0	2	0	0	3	0	3	0	0	2
Lymph node, mediastinal (No. Examined)	2	3	3	2	2	0	2	2	2	1
Vacuolation; macrophage	(0)	(3)	(0)	(1)	(2)	(0)	(2)	(1)	(2)	(1)
Minimal	0	0	0	1	0	0	0	0	2	0
Mild	0	3	0	0	2	0	1	1	0	0
Moderate	0	0	0	0	0	0	1	0	0	1
Lymph node, mesenteric (No. Examined)	4	4	4	4	4	4	3	4	4	3
Vacuolation; macrophage	(0)	(3)	(0)	(0)	(4)	(0)	(3)	(0)	(0)	(3)
Minimal	0	2	0	0	1	0	1	0	0	1
Mild	0	1	0	0	1	0	2	0	0	2
Moderate	0	0	0	0	2	0	0	0	0	0
Lymph node, tracheobronchial (No. Examined)	0	1	1	1	1	0	1	0	0	3
Vacuolation; macrophage	(0)	(1)	(0)	(0)	(1)	(0)	(1)	(0)	(0)	(3)
Minimal	0	1	0	0	0	0	1	0	0	1
Mild	0	0	0	0	1	0	0	0	0	2

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Group 2 is vehicle (Captisol) control group.

Source: Applicant's Table, SSN-01273, pp.46-48.

Table 103: SBECD-related histopathologic findings after the recovery period in the 28-day dog repeat dose study

	Males				Females			
Group	**1**	**2[b]**	**4**	**5**	**1**	**2[b]**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**36**	**72**	**0**	**0**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**1800**	**3600**	**0**	**3600**	**1800**	**3600**
No. Animals Examined	**2**	**2**	**2**	**2**	**2**	**2**	**2**	**2**
Kidney (No. Examined)	2	2	2	2	2	2	2	2
Vacuolation; tubular	(0)[a]	(2)	(1)	(0)	(0)	(0)	(0)	(0)
Minimal	0	2	1	0	0	0	0	0
Vacuolation; urothelial; pelvis	(0)	(2)	(2)	(2)	(0)	(2)	(2)	(2)
Minimal	0	0	1	0	0	1	2	2
Mild	0	2	1	2	0	1	0	0
Vacuolation, macrophage; interstitial	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
Minimal	0	0	0	0	(0)	(1)	(0)	(0)
Lymph node, iliac (No. Examined)	1	2	2	2	2	1	2	2
Vacuolation; macrophage	(0)	(2)	(2)	(2)	(0)	(1)	(2)	(2)
Minimal	0	0	0	1	0	0	0	0
Mild	0	1	2	1	0	1	2	1
Moderate	0	1	0	0	0	0	0	1
Lymph node, inguinal (No. Examined)	0	0	1	0	0	0	0	0
Vacuolation; macrophage	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
Mild	0	0	1	0	0	0	0	0
Lymph node, mandibular (No. Examined)	0	0	1	0	0	0	1	0
Vacuolation; macrophage	(0)	(0)	(1)	(0)	(0)	(0)	(1)	(0)
Mild	0	0	1	0	0	0	1	0
Lymph node, mediastinal (No. Examined)	2	0	1	0	0	1	1	0
Vacuolation; macrophage	(0)	(0)	(1)	(0)	(0)	(1)	(1)	(0)
Mild	0	0	1	0	0	1	1	0
Lymph node, popliteal (No. Examined)	0	0	0	1	0	0	0	0
Vacuolation; macrophage	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)
Mild	0	0	0	1	0	0	0	0
Site, infusion (No. Examined)	1	0	1	2	1	1	0	2
Vacuolation; macrophage	(0)	(0)	(1)	(1)	(0)	(1)	(0)	(2)
Minimal	0	0	1	1	0	0	0	1
Mild	0	0	0	0	0	1	0	1

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Group 2 is vehicle (Captisol) control group.

Table 104: Infusion site histopathologic findings in the 28-day dog repeat dose study

	Males					Females				
Group	**1**	**2[b]**	**3**	**4**	**5**	**1**	**2**	**3**	**4**	**5**
SAGE-547 Dose (mg/kg/day)	**0**	**0**	**12**	**36**	**72**	**0**	**0**	**12**	**36**	**72**
Captisol Dose (mg/kg/day)	**0**	**3600**	**600**	**1800**	**3600**	**0**	**3600**	**600**	**1800**	**3600**
No. Animals Examined	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**4**	**3**
Infusion site (No. Examined)	4	4	4	4	4	4	3	4	4	3
Inflammation, vascular/perivascular	(2)[a]	(4)	(2)	(1)	(2)	(0)	(1)	(1)	(1)	(1)
Minimal	1	0	0	0	0	0	0	0	0	0
Mild	0	1	1	0	1	0	0	0	0	0
Moderate	1	3	1	1	1	0	1	0	1	1
Marked	0	0	0	0	0	0	0	1	0	0
Thrombosis	(2)	(0)	(2)	(3)	(1)	(3)	(1)	(1)	(4)	(0)
Mild	1	0	0	2	1	3	0	0	3	0
Moderate	0	0	0	1	0	0	1	1	0	0
Marked	1	0	2	0	0	0	0	0	1	0

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Group 2 is vehicle (Captisol) control group.
Source: Applicant's Table, SSN-01273, p.49.

A Fertility and Early Embryonic Development to Implantation Study with SAGE-547 by Intravenous Infusion in Male Rats/Study No. SSN-01274

Observations and Results

Mortality

Table 105: Summary of unscheduled deaths in fertility and early embryonic development study in male rats

Animal No.	Dose Level (mg/kg/day)	Study Day	Status[a]	Clinical Signs Noted Prior to Death/Euthanasia	Noteworthy Microscopic Observations at IS[b]	Cause of Death
1006	Vehicle control	40	UE	Abnormal gait, limited usage of hindpaws and forelimb, decreased activity, dehydration, thin, respiratory rate increased	Moderate inflammation vascular/perivascular	Inflammation at IS
2016	10	15	UE	Abnormal gait, limited usage of right hindpaw, decreased activity, prominent backbone, dehydrated, partly closed eyes, respiratory rate increased	Moderate inflammation vascular/perivascular with bacteria	Inflammation at IS with bacteria
3004	30	21	UE	Decreased activity, dehydration, weak, respiratory rate increased	Minimal inflammation vascular/perivascular	Undetermined

Animal No.	Dose Level (mg/kg/day)	Study Day	Status[a]	Clinical Signs Noted Prior to Death/Euthanasia	Noteworthy Microscopic Observations at IS[b]	Cause of Death
3005	30	22	UE	Decreased activity, prominent backbone, dehydration, ungroomed fur, weak, partly closed eyes, prostrate	Minimal inflammation vascular/perivascular with bacteria	Inflammation at IS with bacteria
3008	30	25	FD	-	Minimal inflammation vascular/perivascular	Undetermined
3019	30	31	UE	Decreased activity, prominent backbone, dehydrated, skin pallor, thin, weak, partly closed eyes, labored breathing, respiratory rate decreased, prostrate	Minimal inflammation vascular/perivascular	Undetermined
3020	30	18	UE	Decreased activity, prominent backbone, cold to touch, muscle tone decreased, ungroomed fur, skin pallor, thin, partly closed eyes	Minimal inflammation vascular/perivascular with bacteria	Inflammation at IS with bacteria
4003	45	38	UE	Decreased activity, prominent backbone, muscle tone decreased, skin pallor, partly closed eyes, labored breathing, prostrate	-	Undetermined
4007	45	29	UE	Decreased activity, prominent backbone, limited usage of hindlimbs, swollen soft hindlimbs, skin blue inguinal, dehydrated, fur ungroomed, weak, labored breathing, respiratory rate increased	Marked inflammation vascular/perivascular with bacteria	Inflammation at IS with bacteria
4019	45	28	UE	Decreased activity, weak, dehydrated, respiratory rate increased, thin	Severe inflammation vascular/perivascular	Inflammation at IS
4020	45	32	UE	Limited usage of hindpaw, abnormal gait, swollen firm hindpaw, dehydrated, weak, partly closed eyes, respiratory rate increased	Marked inflammation vascular/perivascular with bacteria	Inflammation at IS with bacteria
4021	45	33	UE	Decreased activity, limited usage of hindpaw, abnormal gait, swollen firm hindpaw/hindlimb, weak, dehydrated, partly closed eyes, respiratory rate decreased and irregular, prostrate	Moderate inflammation vascular/perivascular	Inflammation at IS

[a] UE = Unscheduled euthanasia; FD = Found dead;
[b] IS = Infusion Site
- Indicate there were no noteworthy microscopic observations.
Source: Applicant's Table, SSN-01274, pp.35-36.

Necropsy

Minimal to mild vacuolation of macrophages in the interstitial tissue of the testis and epididymis were observed in control and HD groups (LD and MD groups were not evaluated microscopically) and is most likely vehicle related; however, because LD and MD groups were not examined microscopically a dose-response cannot be established (Table 105).

Table 106: Summary of SBECD-related microscopic findings

	Males			
Group	1	2	3	4
SAGE-547 Dose (mg/kg/day)	0	10	30	45
Captisol Dose (mg/kg/day)	2250	500	1500	2250
No. Animals Examined	21	21	17	17
Testis (No. Examined)	(10)	(0)	(0)	(14)
Vacuolation; macrophage	(10) [a]	-	-	(14)
Minimal	10	-	-	14
Epididymis (No. Examined)	(10)	(0)	(0)	(14)
Vacuolation; macrophage	(10)	-	-	(14)
Mild	10	-	-	14
Infusion Site (No. Examined) [b]	(10)	(10)	(2)	(9)
Vacuolation; macrophage	(10)	(8)	(2)	(9)
Mild	0	2	0	0
Moderate	0	4	0	4
Marked	10	2	2	5

[a] Numbers in parentheses represent the number of animals with the finding.
[b] Only animals with mass at the infusion site were evaluated histologically.
Source: Applicant's Table, SSN-01274, p.42.

Pale discoloration and enlargement of the kidney were observed in the control and HD group, but not in the LD or MD group. Because controls and HD group received the highest dose of SBECD (2250 mg/kg/day compared to 500 and 1500 mg/kg/day for LD and MD), the findings in the kidney are most likely vehicle related. There were no histopathological findings in the kidney.

Infusion site findings: Macroscopic findings at the infusion site (for example, mass, swelling, thick) were observed in all groups with similar incidence and correlated microscopically with vascular/perivascular inflammation. The lesion was characterized by accumulation of neutrophils admixed with fibrin and/or necrotic cellular debris in the center of the vein. The wall of the vein and surrounding tissues were effaced/infiltrated by various amounts of macrophages with variable number of lymphocytes and plasma cells. Bacteria were noted within the cellular debris/center of the lesion and there was moderate thrombosis in several rats. Mild to marked macrophage vacuolation infiltrating the tissue within and around the vascular/perivascular inflammation were observed in most rats with masses at the infusion site (Table 105). Findings at the infusion site could be vehicle related or related to the continuous infusion; however, because there was no saline control to compare to the vehicle group it is unclear from this study. Nevertheless, results from the 28-day rat general toxicology study suggests that the inflammation and masses at the infusion site are procedure related, while the vacuolation is vehicle related.

Study of Fertility and Early Embryonic Development to Implantation of SAGE-547 by Intravenous Infusion in Female Rats/Study No. SSN-01271

Necropsy
Macroscopic findings were limited to findings at the infusion site (caudal vena cava), which were generally of low incidence, present in all groups including the controls (without a difference in incidence across groups), and were thought to be related to the experimental

procedure and/or vehicle. Enlarged iliac lymph nodes were observed in 1/22 controls, 1/22 MD, and 4/22 HD and correlated with macroscopic findings at the infusion site; therefore, they were considered secondary.

Offspring
The overall incidence of litters and fetuses with malformations in all dose groups were within the laboratories historical control range (affected litters 0 – 14%; affected fetuses 0 – 1%). There were no external variants noted. The overall incidence of litters and fetuses with visceral variants were within the laboratories historical control range (affected litters 0 – 33%; affected fetuses 0 – 4%). The overall incidence of litters and fetuses with skeletal variants were within the laboratories historical control range for test article-dosed groups (affected litters 46 – 100%; affected fetuses 9 – 58%); however, the overall incidence of fetuses with skeletal variants was slightly above the historical control range for the control group. This was due to a high number of fetuses with incomplete ossification of the parietal and interparietal bones in the skull.

Table 107: Offspring data from rat embryo-fetal development study

Parameter	0 mg/kg	15 mg/kg	30 mg/kg	60 mg/kg
Total number of litters examined	21	22	21	21
Number of fetuses examined	280	277	268	285
Fetuses with malformations (litters)	2(1) 0.7%(4.8%)	1(1) 0.4%(4.5%)	1(1) 0.4%(4.8%)	3(2) 1.1%(9.5%)
Fetuses with visceral variants (litters)	3(2) 2%(9.5%)	3(3) 2.2%(14%)	2(2) 1.5%(9.5%)	1(1) 0.7%(4.8%)
Fetuses with skeletal variants (litters)	85(19) 61%(90%)	48(17) 35%(77%)	52(18) 39%(86%)	61(18) 43%(86%)

A Dosage Range-Finding Embryo-fetal Development Study of SAGE-547 by Continuous Infusion in Rabbits/Study No. SSN-755

A preliminary dose range finding study for female New Zealand White rabbits was conducted in two phases: 1) toxicity phase (n= 3/group) and 2) pregnancy phase (n = 6/group). For the toxicity phase, non-pregnant rabbits were dosed with 0, 15, 30, and 60 mg/kg/day by continuous IV infusion for 14 days. Because of severe clinical signs, the dose was lowered from 60 mg/kg to 45 mg/kg on Day 3 and the group was terminated on Day 4. Clinical signs began ~30 minutes after dosing began and included decreased activity, partly closed eyes, moderate to severe uncoordinated gait, repetitive behavior (head and eye movements from left to right), limited usage of hind limbs, tremors and/or lying on side. The rabbits were unresponsive and unable to eat or drink. When dosing was stopped, the rabbits returned to normal; however, the rabbits exhibited the same clinical signs ~2 hours after dosing started with 45 mg/kg. The 45 mg/kg was considered above an MTD. Severe uncoordinated gait was noted at 30 mg/kg/day during the first 2 days of dosing and decreased activity was noted at 15 and 30 mg/kg from Day 1 to Day 8. At 15 mg/kg/day one rabbit (No. 2602) showed respiratory changes on Day 7, and a second rabbit (No. 2503) had decreased activity, weakness, respiratory changes and prostration

beginning on Day 6 and was euthanized on Day 8. There was no effect on body weight or food consumption at ≤30 mg/kg.

For the pregnancy phase, pregnant rabbits were dosed with 0, 15, 30, 45, and 7.5 mg/kg/day by continuous IV infusion from gestational day (GD) 7 – 19 (infusion pump stopped on morning of GD20). Because of severe clinical signs (severe pharmacologic effects), the 45 mg/kg dose group was terminated on PND8. Clinical signs included decreased activity, partly closed eyes, uncoordinated gait, decreased muscle tone, tremors, lying on side, chewing action, repetitive behavior (head and eye movements from left to right), decreased respiratory rate, and for one animal, irregular respiratory rate. The rabbits were unresponsive and unable to eat or drink. Dose-dependent decreased activity was observed at ≥7.5 mg/kg. A convulsion occurred in one 30 mg/kg dosed female ~4 hours after dosing stopped on GD 29 while the doe was being manipulated for TK sampling. Body weight gain and food consumption were not affected during dose administration; however, decreased body weight gain (↓~6%) and food consumption (↓93% at 30 mg/kg) compared to controls were observed at ≥7.5 mg/kg during the post dose period (GD 20 – 29). There was a slight increase in the number of late resorptions and post-implantation loss at 30 mg/kg. Malformations were observed in 1 fetus (1 litter) at 0 mg/kg, 2 fetuses (2 litters) at 15 mg/kg, and 4 fetuses (2 litters) at 30 mg/kg (Table 107). TK parameters are shown in Table 108. Based on the results of this study, doses of brexanolone at 7.5, 15, and 30 mg/kg/day were selected for the main study reviewed in detail in this section.

Table 108: Summary of malformations observed in pregnant rabbits dosed with brexanolone from gestational day 7 – 19

Malformation	0 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg
Thinning of the median or entire region of the diaphragm	1 (1)			3(1)
dilated ascending aorta, stenosis of the pulmonary trunk or aortic arch, and membranous heart septum defect			1(1)	1(1)
shortened digits of the left hindpaw and polydactyly of the fore- and hindpaws			1(1)	

Table 109: Mean TK parameters in female rabbit on GD7

Dose (mg/kg)	T_{max} (hr)	C_{max} (ng/mL)	$AUC_{(24hr)}$ (ng·hr/mL)	$AUC_{(0-312)}$ (ng·hr/mL)	Css (ng/mL)
7.5	251 ± 136	91 ± 15.0	1920 ± 240	25000 ± 3060	79 ± 10
15	72.0 ± 118	201 ± 18.3	4400 ± 437	57300 ± 5670	182 ± 18
30	166 ± 160	466 ± 50.6	9800 ± 1090	128,000 ± 14200	406 ± 45
45	20.0 ± 6.2	727 ± 195	NC	NC	NC

NC = Not Calculated

Dose (mg/kg)	$C_{(312h)}$ (ng/mL)	$T_{1/2}$ (hr)	CL (mL/hr/kg)	Vd (mL/kg)
7.5	90.0 ± 14.2	8.09 ± 1.88	303 ± 38.5	3490 ± 628
15	177 ± 19	9.80 ± 4.22	260 ± 24	3590 ± 1450
30	409 ± 60.2	6.61 ± 2.17	237 ± 30	2260 ± 856
45	NC	NC	NC	NC

NC = Not Calculated

Source: Applicant's Table, SSN-755, p.32.

A Continuous Intravenous Infusion Embryo-fetal Development Study of SAGE-547 in Rabbits/Study No. SSN-825

Observations and Results

Offspring

There were no test article-related malformations (Table 109 and Table 110). The overall incidence of litters and fetuses with malformations in all dose groups were within the laboratories historical control range (affected litters 0 – 27%; affected fetuses 0 – 6.1%). There was no test article-related effect on external or visceral variants. The overall incidence of litters and fetuses with external and visceral variants were within the laboratories historical control range (affected litters 0 – 56%; affected fetuses 0 – 12.3%). The overall incidence of litters and fetuses with skeletal variants were within the laboratories historical control range (affected litters 18 – 100%; affected fetuses 2 – 62%).

Table 110: Offspring data from rabbit embryo-fetal development study

Parameter	0 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg
Total number of litters examined	22	20	16	12
Number of fetuses examined	196	156	126	75
Fetuses with malformations (litters)	0	4(4) 2.6%(20%)	1(1) 0.8%(6.3%)	0
Fetuses with external and visceral variants (litters)	4(4) 2%(18%)	5(4) 3.2%(20%)	1(1) 0.8%(6.3%)	2(2) 2.7%(17%)
Fetuses with skeletal variants (litters)	44(18) 22%(81%)	45(19) 29%(95%)	53(14)* 42%(88%)	25(10) 33%(83%)

* $p \leq 0.001$

Table 111: Summary of malformations observed in rabbit embryo-fetal development study

Malformation	0 mg/kg	7.5 mg/kg	15 mg/kg	30 mg/kg
truncus arteriosus and an absent interventricular septum			1(1)	
hydrocephaly		1(1)		
aortic arch dilation, stenosis of the pulmonary trunk and membranous ventricular septum defect		1(1)		
gastroschisis and herniated abdominal muscles		1(1)		
omphalocele		1(1)		

A Continuous Intravenous Infusion Pre and Postnatal Study of SAGE-547 in the Rat/Study No. SSN-01263

Observations and Results

F_0 Dams

Mortality: Dams that were found dead or were preterminally euthanized with undetermined cause of death were all pregnant (Table 111). Infusion site masses could be a contributing factor to the death; however, similar or larger infusion site masses were present in rats that survived to terminal euthanasia. Litters from dams that were found dead during the lactation period were euthanized.

Table 112: F_0 Dams with undetermined cause of death in rat pre- and postnatal development study

Dose	No.	Day of Death		Clinical Signs
0 mg/kg	158	GD 21	Found dead	
	166	GD 22	Found dead	
	151	PND 16	Found dead	
	156	PND 20	Unscheduled	Decreased activity, dehydrated, prominent backbone, hunched posture, thin, weak, labored breathing, eyes partly closed, pallor skin, fur erect
30 mg/kg	355	GD 20	Found dead	
60 mg/kg	451	GD 13	Unscheduled	Abnormal gait, decreased activity, prominent backbone, cold to touch, dehydrated, hunched posture, pallor skin, fur erect
	474	PND 0	Unscheduled	Decreased activity, loss of consciousness, cold to touch, lying on side, weak, deep and labored breathing, red liquid and mucoid discharge from vagina and vulva
	462	PND 14	Found dead	

Clinical Signs: There were no test article-related clinical signs. Adverse clinical signs were noted in dams of all groups (including the control) and included decreased activity, prominent backbone, cold to touch, dehydration, hunched posture, labored breathing, thinness and/or skin pallor. Most the dams noted with these clinical signs had a large infusion site mass, which could contribute to the poor condition. These signs have also been observed in other tox studies with rats.

Uterine Content: As mentioned previously; 1 control, 1 LD, and 1 HD were euthanized due to signs of dystocia. All had infusion site masses, which may have contributed to their inability to litter by impeding the birth canal. In addition; 2 control, 4 LD, 3, MD, and 3 HD had no remaining pups on PND 0 or 1; 1 control, 2 MD, and 1 HD dams failed to litter; and 1 control was not pregnant. Because there was no dose-dependency and findings occurred in all groups it is unlikely test article or vehicle related.

Necropsy: Other macroscopic findings noted in all groups, including vehicle, were infusion site mass, spleen enlargement, lymph node enlargement (iliac, renal, mediastinal and/or mandibular lymph nodes) and adrenal gland enlargement (Table 112). However, except for adrenal enlargement, there was no dose vehicle- or test article-dose response for these findings. The infusion site masses were quite large (most masses 25x10x6 mm or greater) and many extended to the kidney, liver, and/or invaded into the underlying skeletal muscle regardless of SBECD dose. Green material consistent with bacterial infection was noted in the infusion site masses of 1 control, 1 LD, and 4 HD. The spleen and lymph node enlargement is consistent with systemic inflammation from the infusion site masses. The adrenal gland enlargement and the small thymus is likely from the stress of the systemic inflammation.

Table 113: Macroscopic findings in F_0 dams in the pre- and postnatal development study

	Females			
Group	**1**	**2**	**3**	**4**
SAGE-547 Dose (mg/kg/day)	**0**	**10**	**30**	**60**
Captisol Dose (mg/kg/day)	**3000**	**500**	**1500**	**3000**
No. Animals Examined	**24 (15/9)[a]**	**24 (19/5)[a]**	**24 (18/6)[a]**	**24 (16/8)[a]**
Infusion site				
Mass	15 (7/8)	15 (12/3)	13 (10/3)	17 (11/6)
Spleen				
Enlargement	7 (2/5)	5 (2/3)	5 (1/4)	7 (2/5)
Lymph node				
Enlargement	11 (2/9)	15 (11/4)	8 (4/4)	13 (5/8)
Adrenal gland				
Enlargement	14 (8/6)	5 (4/1)	6 (5/1)	11 (6/5)
Thymus				
Small	3 (0/3)	1 (1/0)	0 (0/0)	3 (0/3)

[a] Numbers in parentheses are terminal euthanasia animals/early death animals.
Source: Applicant's Table, SSN-01263, p.57.

Toxicokinetics: Test article was not detected in plasma samples of 1 LD (No. 256), 1 MD (No. 356), and 1 HD (No. 456) dam on PND 4 and 1 LD (No. 267) dam on PND 20 in addition to control F_0 dams. Test article was not detected in F_1 pups plasma samples, except for 1 LD litter from dam No. 256 (46.6 ng/mL) and 1 MD litter from dam No. 356 (226 ng/mL) on PND 4. Samples were re-assayed and these results were confirmed. The Applicant states that there is no documentation of a mix-up with the sample tubes at time of blood collection and/or processing; however, it is biologically implausible that dam Nos. 256 and 356, that were bled while on continuous infusion, would have no quantifiable plasma levels, while their pups had measurable levels at the same times, contrary to all other litters on PND 4. In addition, no test article was detected in pup plasma on PND 20. Therefore, the observation of plasma levels in those litters on PND 4 could be an experimental error that could not be explained. This reviewer does not consider this as a major issue to compromise the results obtained from this study.

Table 114: Plasma concentration of brexanolone in F_0 Dams

		Concentration (ng/mL)	
Group	**Animal ID**	**LD4 H2-3**	**LD20 H2-3**
2/ 10 mg/kg/day	256	< LLOQ	41.7
	258	6.92	5.66
	259	29.6	33.7
	262	29.1	32.0
	267	57.9	< LLOQ
	Mean	24.7 (30.9[a])	22.6 (28.2[a])
	S.D.	22.8 (20.9[a])	18.5 (15.6[a])
3/ 30 mg/kg/day	356	< LLOQ	122
	361	126	82.2
	364	87.5	35.8
	366	158	98.5
	371	149	103
	Mean	104 (130[a])	88.3
	S.D.	64.3 (31.4[a])	32.6
4/ 60 mg/kg/day	456	< LLOQ	216
	463	242	155
	465	342	263
	466	393	291
	475	299	205
	Mean	255 (319[a])	226
	S.D.	153 (64.1[a])	52.7

LLOQ Lower limit of quantitation (theoretical concentration 5.00 ng/mL). LD Lactation Day; H2-3 = Hours 2-3 post start of infusion on that day.
[a] < LLOQ value not included in statistical calculations.
Source: Applicant's Table, SSN-01263, p.52.

Impurities

(b) (4): Bacterial Reverse Mutation Assay in Salmonella typhimurium and Escherichia coli/Study No. SSN-01667
Key Study Findings
- (b) (4) was negative for mutagenicity in bacterial cells in a valid Ames test.

GLP compliance: Yes
Test system: *Salmonella typhimurium* strains TA1535, TA1537, TA98, and TA100 and *Escherichia coli* strain WP2 *uvrA*; doses ≤ 5000 µg/plate in DMSO; +/- S9
Study is valid: Yes
Study reviewed by: Dr. Baishali Kanjilal

A 14-Day Intravenous (1 Hour) Infusion Toxicity Study of (b) (4) in Sprague Dawley Rats/Study No. SSN-01667
Key Study Findings
- No (b) (4)-related findings were observed for any endpoints at doses up to 0.15 mg/kg/day for 14-days.

GLP compliance: Yes

Methods: [redacted] in 250 mg/mL SBECD were co-administered to male and female rats at [redacted] mg/kg/day [redacted] via IV infusion (1 hour/day) for 14 days. Standard toxicology endpoints were used.
Study reviewed by: Dr. Julie Frank

22.4. OCP Appendices (Technical documents supporting OCP recommendations)

22.4.1. Summary of Bioanalytical Method Validation

LC-MS/MS bioanalytical methods were developed and validated according to FDA and EMA guidances (FDA 2001, EMA 2012).

The bioanalytical methods utilized for the quantitation of brexanolone in human plasma, urine, and breast milk were demonstrated to be accurate, precise, selective, and robust. Independent lots of blank matrix were tested and found to have no interference with the quantitation of brexanolone in the matrices validated. Evaluation of stability was carried out at sample collection, sample preparation, and sample analysis, as well as the storage conditions used to ensure that there was no effect on the concentration of the analyte.

In the human plasma methods, [redacted]

Bioanalytical methods were developed and validated by [redacted] or the analysis of brexanolone in human plasma and urine samples. Bioanalytical methods for determination of brexanolone in human breast milk were developed and validated by [redacted]. The methods utilized [redacted]

In addition to brexanolone, methods to measure SBECD and free/total phenytoin in human plasma samples were developed and validated by (b) (4)

Validation Summary for Determination of Brexanolone in Human Plasma by LC-MS/MS

Validation Parameter	SSN-597 (b) (4)	SSN-01481 (b) (4)
Validation Dates *	27 Sep 2013 – 18 Nov 2016	13 Aug 2015 – 24 Aug 2016
Matrix (Anticoagulant)	Human plasma (K_2EDTA)	Human plasma (K_2EDTA)
Assay Range (ng/mL)	1.00 to 500	1.00 to 500
Reference Standard	SAGE-547 Lot # 8	SAGE-547 Lot # 407-14-01-58
Internal Standard	SGE-135 Lot # Z370P24	SAGE-547-d4 Lot # Z370P38
Reference Curve Range (ng/mL)	1.00, 2.00, 10.0, 20.0, 100, 200, 400, 500	1.00, 2.00, 10.0, 40.0, 100, 300, 450, 500
QC Levels (ng/mL)	1.00, 3.00, 150, 380	1.00, 3.00, 35.0, 400
LLOQ (ng/mL)	1.00	1.00
ULOQ (ng/mL)	500	500
Dilutional Integrity	1000 ng/mL (100-fold dilution)	2500 ng/mL (10-fold dilution)
Intra-Assay Precision (%CV) and Accuracy (% RE)	LLOQ: %CV: 6.4 – 13.2; %RE: -7.2 – 8.0 LQC, MQC and HQC: %CV: 1.5 – 4.4; %RE: 0.7 – 7.7	LLOQ: %RSD: 4.9 – 12.5; %Bias: -5.8 – 1.0 LQC, MQC and HQC: %RSD: 2.0 – 7.8; %Bias: -2.0 – 6.3
Inter-Assay Precision (%CV) and Accuracy (% Diff. from Nominal)	LLOQ: %CV = 11.2; %RE = 1.0 LQC, MQC and HQC: %CV: 2.4 – 4.7; %RE: 2.7 – 5.3	LLOQ: %RSD = 8.2; %Bias = -1.9 LQC, MQC and HQC: %RSD: 3.1 – 5.2; %Bias: 0.9 – 3.8
Freeze/Thaw Stability in Matrix	Four Cycles at -20° C	Five Cycles at -10 - -30° C Five Cycles at -60 - -80° C
Analyte Stability in Frozen Matrix (LTS)	1077 days at -20° C 65 days at -80° C	109 days at -10 - -30° C 376 days at -60 - -80° C
Whole Blood Stability	2 hours on ice	2 hours at room temperature and on wet ice
Analyte Stability in Matrix at Room Temperature	5 hours	24 hours
Matrix Factor (MF)	1.03 (LQC) and 0.991 (HQC)	ISTD normalized MF: 0.996 (LQC) and 0.966 (HQC)

Validation Summary for Determination of Brexanolone in Human Urine and Breast Milk by LC-MS/MS

Validation Parameter	SSN-01420 (13-8583) (b) (4)	SSN-01419 (14-8716) (b) (4)	SSN-01482 (8323543) (b) (4)
Validation Dates *	05 Aug 2014 – 25 Mar 2015	30 July 2015 – 15 Jan 2016	10 Sep 2015 – 03 Dec 2016
Matrix	Human breast milk	Human urine	Human urine
Assay Range (ng/mL)	5.00 – 2500	1.00 –500	1.00 – 500
Reference Standard	SAGE-547 Lot # 8	SAGE-547 Lot # 407-14-01-58	SAGE-547 Lot # 407-14-01-58
Internal Standard	SGE-135 Lot # Z370P24	SGE-135 Lot # Z370P24	SAGE-547-d4 Lot# Z370P38
Reference Curve Range (ng/mL)	5.00, 10.0, 50.0, 100, 500, 1000, 2000, 2500	1.00, 2.00, 10.0, 20.0, 100, 200, 400, 500	1.00, 2.00, 10.0, 40.0, 100, 300, 450, 500
QC Levels (ng/mL)	5.00, 15.0, 750, 1900	1.00, 3.00, 150, 380	1.00, 3.00, 35.0, 400
LLOQ (ng/mL)	5.00	1.00	1.00
ULOQ (ng/mL)	5000	500	500
Dilutional Integrity	5000 ng/mL (50-fold dilution)	1000 ng/mL (10-fold dilution)	2500 ng/mL (10-fold dilution)
Intra-Assay Precision (%CV) and Accuracy (% RE)	LLOQ: %CV: 7.0 – 7.8; %RE: 7.0 – 12.2 LQC, MQC and HQC: %CV: 1.5 – 4.4; %RE: -5.3 – 7.4	LLOQ: %CV: 3.7 – 11.4; %RE: -9.9 – 6.0 LQC, MQC and HQC: %CV: 1.6 – 5.7; %RE: -5.0 – 8.7	LLOQ: %RSD: 6.1 – 7.3; %Bias: -3.7 – 4.0 LQC, MQC and HQC: %RSD: 1.2 – 5.9; %Bias: -5.3 – 5.0

Validation Parameter	SSN-01420 (13-8583) (b) (4)	SSN-01419 (14-8716) (b) (4)	SSN-01482 (8323543) (b) (4)
Inter-Assay Precision (%CV) and Accuracy (% Diff. from Nominal)	LLOQ: %CV = 7.2; %RE = 9.0 LQC, MQC and HQC: %CV: 2.4 – 5.9; %RE: -1.2 – 4.2	LLOQ: %CV: 9.6; %RE: -1.9 LQC, MQC and HQC: %CV: 2.7 – 4.3; %RE: -4.0 – 7.7	LLOQ: %RSD: 7.0; %Bias: -0.2 LQC, MQC and HQC: %RSD: 2.0 – 5.9; %Bias: -0.6 – 0.3
Freeze/Thaw Stability in Matrix	Four cycles at -20° C	Four cycles at -20° C	Five cycles at -10 to -30° C Six cycles at -60 to -80° C
Analyte Stability in Frozen Matrix (LTS)	651 days at -20° C	157 days at -20° C	85 days at -10 - -30° C 273 days at -60 - -80° C
Analyte Stability in Matrix at Room Temperature	22 hours and 51 minutes	20 hours	24 hours
Matrix Factor (MF)	1.02 (LQC) and 0.992 (HQC)	0.974 (LQC) and 0.988 (HQC)	ISTD normalized MF: 0.938 (LQC) and 0.969 (HQC)

* Experimental dates
Abbreviations: CV = coefficient of variation; HQC = high quality control; ISTD = internal standard; K_3EDTA or K_3EDTA = potassium ethylenediaminetetraacetic acid; LLOQ = lower limit of quantitation; LQC = low quality control; LTS = long term stability; MF = matrix factor; MHQC = medium-high quality control; MQC = medium quality control; QC = quality control sample; RE = relative error; RSD = relative standard deviation; ULOQ = upper limit of quantification.

Validation Summary for Determination of SBECD and Phenytoin in Human Plasma by LC-MS/MS

Validation Parameter	2891 (Captisol, (b) (4)	RGKI2 (Total phenytoin, PPD)	RGKJ2 (Free Phenytoin, PPD)
Validation Dates [a]	16 Jan 2015 – 12 Aug 2016	09 Jun 2016 – 28 Sep 2017	15 Jun 2016 – 29 Sep 2017
Matrix (Anticoagulant)	Human plasma (K_2EDTA) [b]	Human plasma (Sodium Heparin)	Human plasma (Sodium Heparin)
Assay Range	2.00 – 200 µg/mL	20.0 –10,000 ng/mL for total phenytoin	20.0 – 2000 ng/mL for free phenytoin
Reference Standard	Captisol RS-04A-050026	Phenytoin Lot # J0E090	Phenytoin Lot # J0E090
Internal Standard	Captisol-G Lot # CD-74-72	Phenytoin-d_5 Lot # BDG 7430.1	Phenytoin-d_5 Lot # BDG 7430.1
Reference Curve Range	2.00, 5.00, 10.0, 20.0, 50.0, 100, 150, 200 µg/mL	20.0, 40.0, 75.0, 250, 800, 3000, 8000, 10,000 ng/mL	20.0, 30.0, 50.0, 125, 300, 750, 1600, 2000 ng/mL
QC Levels	2.00, 6.00, 80.0, 170 µg/mL	50.0, 125, 450, 1500, 7500 ng/mL	20.0, 40.0, 80.0, 200, 500, 1500 ng/mL
LLOQ	2.00 µg/mL	20.0 ng/mL	20.0 ng/mL
ULOQ	200 µg/mL	10,000 ng/mL	2000 ng/mL
Dilutional Integrity	800 µg/mL (10-fold dilution)	450 ng/mL (2.5-fold dilution); 20,000 ng/mL (10-fold dilution)	4000 ng/mL (5-fold dilution)
Intra-Assay Precision (%CV) and Accuracy (% RE)	LLOQ: %CV: 9.0; %RE: 15 LQC, MQC and HQC: %CV: 2.6 – 8.7; %RE: -0.5 – 11	LLOQ: %CV: 1.55 – 4.92; %RE: 0.133 – 7.23 LQC, LMQC, MQC and HQC: %CV: 1.96 – 5.04; %RE: -0.0843 – 7.15	LLOQ: %RSD: 1.89 – 2.31; %Bias: 6.04 – 9.88 LQC, LMQC, MQC, MHQC, and HQC: %CV: 0.346 – 26.1; %RE: -4.24 – 8.44
Inter-Assay Precision (%CV) and Accuracy (% Diff. from Nominal)	K_2EDTA: NA Sodium heparin plasma [b]: LLOQ: %CV = 14; %RE = 8.0 LQC, MQC and HQC: %CV: 5.7 – 9.8; %RE: -4.2 – 2.0	LLOQ: %CV: 3.68 – 4.45; %RE: 4.49 – 4.79 LQC, MQC and HQC: %CV: 2.94 – 4.60; %RE: 1.14 – 5.29	LLOQ: %RSD: 2.50; %Bias: 8.25 LQC, LMQC, MQC, MHQC, and HQC: %CV: 1.00 – 12.2; %RE: 2.60 – 7.72
Freeze/Thaw Stability in Matrix	Five cycles at -80° C	Five cycles at -20° C or -70° C	Five cycles at -20° C or -70° C

Validation Parameter	2891 (Captisol, (b) (4)	RGKI2 (Total phenytoin, PPD)	RGKJ2 (Free Phenytoin, PPD)
Analyte Stability in Frozen Matrix (LTS)	578 days at -20° C 386 days at -80° C	478 days at -20° C or -70° C	473 days at -20° C or -70° C
Whole Blood Stability	K_2EDTA plasma [b]: NA Sodium heparin plasma: 2 hours at 2–8° C	2 hours at room temperature or on ice	NA
Analyte Stability in Matrix at Room Temperature	22 hours	25 hours	26 hours at room temperature in human plasma and plasma filtration; 1 hour at 37 °C in human plasma
Matrix Factor (MF)	K_2EDTA plasma [b]: NA Sodium heparin plasma: 0.95 (LQC) and 1.1 (HQC)	Lot-to-lot response consistency was demonstrated	Lot-to-lot response consistency was demonstrated for free Phenytoin

[a] Experimental dates
[b] The method was initially validated for human sodium heparin plasma, and partial validated for human K_2EDTA plasma. The study sample was collected with K_3EDTA. The validation data for K_2EDTA plasma is shown in this table.

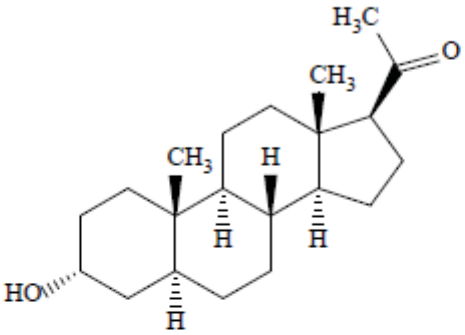
Abbreviations: CV = coefficient of variation; HQC = high quality control; ISTD = internal standard; K_2EDTA or K_3EDTA = potassium ethylenediaminetetraacetic acid; LLOQ = lower limit of quantitation; LMQC = low-medium quality control; LQC = low quality control; LTS = long term stability; MF = matrix factor; MHQC = medium-high quality control; MQC = medium quality control; NA = not available; QC = quality control sample; RE = relative error; RSD = relative standard deviation; ULOQ = upper limit of quantification.

A stable isotopic-labeled brexanolone was employed as the internal standard for the program. The structures of brexanolone (SAGE-547) and the internal standard (SGE-135) are shown below:

SAGE-547

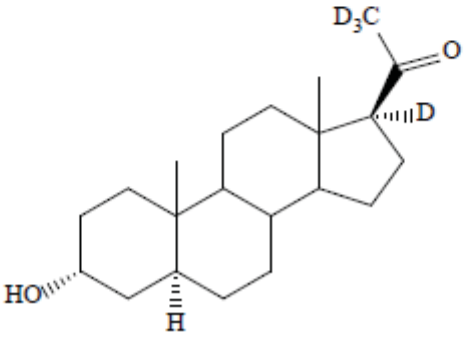
1-(3-hydroxy-10,13-dimethyl-2,3,4,5,6,7,8,9,11,12,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-17-yl)ethanone

Chemical Formula: $C_{21}H_{34}O_2$
Molecular Weight: 318.50



SGE-135 (IS)

Chemical Formula: $C_{21}H_{30}D_4O_2$
Molecular Weight: 322.53



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22.4.2. **Pharmacometrics Review**

Population PK Analyses

The Applicant conducted two population PK analyses. Report 547-clp-108-exploratory-rep.pdf describes the analyses to assess plasma PK and the relationship with breast milk PK acquired from n=12 subjects in Phase 1 Study 108. Report 547-pop-pk.pdf describes analyses to assess plasma PK from 5 clinical studies from Phase 1, Phase 2, and Phase 3.

Population PK in Plasma in PPD Patients (547-pop-pk)

Report 547-pop-pk is titled "*Pharmacokinetic Analysis of Brexanolone Exposure in Patients with Post-Partum Depression*". The Applicant conducted population pharmacokinetic analyses for brexanolone following continuous IV infusion in patients with post-partum depression. The purpose of this analysis is to model the plasma concentration-time profile and assess the impact of covariates on brexanolone PK.

Data from the following clinical studies were included in the analysis:

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Table 115: Studies and Trials Included in the Population PK Analyses

Study ID	Study Information	Dose Regimen	Description of Data
547-CLP-108 (Phase 1b)	An Open-Label Study Evaluating Concentrations of Allopregnanolone Following Administration of SAGE-547 Injection in the Breast Milk of Adult Lactating Women (n = 12)	Continuous IV infusion of SAGE-547 administered as: 30 µg/kg/h for 4 h (H 0 to H 4), 60 µg/kg/h for 20 h (H 4 to H 24), 90 µg/kg/h for 28 h (H 24 to H 52), 60 µg/kg/h for 4 h (H 52 to H 56), 30 µg/kg/h for 4 h (H 56 to H 60)	**Rich PK**: pre-infusion and at 12, 24 (before infusion rate change), 36, 48, 56, 60 (before infusion end), 61, 62, 64, and 72 hours after the start of infusion **Sparse PK**: Day 7
547-PPD-201 (Phase 2a)	An Open-Label Proof-of-Concept Study Evaluation the Safety, Tolerability, Pharmacokinetics, and Efficacy of SAGE-547 Injection in the Treatment of Adult Female Patients with Severe Postpartum Depression (n= 4)	Continuous IV infusion of SAGE-547 administered as: 21.5 µg/kg/h for 4 h (H 0 to H 4), 43 µg/kg/h for 4 h (H 4 to H 8), 64.5 µg/kg/h for 4 h (H 8 to H 12), 86 µg/kg/h for 36 h (H 12 to H 48), 64.5 µg/kg/h for 4 h (H 48 to H 52), 43 µg/kg/h for 4 h (H 52 to H 56), 21.5 µg/kg/h for 4 h (H 56 to H 60)	**Rich PK**: At 30 minutes, and at 1, 2, 3, 4, 6, 12, 24, 36, 40, 44, 48, 60 h after infusion onset, and at 72 h after infusion onset (12 h post-infusion)
547-PPD-202A (Phase 2)	A Multicenter, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study Evaluating the Efficacy, Safety, and Pharmacokinetics of SAGE-547 Injection in the Treatment of Adult Female Subjects with *Severe* Post-Partum Depression (n= 21)	Subjects were randomized in a 1:1 ratio. Continuous IV infusion of blinded study drug (SAGE-547 or PBO) administered as: 30 µg/kg/h for 4 h (H 0 to H 4), 60 µg/kg/h for 20 h (H 4 to H 24), 90 µg/kg/h for 28 h (H 24 to H 52), 60 µg/kg/h for 4 h (H 52 to H 56), 30 µg/kg/h for 4 h (H 56 to H 60)	**Rich PK**: pre-infusion, 4 (just prior to infusion rate change), 8, 12, 24 (just prior to infusion rate change), 30, 36, 48, 60 h after infusion onset, , and at 72 h after infusion onset (12 h post-infusion)
547-PPD-202B (Phase 3)	A Multicenter, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study Evaluating the Efficacy, Safety, and Pharmacokinetics of SAGE-547 Injection in the Treatment of Adult Female Subjects with *Severe* Post-Partum Depression (n=122)	Subjects were randomized to one of three treatment groups (SAGE-547 60 µg/kg/h, SAGE-547 90 µg/kg/h, or PBO) in a 1:1:1 ratio. *60 µg/kg/h* arm: 30 µg/kg/h for 4 h (H 0 to H 4), 60 µg/kg/h for 20 h (H 4 to H 56), 30 µg/kg/h for 4 h (H 56 to H 60) *90 µg/kg/h* arm: Same regimen as used in Study 202A. PBO arms received matching volumetric flow rate and infusion duration as brexanolone arms.	Same as Trial 202A
547-PPD-202C (Phase 3)	A Multicenter, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Study Evaluating the Efficacy, Safety, and Pharmacokinetics of SAGE-547 Injection in the Treatment of Adult Female Subjects with *Moderate* Post-Partum Depression (n=104)	Same as Trial 202A	Same as Trial 202A

Data points were excluded from analyses if one or more of the following conditions were met:

- Absolute residual variability larger than three times the expected residual standard deviation (|CWRES| > 6)
- ≥ 500 ng/mL. There were 123 [8%] samples measuring greater than 500 ng/mL which the Applicant considers not biologically plausible. In addition, these tended to be site-specific, and might have been the result of an incorrect PK sampling procedure.
- PK samples that were between 100 and 500 ng/mL, if, after scrutiny, were identified as kinetically implausible defined as observed concentration being ≥ 4 x the predicted concentration (based on preliminary modeling from data on file) and if there was no adverse event associated with it. There were 17 (1.04%) samples meeting this criteria (2 were excluded a prior due to suspected sampling errors).

The Applicant states that several observations were inconsistent with the expected pattern based on recorded dosing; however, these were still retained in the dataset (provided the above conditions were met).

[*Reviewer comment: The Applicant's rationale for excluding PK samples is acceptable. However, the proportion of PK samples that were excluded was unexpectedly high (8%).*]

The PK dataset included PK samples from 156 subjects. There were 1337 PK samples acquired post-dosing, of which 46 were below the lower limit of quantification (BLQ). There were 152 PK samples acquired pre-dosing of which all 152 were BLQ. At 120 hours after infusion onset (60 hours *after* the completion of the 60-hour infusion), a total of 30 PK samples were above the lower limit of quantification.

Model Description

Structural Model: The base structural model is a 2-compartment model. PK parameters include CL, V1, V2, and Q.
Allometric Scaling: CL, V1, Q, and V2 had allometric scaling applied using body weight normalized to 82.9 kg, the population median body weight.
Inter-individual variability: exponential
Residual variability: proportional error model
Covariates: No covariates were included in the final model. The Applicant performed stepwise covariate modeling to investigate covariate effects. Potential CL covariates tested for were age, albumin, ALP, ALT, AST, bilirubin, BMI, creatinine clearance (CL_{CR}), and race. Potential covariates tested on V1 as well as V2 included age and BMI. BMI was also tested as a covariate on Q. In addition to pre-specified covariate analyses, concomitant antidepressants were determined by graphical assessment to have no impact on brexanolone C_{max} or $AUC_{0-\infty}$.

Final model parameter estimates are shown in the table below.

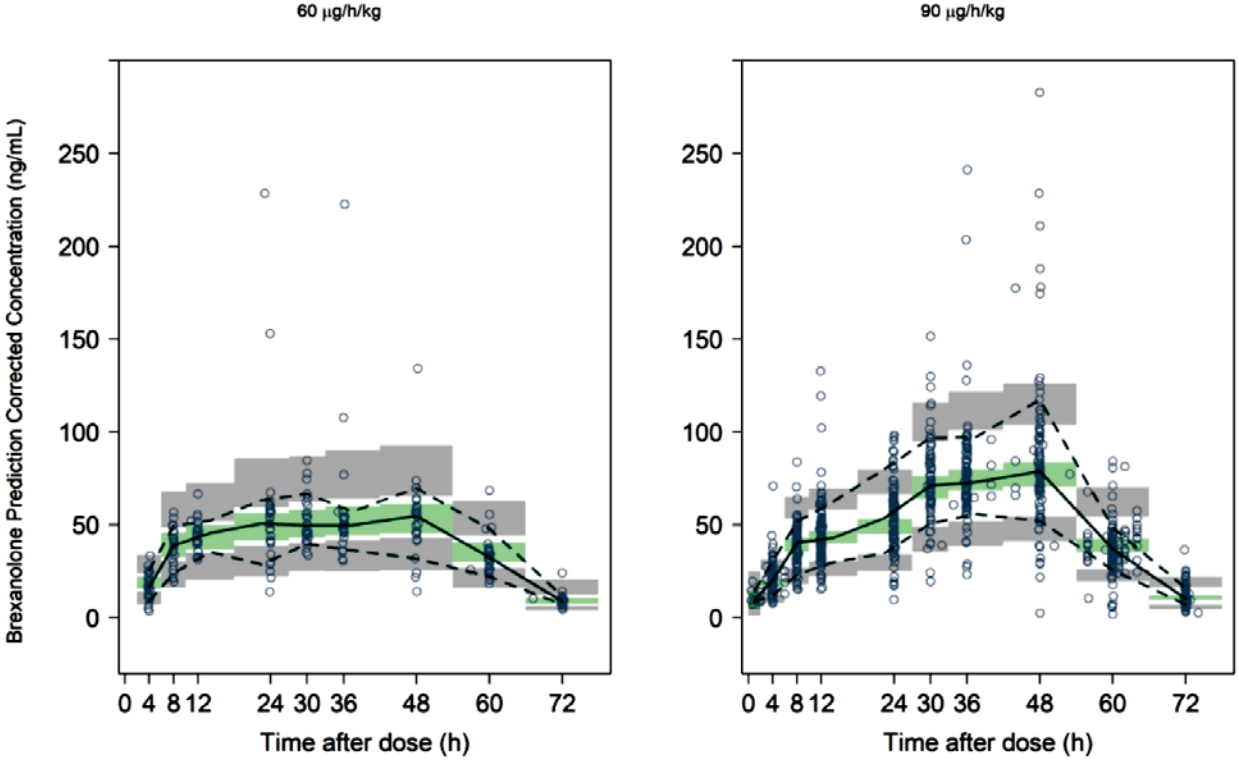
Table 116: PK Parameter Estimates for Final PK Model (Run 1038) in Patients with Post-Partum Depression

Parameter	Alias	Estimate	Relative SE (%)	95% CI
θ_1	CL ($L \cdot h^{-1}$)	89.8	1.8	(86.6 - 93.1)
θ_2	V_1(L)	117.	22.9	(64.6 - 170)
θ_3	Q ($L \cdot h^{-1}$)	37.9	7.7	(32.2 - 43.6)
θ_4	V_2 (L)	470.	5.9	(415 - 524)
θ_5	Proportional residual variability	0.272	4.1	(0.25 - 0.294)
$\omega_{1.1}$	ω^2_{CL}	0.0435	21.3	(0.0254 - 0.0617)
$\omega_{2.1}$	$\omega^2_{CL,V1}$	0.106	25.9	(0.0521 - 0.159)
$\omega_{2.2}$	ω^2_{V1}	1.15	28.1	(0.515 - 1.78)

Source: Sequence 0001, 547-pop-pk.pdf, page 37 of 151

The IIV for Cl and V1 were estimated to be 21.1% CV and 147% CV, respectively.

Figure 31: Visual Predictive Check for Final PPK Model (Run 1038) By Treatment Arm



Source: Sequence 0001, 547-pop-pk.pdf, page 43 of 151

[*Reviewer comment: The DV vs PRED and DV vs IPRE plots provided in the study report (not shown) do not present any obvious signs of systematic bias. However, these plots demonstrate a modest number of PK samples in the range of 50 to 100 ng/mL which are underpredicted.*

The CWRES vs PRED plot provided in the study report (not shown) shows no systematic bias for the 90 µg/kg/h dose level and modest underprediction for the 60 µg/kg/h dose level from 20 to 40 ng/mL. However, the underprediction in that concentration range may be due to the modest number of PK samples in that concentration range for the 60 µg/kg/h dose group.

The VPC indicates that the central tendency of the predictions do not show any systematic bias except for under-predictions at the 24-hours after infusion onset and only in the 90 µg/kg/h patient group. The highest end of the exposure distribution across the population tends to be modestly overpredicted particularly in the 60 µg/kg/h patient group and to a lesser extent in the 90 µg/kg/h patient group. There is also a modest under prediction of the lowest end of the exposure distribution across the population for both the 60 µg/kg/h patient group as well as the 90 µg/kg/h patient group.

Overall, the population PK model for brexanolone represents the central tendency of the PK data well and is less accurate at predicting the extreme plasma concentration values.

There are no label statements based on population PK modeling using this population PK model. There are label statements proposed for section 8.2 Lactation based on another population PK model, the model built from plasma and milk PK data from Study 108 (please refer to the section regarding Population PK in Milk in PPD Patients for details).]

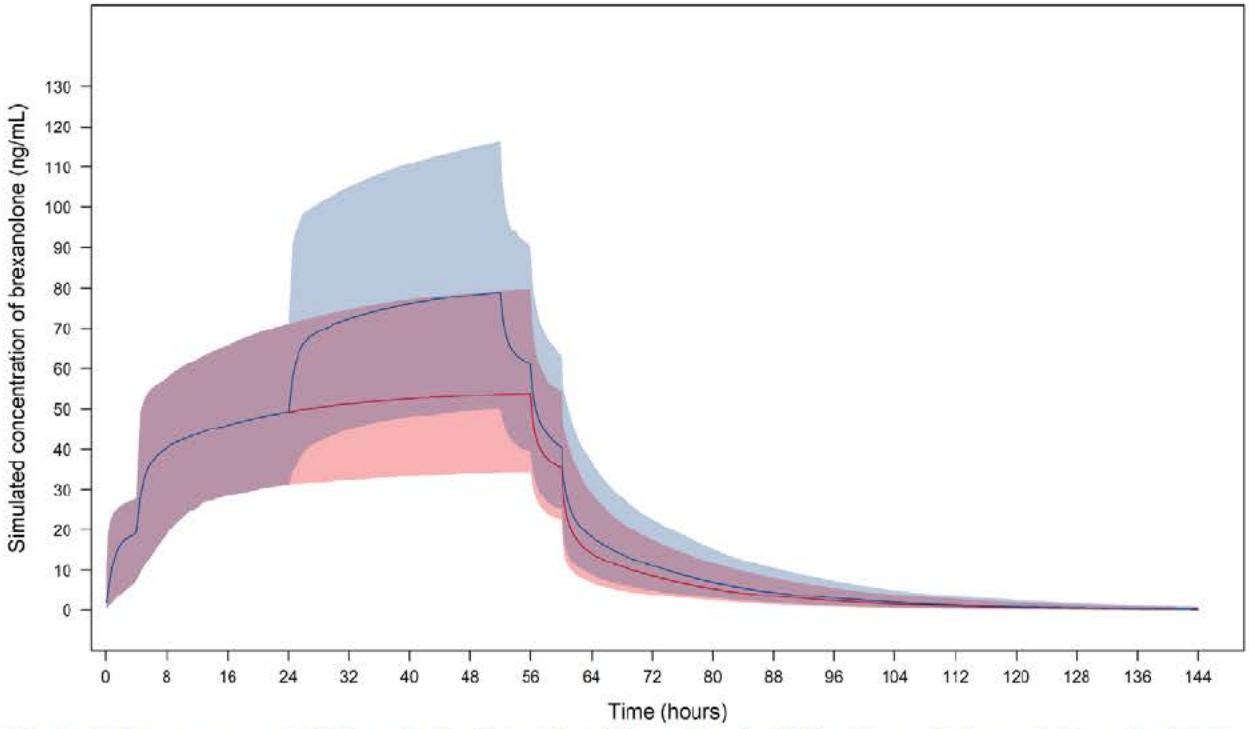
Simulated Exposure at Low and High Dose Levels

The Applicant conducted PK simulations to assess the distribution of expected plasma brexanolone exposures throughout the proposed 60-hour regimen for each of the studied dosing regimens;

Low dose regimen	High dose regimen	Time period
30 µg/kg/h	30 µg/kg/h	0-4 hours
60 µg/kg/h	60 µg/kg/h	4 to 24 hours
60 µg/kg/h	**90 µg/kg/h**	**24 to 52 hours**
60 µg/kg/h	60 µg/kg/h	52 to 56 hours
30 µg/kg/h	30 µg/kg/h	56 to 60 hours

The results of the Applicant's PK simulations are shown in **Figure 32** below.

Figure 32: Simulated 5th, 50th, and 95th percentile of simulated brexanolone Plasma Concentration over time Following Administration with the [redacted] (b) (4) 60 µg/kg/h [redacted] (b) (4) (Red) and the 90 µg/kg/h [redacted] (b) (4) (Blue)



The shaded area represents 95% prediction interval and the median (solid line) for each time point in a simulated population of 1000 individuals.

Source: Sequence 0001, 547-pop-pk.pdf, page 53 of 151

[*Reviewer comment: The Applicant utilized the PK simulations presented in* ***Figure 32*** *above to inform dose selection in their Phase 3 program. Please refer to section 3.3.2 for details.*]

Population PK in Milk in PPD Patients (547-CLP-108-exploratory-rep)

Report 547-clp-108-exploratory-rep is titled "*Exploratory Report: Model Development for Brexanolone in Milk, Study CLP-108*".

The Applicant conducted analyses to examine the relationship between plasma and breast milk concentrations of brexanolone. Data used in the analysis were collected from study 547-CLP-108. The study information is summarized in **Table 116** below.

Table 117: Summary of Study 108 Trial Features

Study ID	Study Information	Dose Regimen	Description of Data
547-CLP-108 (Phase 1b)	An Open-Label Study Evaluating Concentrations of Allopregnanolone Following Administration of SAGE-547 Injection in the Breast Milk of Adult Lactating Women (n = 12)	Continuous IV infusion of SAGE-547 administered as: 30 µg/kg/h for 4 h (H 0 to H 4), 60 µg/kg/h for 20 h (H 4 to H 24), 90 µg/kg/h for 28 h (H 24 to H 52), 60 µg/kg/h for 4 h (H 52 to H 56), 30 µg/kg/h for 4 h (H 56 to H 60)	**Rich PK**: pre-infusion and at 12, 24 (before infusion rate change), 36, 48, 56, 60 (before infusion end), 61, 62, 64, and 72 hours after the start of infusion **Sparse PK**: Day 7

[*Reviewer comment: The infusion regimen administered in Study 108 is identical to the regimen utilized in Phase 3 and proposed by Applicant for use in the label.*]

There were a total of 144 plasma samples and 337 breast milk samples from the n=12 women enrolled in Study 108.

Model Description

Structural Model: The base structural model is a 2-compartment model. PK parameters include CL, V1, V2, and Q.
Allometric Scaling: CL, V1, Q, and V2 had allometric scaling applied using body weight normalized to 80.25 kg, the population median body weight
Inter-individual variability: exponential
Residual variability: proportional
Covariates: None tested

Final model parameter estimates and key diagnostic plots are presented in **Table 117** below.

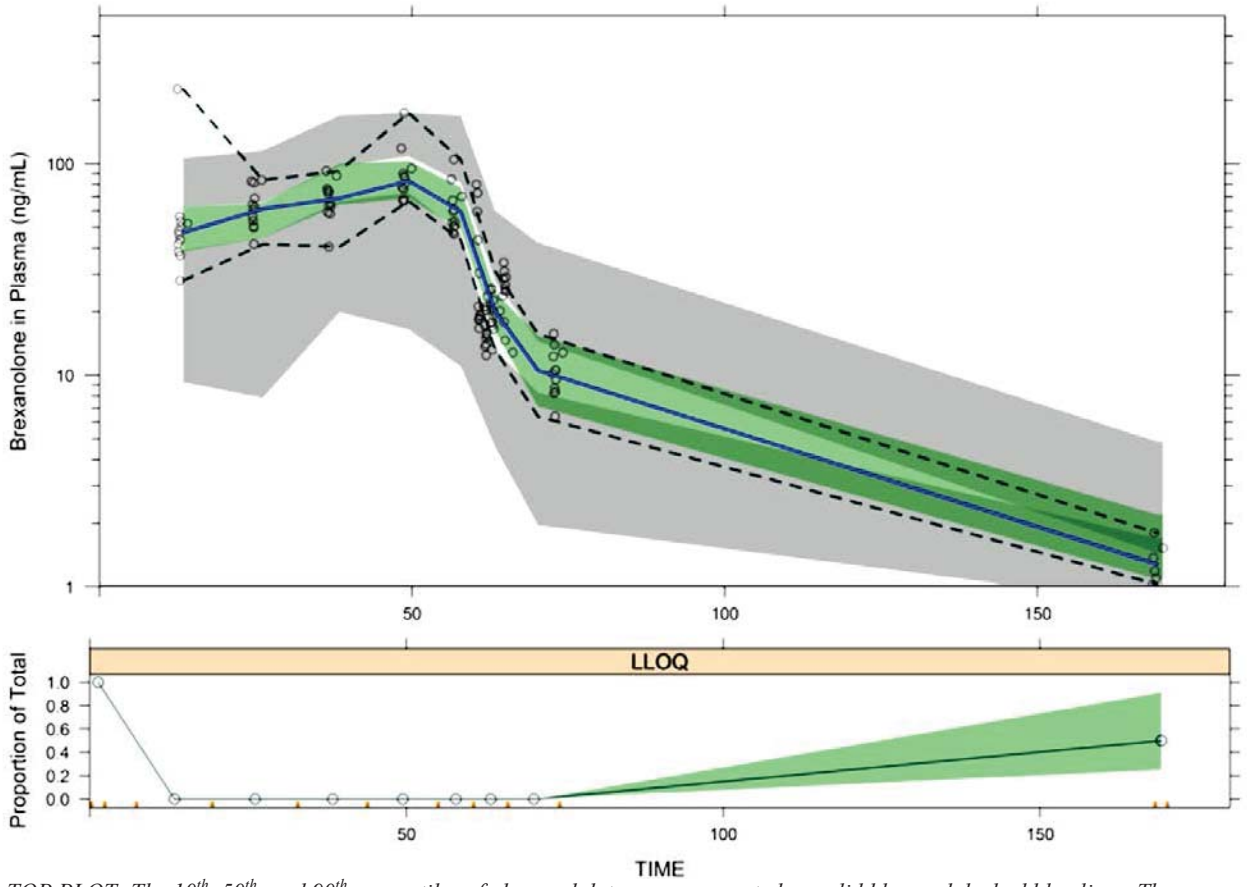
Table 118: PK Parameter Estimates for Final PK Model (Run 167) Linking Brexanolone Plasma Concentration to Brexanolone Breast Milk Concentration in Post-Partum Healthy Volunteers

Parameter	Estimate	CV.perc	SE	estimated	CI95	transformed
Cl	73.5	.....	.....	estimated	(35.5 - 152)	log
V1	123.	.....	.....	estimated	(53.3 - 284)	log
Q	23.4	.....	.....	estimated	(6.8 - 80.7)	log
V2	701.	.....	.....	estimated	(476 - 1030)	log
Kp(milk:plasma)	1.36	.....	.....	estimated	(0.858 - 2.16)	log
Prop - Var	0.290	.....	.....	estimated	(0.256 - 0.327)	log
Cl - eta	0.0287	204.6	0.0588	estimated	(-0.0865 - 0.144)	no
Q - eta	0.0896	295.5	0.265	estimated	(-0.429 - 0.608)	no

Source: Sequence 0001, 547-clp-108-exploratory-rep.pdf, page 11 of 70

Key diagnostic plots are presented below.

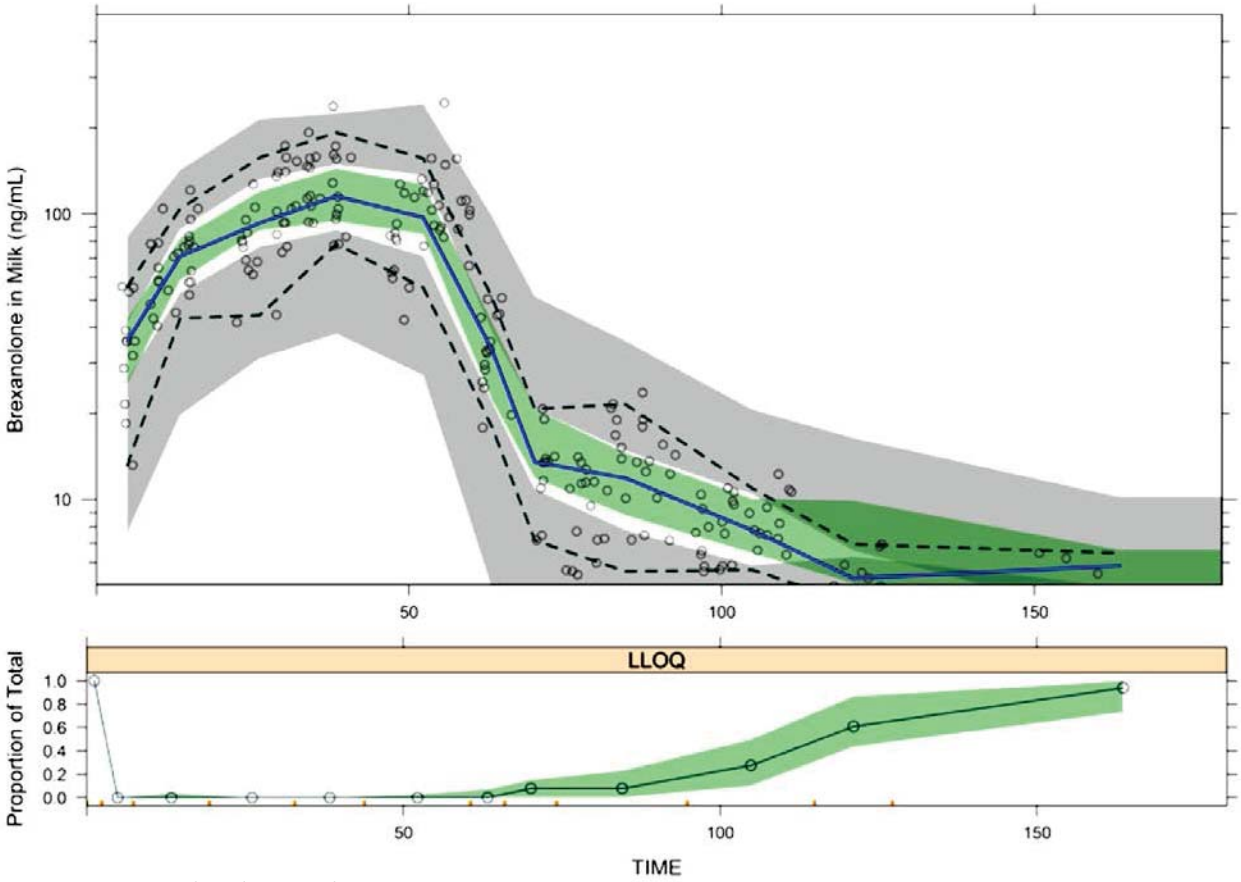
Figure 33: Visual Predictive Check for Plasma Brexanolone - Final Model (Run 167) - Study 108



TOP PLOT: The 10th, 50th, and 90th percentiles of observed data are represented as solid blue and dashed blue lines. The simulated data are represented by the shaded regions. The 95% CI of the 10th, 50th, and 90th percentiles of the simulated data are displayed as the top grey region (CI for the 90th percentile), the middle green region (CI for the 50th percentile) and bottom grey region (CI for the 10th percentile). The darker and darkest shades of green refer to regions where the green region overlaps with one or both grey regions, respectively.
BOTTOM PLOT: The blue line represents the probability that the measurement is BLQ. The green shaded area represents the 95% CI of the estimated probability.

Source: Sequence 0001, 547-clp-108-exploratory-rep.pdf, page 12 of 70

Figure 34: Visual Predictive Check for Milk Brexanolone - Final Model (Run 167) - Study 108



TOP PLOT: The 10th, 50th, and 90th percentiles of observed data are represented as solid blue and dashed blue lines. The simulated data are represented by the shaded regions. The 95% CI of the 10th, 50th, and 90th percentiles of the simulated data are displayed as the top grey region (CI for the 90th percentile), the middle green region (CI for the 50th percentile) and bottom grey region (CI for the 10th percentile). The darker and darkest shades of green refer to regions where the green region overlaps with one or both grey regions, respectively.
BOTTOM PLOT: The blue line represents the probability that the measurement is BLQ. The green shaded area represents the 95% CI of the estimated probability.

Source: Sequence 0001, 547-clp-108-exploratory-rep.pdf, page 12 of 70

[*Reviewer comments: The reviewer compared the plasma PPK model parameters for the single-study PPK analyses (model 167; Study 108) with the plasma PPK model from the multi-study PPK analysis built using Phase 1, Phase 2, and Phase 3 PK data (model 1038; Studies 108, 201, 202A, 202B, 202C). The PK estimates from Run 1038 are used as the basis for comparison of the plasma PK parameter estimates from the two plasma PPK models (see*

***Table* 118** *below).*

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Table 119: Comparison of Plasma PK Parameter Estimates From Run 167 (Study 108) and Run 1038 (5 Studies)

PK Parameter	Run 1038 (Studies 108, 201, 202A, 202B, 202C; N=156)	Run 167 (Study 108; N=12)	% diff from Run 1038
CL	89.8 L/h	73.5 L/h	- 18%
V1	117 L	123 L	+ 5%
Q	37.9 L/h	23.4 L/h	- 38%
V2	470 L	701 L	+ 49%

Overall, the V1 parameter estimate for Model 1038 appears comparable to Model 167. However, the Q and V2 estimates differ by -38% and +49%, respectively. The differences in parameter estimates in Run 167 comapred to Run 1038 are likely due to the smaller sample size in Run 167 compared to Run 1038.

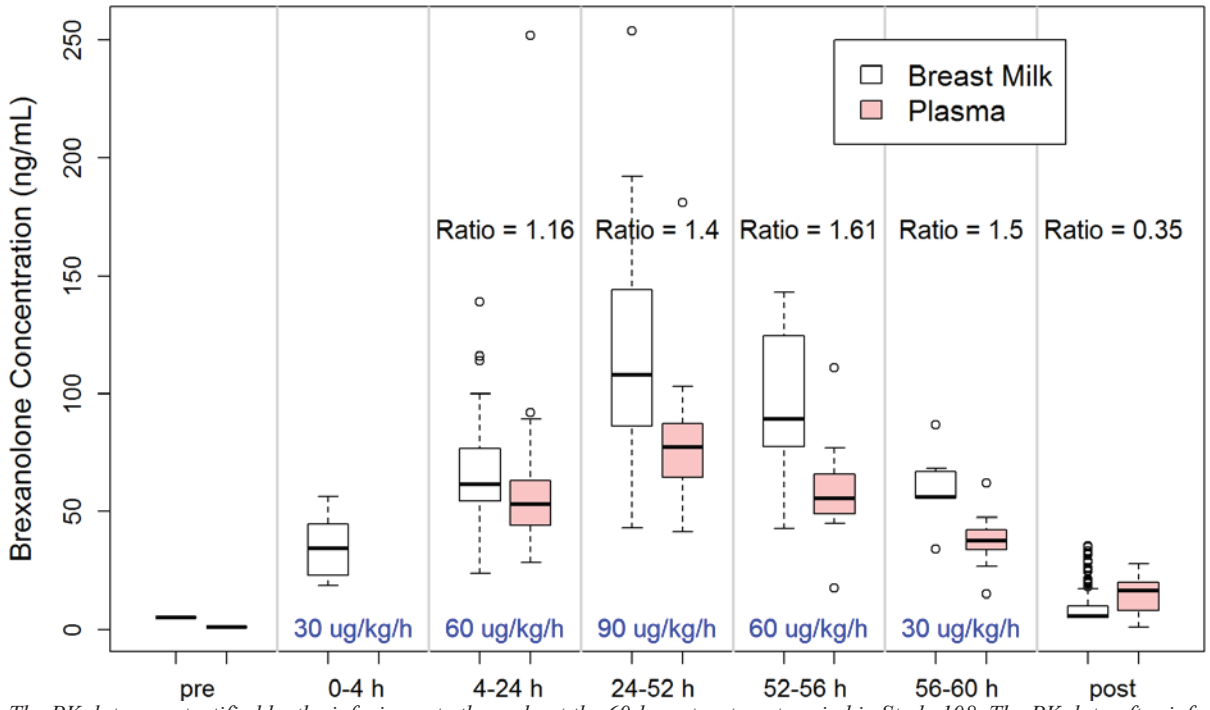
The VPC plot for plasma PK concentrations (figure not included in this review) shows that the model is able to capture the central tendency of the observed data well. However, the extreme values of the simulated data (e.g. 10th percentile and 90th percentile) demonstrate lower precision and generally do not represent the observed data as well.

The same observations are apparent for the VPC for brexanolone in milk. ***Overall, the model appears to capture the central tendency of brexanolone milk concentration profile but is less accurate at predicting extreme milk concentration values****.*

Estimating Milk:Plasma Ratio

The approach of modeling the milk brexanolone concentration as a proportion of the plasma brexanolone concentration (using a partition coefficient) assumes a constant concentration ratio for brexanolone in milk:plasma. However, the observed PK data demonstrate variability in the milk:plasma concentration ratio throughout and after the infusion duration. However, during the period of maximum brexanolone infusion, the Applicant's milk:plasma ratio estimate of 1.36 is comparable to the Reviewer's estimate of the ratio of observed milk concentration to observed plasma concentration (see ***Figure 35*** *below).*

Figure 35: Plot of Brexanolone Concentration in Milk and Plasma During and After Infusion in Study 008



The PK data are stratified by the infusion rate throughout the 60-hour treatment period in Study 108. The PK data after infusion are grouped together (60-168 hours). The white and red box plots represent the brexanolone concentration in milk and plasma across all subjects across all time points collected within the specified time interval for milk and plasma, respectively. The "Ratio" represents the value of the median concentration of brexanolone in milk across all subjects and all samples collected within the specified time interval divided by the median for plasma within the same time interval.

The variability in the ratio at different periods over time is likely a factor contributing to the width of the 95% CI of the ratio estimate (ratio estimate is 1.36, 95% CI is 0.858 to 2.16). Use of the 1.36 ratio may lead to modest over or underprediction for any PK simulations of the up-titration or down-titration period. However, the 1.36 ratio appears to be a reasonable estimate for the prediction of the milk concentration during the period of maximum infusion rate.]

Estimating Relative Infant Dose from Breast Milk

The applicant utilized the results of the population PK analyses report from study 108 to derive a relative infant dose (RID). The methodology was that laid out by Bennett and Notarianni (1996)[1] where [relative dose in milk] = [milk conc] * [milk vol per day] * 100% / [maternal daily dose]. The Applicant's RID estimates are summarized in **Table 119** below.

Table 120: Applicant's Calculation of Maximum Brexanolone Relative Infant Dose (RID)

	Observed Plasma $AUC_{24\text{-}48}$, ng*h/mL	Predicted Milk $AUC_{24\text{-}48}$, ng*h/mL (a)	Predicted Average Brexanolone in Milk from 24-48h, ng/mL (b)	Predicted Daily Dose in Milk, ng/kg/day (c)	Predicted RID, % (d)
Minimum	462	628	26.18	3927	0.1818%
Median	1760	2394	99.73	14960	0.6926 %
Maximum	3410	4638	193.2	28980	1.342 %

*a Computed as 1.36*Plasma AUC*
b Milk AUC divided by 24 hours
c Based on feeding rate of 150 mL/kg/day
*d Computed as infant dose divided by maternal dose (90 µg/kg/h*24 h) *100% (Bennett 1996)*

Source: Sequence 0001, summary-clin-pharm.pdf, page 56-57 of 76

The computation of relative infant dose starts with the observed plasma $AUC_{24h\text{-}48h}$ value which occurs during the time period when the infusion rate is at the maximum value of 90 µg/kg/h. Using the maximum observed $AUC_{24h\text{-}48h}$ value of 3410 ng*h/mL, and the milk:plasma partition coefficient of 1.36 (estimated from PPK modeling), the Applicant estimates a milk $AUC_{24h\text{-}48h}$ value of 4638 ng*h/mL (3410*1.36 = 4637.6 = 4638). The milk AUC is used to derive the average brexanolone concentration in milk over the time period of 193.2 ng/mL (48-24 = 24 hours duration; 4638/24 = 193.2). Using 150 mL/kg/day as an estimate of daily breast milk consumption for an infant, the daily dose of brexanolone expected to enter the infants mouth via breast milk is 28980 ng/kg/day (193.2 * 150 = 28980). The expected oral brexanolone dose for infants of 28980 ng/kg/day (assuming 100% bioavailability) is then divided by the maximum adult infusion rate of 90 µg/kg/h to obtain the RID of 1.342% [28980 / (90*24*1000) * 100% = 1.342%].

The Applicant points out that while the oral bioavailability in infants is unknown, absolute oral bioavailability in adults is <5%. The Applicant concludes that as the RID is <10% when assuming 100% bioavailability, the risk exposing infants to brexanolone via breast milk at the proposed dose regimen is acceptable.

[*Reviewer comment: The Applicant estimates a 193.2 ng/mL as the maximum brexanolone concentration in breast milk during the period where the infusion is at the maximum rate of 90*

[1] Bennett PN, Notarianni LJ, Risk from drugs in breast milk: an analysis by relative dose. Br J Clin Pharmacol., 1996; 42:P673-4. Abstract.

µg/kg/day. The maximum milk concentration is estimated by obtaining the observed maximum plasma AUC during 24-48 hours, diving by 24 hours to obtain a maximum plasma concentration during 24-48 hours, and multiplying by the 1.36 milk:plasma ratio to estimate the maximum milk concentration during this time period.

The reviewer conducted a sensitivity analysis of the RID estimate. The Applicant utilized their observed plasma exposure and estimated milk:plasma ratio to estimate milk exposure. The maximum predicted milk exposure of 193.2 ng/mL was predicted based on the maximum observed plasma AUC during the period of 90 µg/kg/h infusion rate. However, as the proposed dose regimen for the label is identical to the dose regimen utilized in Study 108, and since milk concentration was measured in Study 108, the reviewer assessed the effect of using the observed milk concentration (rather than predicted milk concentration) to estimate the RID.

The reviewer derived RID estimates based on the observed maximum, observed median, and observed minimum milk concentrations from study 108. Other than using the observed minimum, median, and maximum milk concentrations observed from 24-52 hours after infusion start, the Reviewer utilized the same RID estimation method as the Applicant. The results are shown in ***Table 120*** *below.*

Table 121: Reviewer's Calculation of Maximum Brexanolone Relative Infant Dose (RID)

	Observed Average Brexanolone in Milk from 24-52 hours after infusion start, ng/mL (b)	Predicted Daily Brexanolone Dose in Milk, ng/kg/day (a)	Predicted RID, % (b)
Minimum	43.3	6495	0.3%
Median	108	16200	0.75%
Maximum	254	38100	1.76%

a Based-on feeding rate of 150 mL/kg/day
*b Computed as infant dose divided by maternal dose (90 µg/kg/h*24 h) *100% (Bennett 1996).*

The maximum observed milk concentration across all subjects and across all time points is 254 ng/mL and occurs in patient 2006 at approximately 35.6 hours after infusion start (during the 90 µg/kg/h infusion time period). The apparent worst-case scenario is RID of 1.76% which represents the highest observed milk concentration in the entire study which occurred while the infusion was at the maximum rate of 90 µg/kg/h (see the computation below used to derive the maximum predicted RID cited in ***Table 120****).*

$$\%RID = \frac{\frac{254\ ng}{mL} \times \frac{150\ mL}{kg \times day}}{\frac{90\ \mu g}{kg \times hour} \times \frac{24\ hours}{1\ day} \times \frac{1000\ ng}{1\ \mu g}} \times 100\% = 1.76\%$$

Overall, the 1.34% RID estimate obtained using the Applicant's predicted milk concentration is comparable to the 1.76% RID estimate obtained using the maximum observed milk concentration.

Considering the width of the 95% CI of the 1.36 ratio used to derive the Applicant's RID (0.858 – 2.16) as well as the effect of using maximum observed milk concentration versus maximum predicted milk concentration, the reviewer is recommending that the RID listed in the label (1.34%) be changed to "1% to 2%". If neonates or infants have comparable oral bioavailability from milk to adults with the oral brexanolone formulation (i.e., <5%), effective relative infant dose could be < 0.05% to 0.1%.

The administration of SBECD to infants via the breast milk is not expected to pose a clinically-relevant safety or tolerability issues. Please refer to section 6.3.2.3. ***Should patients receiving brexanolone treatment continue breast feeding?*** *for details.*]

22.4.3. **Summary of Clinical Pharmacology Studies**

CLINICAL PHARMACOLOGY STUDY REVIEW

Mass Balance Study

Study # 547-CLP-101	**Study Period:** 15-June-2015 to 23-July-2015
NDA 211371_Zulresso_Brexanolone IV infusion	
Title	A Phase 1 Study to Investigate the Metabolism and Excretion of [14C]-SAGE-547 Following Single Intravenous Dose Administration in Healthy Male Subjects
Objectives:	**Primary:** • To determine mass balance and routes of elimination of [14C]-SAGE-547 following intravenous (IV) administration of a single infusion, target dose 90 µg/kg/h (containing approximately 0.0048 µCi/µg), over a period of 4 hours of [14C]-SAGE-547 in male subjects. • To assess the pharmacokinetics (PK) of SAGE-547 and its metabolites following a single target dose of 90 µg/kg/h of SAGE-547 over a period of 4 hours. The dose contained approximately 0.0048 µCi/µg using [14C]-SAGE-547. • To determine the whole blood, plasma, urine, and feces concentrations of total radioactivity. **Secondary:** • To characterize and identify metabolites of [14C]-SAGE 547 in plasma, urine, and feces; • To further determine the safety and tolerability of a single IV dose of [14C]-SAGE-547 in male subjects.
Study Design:	

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This study was an open-label, nonrandomized, metabolism and excretion study to investiga mass balance of [^{14}C]-SAGE-547 administered as a single IV infusion, with a target dose of 90 µg/kg/h (containing approximately 0.0048 µCi/µg), over a period of 4 hours. Eight healt male subjects were enrolled in the study at a single clinical site to ensure a minimum of six subjects completed the study. A schematic of the study design is presented in Table 2. The protocol is provided in Appendix 16.1.1.

Potential subjects were screened to assess their eligibility to enter the study within 28 days (Days -28 to -1) prior to dosing on Day 1. For all subjects, routine screening procedures we performed, as outlined in Table 3.

Subjects were confined at the clinical site from the time of check-in (Day -1) until clinic discharge/early termination (ET) for a minimum confinement of 7 days postdose (Day 8) ar maximum confinement of 13 days postdose (Day 14). Clinic discharge/ET was based on subjects meeting the following discharge criteria:

- Plasma radioactivity reached levels below the limit of quantitation (BLQ) in two consecutive samples, and;
- Greater than or equal to 90% of the dose was recovered, or;
- Less than or equal to 1% of the radioactive dose was recovered in urine and fece two consecutive 24-hour collection intervals;

Sample collection and subject confinement continued until discharge criteria were met.

On Day 1, subjects were administered a single IV infusion of [^{14}C]-SAGE-547, with a target dose of 90 µg/kg/h (containing approximately 0.0048 µCi/µg), over a period of 4 hours. Bloc and other matrices for PK analysis were collected at the time points specified in Table 3.

Safety assessments, including physical examinations, 12-lead electrocardiograms (ECGs), vit signs, How Do You Feel? inquiries, and clinical laboratory evaluations, were performed at screening, at specified times during the study, and/or at clinic discharge/ET.

Table 2: Study Design

Screening	Check-in	Dosing	PK/Radioactivity Sampling	Clinic Discharg Early Terminati
Days -28 to -2	Day -1	Day 1	Day 1 to Day 8/14	Day 8 to Day 1
	← Confinement →			

[a] Subjects were discharged from the clinic starting on Day 8 if plasma radioactivity reached levels below the lir quantitation in two consecutive samples, and if ≥90% of the dose was recovered or if ≤1% of the radioactive d was recovered in urine and feces for two consecutive 24-hour collection intervals. Subjects that did not meet discharge criteria stayed in the clinic until either they had met discharge criteria or until the maximum stay on Day 14.

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Product Used:

Test product, dose and mode of administration, batch number:
Subjects received a single IV infusion of [^{14}C]-SAGE-547 at a dose of 90 µg/kg/h (containing approximately 0.0048 µCi/µg), over a period of 4 hours. Unit doses were formulated from sulfobutyl ether beta-cyclodextrin (SBECD), SAGE-547 Injection 5 mg/mL (Lot No. B130545), [^{14}C]-SAGE-54 (aka 4-[^{14}C]-SAGE-547) (Lot No. 60232MAY15-01), and Sterile Water for Injection.

Route of Administration	IV infusion

PK Sampling Times and Parameters	Blood samples for PK analysis of SAGE-547 in plasma were collected at Day 1 (predose); and at 2, 3, 4 (just prior to the end of infusion, while the pump was still on), 4.5, 5, 6, 8, 12, 24, 36, and 48 hours after the start of infusion; and approximately every 24 hours until Clinical Discharge/ET. Blood samples for PK analysis of total radioactivity in whole blood and plasma were collected at Day 1 (predose); and at 2, 3, 4 (just prior to the end of infusion, while the pump was still on), 4.5, 5, 6, 8, 12, 24, 36, and 48 hours after the start of infusion; and approximately every 24 hours until Clinical Discharge/ET. Blood samples for metabolite profiling and ID were collected at Day 1 (predose); and at 2, 3, 4 (just prior to the end of infusion, while the pump was still on), 4.5, 5, 6, 8, 12, 24, 36, and 48 hours after the start of infusion; and approximately every 24 hours until Clinical Discharge/ET. Urine samples were collected at Day 1 (predose, the last void within 1 hour prior to dosing); at 0 to 6, 6 to12, and 12 to 24 hours postdose; and approximately every 24 hours until Clinical Discharge/ET. Fecal samples were collected predose (Check-in [Day -1] from -24 to 0 hours); Day 1 at 0 to 6, 6 to12, and 12 to 24 hours postdose; and approximately every 24 hours until Clinical Discharge/ET.

	Blood, urine, and feces were collected at specified timepoints or intervals starting on Day -1, a applicable, through clinical discharge/early termination. The following PK parameters were calculated, whenever possible, based on the plasma concer of SAGE-547 and total radioactivity concentrations in whole blood and plasma: maximum obs concentration (C_{max}), time to maximum concentration (t_{max}), area under the concentration-time from Hour 0 to the last measurable concentration (AUC_{0-t}), area under the concentration-time from Hour 0 extrapolated to infinity ($AUC_{0-\infty}$), apparent terminal elimination rate constant (λ_z) apparent terminal elimination half-life ($t_{½}$), total clearance (CL; SAGE-547 only) volume of distribution during the terminal phase (V_z; SAGE-547 only), and volume of distribution at stea (V_{ss}; SAGE-547 only). Whole blood-to-plasma ratios were calculated to determine partitioning of total radioactivity i blood cells. SAGE-547 to total plasma radioactivity ratios was also calculated. For urine SAGE-547 concentrations and total radioactivity concentrations, the following PK parameters were calculated: amount of SAGE-547 or total radioactivity excreted in urine over sampling interval (A_{eu}), cumulative A_{eu} (Total A_{eu}), percent of SAGE-547 total radioactivity d excreted in urine (% F_{eu}), and cumulative (Total % F_{eu}). Renal clearance (CL_R) was also calcu based on the urine concentration of SAGE-547. For fecal total radioactivity concentrations, the following PK parameters were calculated: amo total radioactivity excreted in feces over the sampling interval (A_{ef}), cumulative A_{ef} (Total A_{ef}) of total radioactivity dose excreted in feces (% F_{ef}), and cumulative % F_{ef} (Total % F_{ef}). The PK parameters for the metabolites of [^{14}C]-SAGE-547 were calculated, as deemed approp based on plasma, urine, and fecal concentration levels.
Safety Parameters	**Safety:** Safety and tolerability were assessed by collection and review of adverse events, clinical laboratory evaluations, vital signs, single 12-lead electrocardiograms (ECG), concomitant medications, Stanfo Sleepiness Scale (SSS), and physical examinations.

	The condition of each subject was monitored throughout the study. Subjects were asked a leading question such as "Have there been any changes in your health status since screening/since you were last asked?" at check-in (Day -1), at each postdose vital signs measurement, and at clinic discharge/ET (Table 3). Subjects were also encouraged to volu report adverse events occurring at any other time during the study. All adverse events, and serious adverse events, whether noted on physical examination or reported voluntarily or upon questioning, were recorded throughout the study. The nature, of onset, duration, and severity were documented, together with an Investigator's opinion c relationship to drug administration (not related, possibly related, or related). An adverse event was defined as any untoward medical occurrence experienced by a healt subject, whether or not considered drug-related by the Investigator. A treatment-emergent adverse event was an adverse event that was reported after a dose of study drug. A serious adverse event (by FDA definition) was any adverse drug experience occurring at any dose resulted in any of the following outcomes: • Death; • A life-threatening adverse drug experience (ie, places the subject, in the view of Investigator, at immediate risk of death); • Inpatient hospitalization or prolongation of existing hospitalization; • A persistent or significant disability/incapacity; • A congenital anomaly/birth defect; • Important medical event that may require medical or surgical intervention to pre one of the above outcomes. The severity of each adverse event was categorized as follows: • **MILD** = of little concern to the subject and/or of no clinical significance; was n expected to have any effect on the subject's health or well-being; • **MODERATE** = discomfort enough to cause interference with or change in usu activities; was likely to require medical intervention and/or close follow-up; • **SEVERE** = incapacitating or unable to work or participate in many or all usual activities, was of concern to the subject and/or posed substantial risk to the subj health or wellbeing; was likely to require medical intervention and/or close foll
PK Moieties	Sage-547 and all major circulating metabolites
PD Endpoint(s)	None
Statistical Methods	**Statistical methods:** Descriptive statistics were calculated for PK concentrations and parameters and safety endpoints wh applicable. No formal statistical analyses were planned.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547 and all major circulating metabolites		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- Healthy male between the ages of 18-55 years of age.
- N= 8 subjects

Number of patients (planned and analyzed):

It was planned to study a total of eight subjects in one group to complete a minimum of six subje
Eight subjects entered and completed the study. Data for all subjects entered into the study were
included in the pharmacokinetic (PK) and safety analyses.

Diagnosis and main criteria for inclusion:

Healthy male subjects between ages 18 and 55 years of age with a body mass index between 18 a
30 kg/m^2, inclusive, and weighing ≤100 kg.

Table 1: Demography of subjects

Parameter	SAGE-547 [N=8]
Age (Years)	
n	8
Mean	34.0
SD	9.89
Median	34.5
Minimum	22
Maximum	52
Ethnicity (n [%])	
HISPANIC OR LATINO	3 (37.5)
NOT HISPANIC OR LATINO	5 (62.5)
Race (n [%])	
BLACK OR AFRICAN AMERICAN	2 (25.0)
WHITE	5 (62.5)
OTHER: SOUTH NATIVE AMERICAN	1 (12.5)

Parameter	Visit	N	Mean	SD	Median	Min	
Height (cm)	Screening	8	173.98	9.840	176.00	160.9	1
Weight (kg)	Screening	8	78.94	11.439	80.55	62.7	
	Day -1	8	78.41	11.702	80.80	62.1	
	Clinic Discharge/ET	8	77.63	11.879	80.55	61.7	
Body Mass Index (kg/m2)	Screening	8	25.98	1.821	25.85	24.0	
	Day -1	8	25.80	1.801	25.40	23.8	
	Clinic Discharge/ET	8	25.53	1.816	25.30	23.3	

Data Source: Listing 16.2.4.2
- Abbreviations: ET = Early Termination.

Inclusion Crietria:

For inclusion into the study, subjects were required to fulfill all of the following criteria:

1. Males, between 18 and 55 years of age, inclusive;
2. Within body mass index (BMI) range 18 to 30 kg/m^2, inclusive and ≤100 kg;
3. In good health, determined by no clinically significant findings from medical history, 12-lead ECG, and vital signs;
4. Clinical laboratory evaluations (including chemistry panel [fasted at least 8 hours], complete blood count, and urinalysis) within the reference range for the test laboratory, unless deemed not clinically significant by the Investigator;
5. Negative test for selected drugs of abuse at Screening (did not include alcohol) and at check-in (Day -1) (did include alcohol);
6. Negative hepatitis panel (including hepatitis B surface antigen and hepatitis C virus antibody) and negative human immunodeficiency virus antibody screens;
7. Males were to either be sterile or agree to use from check-in (Day -1) until 90 days following study drug administration one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an intrauterine device with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system (eg, NuvaRing®); a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects were to refrain from sperm donation from check-in (Day -1) until 90 days following study drug administration;
8. Able to comprehend and willing to sign an ICF;
9. Have venous access sufficient to allow for IV infusion and blood sampling as per the protocol; and
10. A minimum of one bowel movement per day.

Exclusion Criteria:

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Any of the following was regarded as a criterion for exclusion from the study:

1. Subject was female;
2. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neurological, or psychiatric disorder (as determined by the Investigator);
3. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator;
4. History of Gilbert's Syndrome;
5. History or presence of an abnormal ECG, which, in the Investigator's opinion, was clinically significant;
6. History of alcoholism or drug addiction within 1 year prior to check-in (Day -1);
7. Use of any tobacco- or nicotine-containing products (including, but not limited to, cigarettes, electronic cigarettes [of any kind], pipes, cigars, chewing tobacco, nicotine patches, nicotine lozenges, or nicotine gum) within 6 months prior to check-in (Day -1) and during the entire study;
8. Participation in more than one other radiolabeled investigational study drug trial within 12 months prior to check-in (Day -1). The previous radiolabeled study drug must have been received more than 6 months prior to check-in (Day -1) for this study and the total exposure from this study and the previous study had to be within the recommended levels considered safe, per US Title 21 CFR 361.1 (ie, less than 5,000 mrem whole-body annual exposure);
9. Exposure to significant radiation (eg, serial x-ray or computed tomography scans, barium meal, current employment in a job requiring radiation exposure monitoring) within 12 months prior to check-in (Day -1);
10. Participation in any other investigational study drug trial in which receipt of an investigational study drug occurred within 5 half-lives or 30 days, whichever was longer, prior to check-in (Day -1) and during the entire study;
11. Use of alcohol-, grapefruit-, or caffeine-containing foods or beverages within 72 hours prior to check-in (Day -1) and during the entire study duration, unless deemed acceptable by the Investigator;
12. Subjects were to refrain from strenuous exercise from 48 hours prior to check-in (Day -1) and during the period of confinement at the clinical research unit (eg, not begin a new exercise program nor participate in any unusually strenuous physical exertion);
13. Use of any prescription medications/products within 14 days prior to check-in (Day -1), unless deemed acceptable by the Investigator;
14. Use of any over-the-counter (OTC), nonprescription preparations (including vitamins, minerals, and phytotherapeutic/herbal/plant-derived preparations) within 7 days prior to check-in (Day -1), unless deemed acceptable by the Investigator;
15. Donation of blood from 30 days prior to Screening through clinic discharge, inclusive, or of plasma from 2 weeks prior to screening through clinic discharge/ET, inclusive;
16. Receipt of blood products within 2 months prior to check-in (Day -1);
17. Any acute or chronic condition that, in the opinion of the Investigator, would limit the subject's ability to complete and/or participate in this clinical study; and
18. Inability to comply with the study protocol.

PK Results

PHARMACOKINETICS RESULTS:

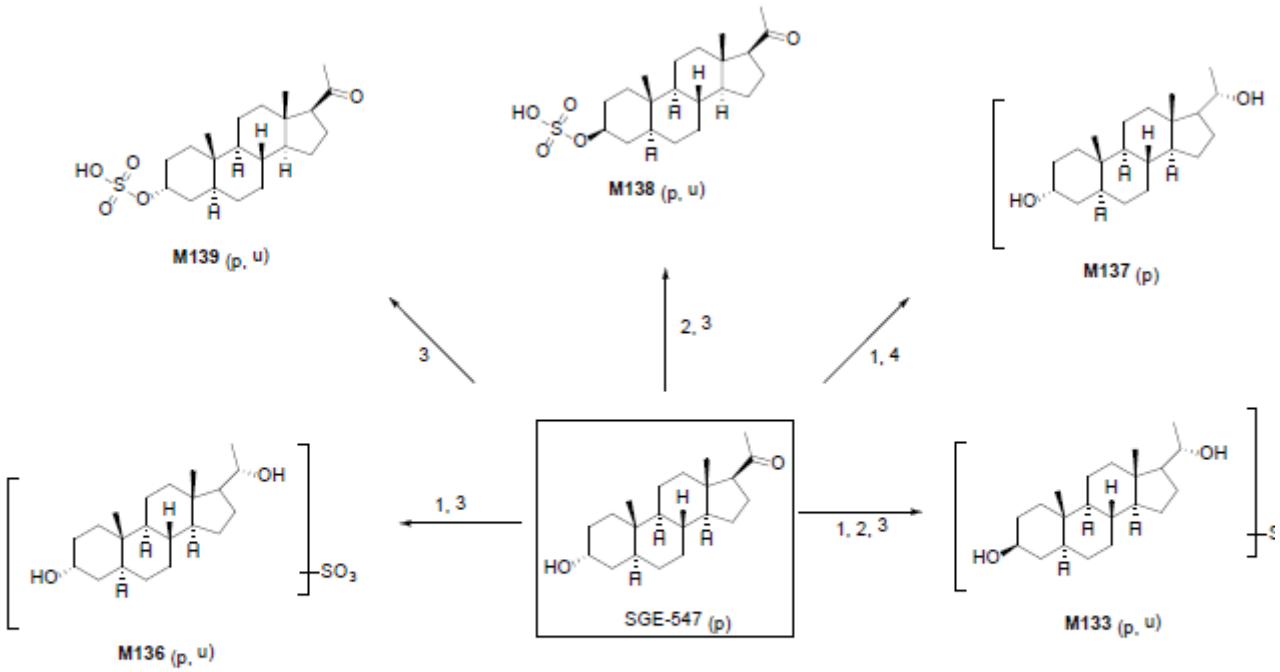
- The overall mean recovery of radioactivity in urine and feces was approximately 89% of the administered radioactive dose, measured over 312 hours from the start of infusion. Most of t administered radioactivity (85.6%) was recovered in the first 144 hours (7 days) after start of infusion.
- Fecal and urinary excretion were comparable indicating no predominate route of elimination SAGE-547; 47.2% and 41.8% of the radioactivity administered was recovered in feces and u respectively, by 312 hours after start of the infusion.
- Urinary excretion of unchanged SAGE-547 was negligible (less than 1% of administered dos
- SAGE-547 was quickly eliminated from plasma, in a biphasic manner, with mean $t_{1/2}$ of approximately nine hours.
- Total radioactivity was slowly eliminated, with mean $t_{1/2}$ of approximately 56 and 45 hours i plasma and whole blood, respectively.
- SAGE-547 represented approximately 2% of total radioactivity in plasma (as assessed by AU indicating that metabolites are a major component of the circulating total radioactivity in pla
- Metabolite profiling and identification results showed that metabolic clearance was the predominant route of elimination of SAGE-547 in humans after an IV infusion due to the prevalence of numerous unidentified metabolites, the low exposure of SAGE-547 in plasma, the absence of SAGE 547 in excreta.
- Low association of radioactivity with blood cells was observed as the mean whole blood-to-p $AUC_{0-\infty}$ ratio was 0.515.

Based on LC-MS/MS analyses of plasma, urine, and feces extracts, SAGE-547 underwe extensive biotransformation in human male subjects after a single IV dose of [^{14}C]-SAG (90 µg/kg/h, 0.0048 µCi/µg) administered over 4 hours to produce at least 45 radioactive components, of which five were identified/characterized by LC-MS/MS.

Stereoselective reduction of the C-20 keto group to its corresponding 20α alcohol with subsequent sulfonation and glucuronidation of either the 3- or 20-position alcohol were t abundant identified biotransformation pathways. Direct sulfonation of SAGE-547 to yie was also evident. The metabolites M133 and M138 showed epimerization of the alcohol This epimerization would involve oxidation of the 3α alcohol to the intermediate ketone, subsequent reduction to the 3β alcohol.

Reduction of the C-20 keto moiety, epimerization at the C-3 hydroxy position, and sulfona SAGE-547 and the corresponding 5α-pregnan-3,20 diol metabolites were the main identifi route of elimination. The prevalence of numerous unidentified metabolites, the low expos SAGE-547 in plasma, and the absence of SAGE-547 in excreta indicate that metabolic cle was the predominant route of elimination of SAGE-547 in humans after an IV infusion.

Figure 4: Proposed Biotransformation Pathways of SAGE-547 in Humans



Safety Results	
Was there any death or serious adverse events?	☐ Yes ☑ No ☐ NA

SAFETY RESULTS:

- A single IV infusion of 90 µg/kg/h [^{14}C]-SAGE-547 over 4 hours was generally well tolerat this group of healthy male subjects.
- There were no serious adverse events or discontinuations due to treatment-emergent adverse events.
- All adverse event s were mild in severity. Mild somnolence was the most commonly report treatment-emergent adverse event (50.0%) and was considered to be related to study drug. Somnolence was commonly experienced as starting during the infusion and continuing for approximately 1 to 3 hours following the end of infusion.
- No clinically significant findings in clinical chemistry, hematology, urinalysis, vital signs, E or physical examinations were noted in this study.
- Most subjects experienced sleepiness as measured by the Stanford Sleepiness Scale with pea levels of sleepiness occurring at 4 hours after start of the infusion.

Table 13: Frequency of Treatment-Emergent Adverse Events by System Organ Clas and Preferred Term (Safety Population)

System Organ Class Preferred Term[a]	SAGE-547 (N=8)	
	No. of Subjects (%)[b]	No. of Event
Any treatment-emergent adverse event	6 (75.0)	19
Gastrointestinal disorders	4 (50.0)	4
Abdominal distension	2 (25.0)	2
Diarrhoea	2 (25.0)	2
General disorders and administration site conditions	1 (12.5)	2
Fatigue	1 (12.5)	1
Vessel puncture site reaction	1 (12.5)	1
Nervous system disorders	5 (62.5)	11
Dizziness	2 (25.0)	2
Headache	2 (25.0)	4
Somnolence	4 (50.0)	5
Respiratory, thoracic and mediastinal disorders	2 (25.0)	2
Nasal congestion	2 (25.0)	2

No. = number.
[a] Subjects with multiple events in the same category are counted only once in that category. Subjects with event more than one category are counted once in each of those categories.
[b] Number of subjects reporting at least one event of type specified. For subjects with "Any treatment-emergent adverse event", the number of subjects reporting at least one event of any type is represented.
[c] The total number of events of the type specified. Subjects can be represented more than once. For "Any Event represents the total number of treatment-emergent adverse events.
Reference: Table 14.3.1.3

The most frequently reported treatment-emergent adverse event was somnolence (50.0%). All remaining treatment-emergent adverse events were reported by one or two subjects each. Additional treatment-emergent adverse events that could be related to sedation included dizziness (two subjects [25.0%]), headache (two subjects [25.0%]) and fatigue (one subject [12.5%]). Events of dizziness may have been a central effect because they were not obviously associated with hypotension.
All treatment-emergent adverse events were mild in severity. All events of somnolence and fatigue were considered related to the study drug, and all events of headache, dizziness, abdominal distension, and diarrhea were considered possibly related to the study drug.

Overall Sponsor Conslusions

- SAGE-547 clearance from plasma is rapid (approximately 72 L/h); it is eliminated from plas a biphasic manner ($t_{1/2}$ of approximately 9 hours). Urinary excretion of unchanged SAGE-54 negligible (less than 1% of administered dose). SAGE-547 appears to be extensively metabc fecal and urinary excretion were comparable indicating no predominate route of elimination radioactivity.
- Metabolite profiling and identification results showed that metabolic clearance was the predominant route of elimination of SAGE-547 in humans after an IV infusion due to the prevalence of numerous unidentified metabolites, the low exposure of SAGE-547 in plasma, the absence of SAGE 547 in excreta.
- SAGE-547 was well-tolerated at a dose of 90 µg/kg/h for 4 hours in healthy male subjects.
- Somnolence was reported as the most common treatment-emergent adverse event (50.0% of subjects) and most subjects experienced sleepiness with peak Stanford Sleepiness Scale scor corresponding to maximum plasma concentrations of SAGE-547.

Reviewer Comments and Conclusions

1. *Study Design: This was an Open Label, Single Center, Single Dose, Phase I study in healthy male subjects to assess the mass-balance and investigate the metabolism and excretion of 14C-SAGE 547 following a single IV infusion. The overall study design was acceptable since:*
    - *It was a single dose study conducted with the requisite amount of radioactive tracer to adequately isolate and quantitate circulating metabolite(s).*
    - *IV dose was chosen because it is the intended route of administration.*
    - *18-55-year-old, healthy adult male were included.*
    - *Adequate number of subjects (N= 8) were included in the study*
    - *The final to-be-marketed formulation of SAGE 547 was used in this study.*

2. *Protocol deviation: No major protocol deviations were reported.*

3. *Data Analysis (i.e., any outliers etc.): There were no outliers and the PK data from all*

subjects were included in the analysis.

4. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

5. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *Healthy adult males between 18-55 years of age were included. Female subjects were excluded from the study to align with regulatory guidance. The regulatory reason for not including female subjects is contained in the "as low as (is) reasonably achievable" principle prescribed by both the US Food and Drug Administration (FDA) and Nuclear Regulatory Commission, (i.e., radiation exposure to female subjects should be kept "as low as reasonably achievable" and if possible, kept at zero potential for radiation exposure by not including females in metabolism and excretion studies and only enrolling/dosing male subjects).*
- *The study excluded subjects who took any prescription medications/products or any OTC, nonprescription preparations (including vitamins, minerals, herbal supplements etc.) within 7 days prior to check-in or during the study. This ensured that the PK of SAGE 547 is not impacted by extrinsic factors.*
- *The study only included healthy subjects and thus excluded subjects with organ disease or organ impairments (i.e., hepatic or renal impairments) that may have either compromised subject safety or interfered with the evaluation of PK, mass balance of SAGE 547.*

6. *Pharmacokinetic findings: We agree with the sponsor's PK, mass balance and metabolite identification analysis and conclusions from the study.*

CLINICAL PHARMACOLOGY STUDY REVIEW

PK Study in Hepatic Impaired

Study # 547-CLP-103	**Study Period:** 23-Dec-2015 to 23-May-2016
NDA 211371_Zulresso_Brexanolone IV infusion	
Title	OPEN-LABEL, NONRANDOMIZED, SINGLE-DOSE, PARALLEL GROUP, SAFETY, TOLERABILITY, AND PHARMACOKINETIC STUDY OF SAGE-547 ADMINISTERED BY INTRAVENOUS INFUSION TO HEALTHY SUBJECTS AND SUBJECTS WITH HEPATIC IMPAIRMENT
Objectives:	The objectives of this study were to evaluate the pharmacokinetic (PK) profile and the safety and tolerability of SAGE-547 in subjects with hepatic impairment compared to healthy subjects.

Study Design:

Methodology: SAGE-547 Injection is a proprietary formulation of allopregnanolone (scientific name), al referred to as brexanolone (USAN). Throughout this study report, "concentrations of SAGE-547" is syno with "concentrations of allopregnanolone".

This was an open-label, nonrandomized, parallel-group study that investigated the safety, tolerability, and profile of SAGE-547 as a single intravenous (IV) infusion administered over a period of 4 hours to subjec mild, moderate, or severe hepatic impairment and healthy subjects with normal hepatic function (control). doses administered were 30 µg/kg/h for 1 hour, followed by 60 µg/kg/h for 1 hour, and followed by 90 µg for 2 hours. Each subject in the control cohort was to be demographically comparable to at least one subj impaired hepatic function with respect to age (± 10 years), sex, and body mass index (BMI; ± 20%). Subj with severe hepatic disease were only to be enrolled after the mild, moderate, and control cohorts complet the interim PK and safety analyses demonstrated that the participation of subjects with severe hepatic imp would not be contraindicated.

There were up to four cohorts in this study. The mild, moderate, and control cohorts were to enroll eight each, and up to eight subjects were to be enrolled in the severe cohort, for a total of up to 32 subjects at m sites in the United States. Subjects were assigned into cohorts based on their level of hepatic function in accordance with the Child-Pugh classification.

Methodology (continued): Following a Screening Period of up to 27 days, subjects who met eligibility c were admitted to the clinical site on Day -1 (Check-in) and received SAGE-547, as a single IV infusion of 30 µg/kg/h for 1 hour, 60 µg/kg/h for 1 hour, and 90 µg/kg/h for 2 hours, on Day 1. SAGE-547 was admi by trained study personnel. Subjects were discharged from the clinical site on Day 3 (if appropriate as determined by the Investigator) and returned for a Follow-Up Visit on Day 7 (± 1 day) postdose. Serial bl samples for determination of plasma concentrations of SAGE-547 were collected prior to dose administra various time points and with each dose escalation through 4 hours (just prior to the end of infusion, while pump was still on), and up to 48 hours after the start of infusion. Urine samples were collected predose, 0 to 6, 6 to 8, 8 to 12 hours postdose, and then from 12 to 24 and 24 to 48 hours postdose.

After the mild, moderate, and control cohorts completed SAGE-547 administration, interim safety and PK analyses were conducted prior to including subjects with severe hepatic impairment in the study. Additio interim data reviews may have occurred at any time, if deemed appropriate based on safety, tolerability, o information. Results of interim analyses may have led to potential changes in the design of the study, incl alterations in PK and/or clinical laboratory blood sampling times; and/or a change in the dose or dosing re given to subjects; or may have led to decisions to exclude a cohort (subjects with severe liver impairment) on overall assessment of safety and PK data. Such changes would not have constituted a protocol deviatic

Number of subject (planned and analyzed):

Up to 32 subjects (eight per cohort) were planned and 32 (8 per cohort) were analyzed.

Diagnosis and main criteria for inclusion: Subjects with hepatic impairment (as classified by Child-Pu scoring criteria [mild: Child-Pugh Grade A, score five to six; moderate: Child-Pugh Grade B, score s nine; or severe: Child-Pugh Grade C, score ten to 15]) or healthy subjects

Test product, dose and mode of administration, batch number:

SAGE-547 Injection 5 mg/mL, diluted with sterile water for injection. Continuous IV infusion at increas doses over 4 hours: 30 µg/kg/h for 1 hour, 60 µg/kg/h for 1 hour, and 90 µg/kg/h for 2 hours. The study number for SAGE-547 was B150493.

Duration of treatment: 4 hours

Table 2: Study Cohorts

Cohort	Description of Hepatic Function[a]	Points from Child-Pugh Assessment	Number of Subjects	Dose of SAGE-547
1	Mild hepatic impairment (good operative risk)	five to six	eight	30 µg/kg/h for 1 hour, 60 µg/kg/h for 1 hour, and 90 µg/kg/h for 2 hours
2	Moderate hepatic impairment (moderate operative risk)	seven to nine	eight	
3	Severe hepatic impairment (poor operative risk)[b]	ten to 15	up to eight	
4	Normal hepatic function (control)	not applicable	eight	

[a] Hepatic function was determined using the Child-Pugh assessment.
[b] Subjects with severe hepatic impairment were only to be enrolled after the mild, moderate, and control cohorts completed and the interim PK and safety analyses demonstrated that the participation of subjects with severe hepatic impairment would not be contraindicated.

Figure 1: Study Design Schematic

	Inpatient Confinement Period			
Screening	Check-in	Treatment (T) and Assessments	Assessments and Clinic Discharge	Follow-up Visit
Days -28 to -2	Day -1	Day 1 (T), Day 2	Day 3	Day 7 (± 1 day)

T = treatment

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Product Used:

Test product, dose and mode of administration, batch number:

SAGE-547 Injection 5 mg/mL, diluted with sterile water for injection. Continuous IV infusion at increas
doses over 4 hours: 30 µg/kg/h for 1 hour, 60 µg/kg/h for 1 hour, and 90 µg/kg/h for 2 hours. The study
number for SAGE-547 was B150493.

Route of Administration	IV infusion
PK Sampling Times and Parameters	Pharmacokinetic blood samples were collected on Day 1, Hour 0 (predose), 30 minutes, approximately 60 minutes (just prior to dose adjustment from 30 µg/kg/h to 60 µg/kg/h), 90 minutes, approximately 120 minutes (just prior to dose adjustment from 60 µg/kg/h to 90 µg/kg/h), 150 minutes, 210 minutes, approximately 240 minutes (just prior to end of infusion, while pump was still on) after the start of infusion, and at 0.5, 1, 2, 3, 4, 8, 12, 20, 32, and 44 hours after the end of the infusion. Additionally, a PK sample was collected just prior to any unplanned dose adjustment (i.e., in the event that a subject became overly sedated or dosing of severe hepatic impairment subjects). If the infusion duration was less than 240 minutes, then a PK sample was to be collected just prior to

	end of infusion, while pump was still on, and then post-infusion samples were to be collected at the same time points after the end of the infusion as specified above. Additional blood samples to determine unbound concentration and fraction unbound of SAGE-547 were collected approximately 60 minutes after the start of infusion (just prior to dose adjustment from 30 µg/kg/h to 60 µg/kg/h) and just prior to the end of the infusion, while the pump was still on (above for PK samples apply, excluding post-infusion time points). Urine samples were collected predose and within 0 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24 (Day 2), and 24 to 48 hours (Day 3 [Clinic Discharge]) postdose.
Safety Parameters	Safety data (including laboratory values and AEs) were reviewed on an ongoing basis throughout the course of the study by the Medical Monitoring team (b) (4) (b) (4) and any findings were discussed with Sage.
PK Moieties	Sage-547
PD Endpoint(s)	None
Statistical Methods	The Safety Population consisted of all subjects who started the infusion of SAGE-547. The PK Population consisted of all subjects who started the infusion of SAGE-547 and had evaluable PK data. Summary statistics and statistical analyses were performed for subjects included in the relevant analysis populations (Safety/PK). Plasma concentrations and PK parameters were summarized by hepatic function status cohort using descriptive statistics; supporting figures were presented, as appropriate. The effect of hepatic impairment in the mild, moderate, and severe cohorts (test cohorts) was compared to the control cohort (reference cohort). The dosenormalized PK parameters AUC0-∞ and Cmax were log transformed (base e) prior to statistical analysis and analyzed using Proc Mixed procedure in SAS®. The model included hepatic function cohorts fixed effect. Least

	squares mean differences were calculated between the subjects with mild, moderate, and severe hepatic impairment and subjects with normal hepatic function. The residual variance from the mixed model was used to calculate the 90% confidence interval for the difference between the test and reference cohorts. These values were back-transformed to give the ratio of geometric least square means of the test cohorts relative to the reference cohort and the 90% confidence interval for the ratio. No adjustment was made for multiplicity. For each hepatic impairment cohort, the cohort was concluded to be bioequivalent to the control cohort if the 90% confidence interval for the ratio of geometric means for the respective hepatic impairment cohort were contained within the interval of 80% to 125% of the reference cohort for both dose-normalized Cmax and AUC0-∞. Treatment-emergent AEs were summarized by hepatic function cohort, severity, and relationship to the study drug. Chemistry, hematology, coagulation, vital sign, ECG, and SSS data were summarized along with the changes from baseline by hepatic function cohort.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547, metabolites were not assessed		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- N= 32 subjects (8 per cohort: 4 cohorts= heathy, mild HI, moderate HI and severe HI)

Number of subject (planned and analyzed): Up to 32 subjects (eight per cohort) were planned and 32 (8 per cohort) were analyzed.
Diagnosis and main criteria for inclusion: Subjects with hepatic impairment (as classified by Child-Pu scoring criteria [mild: Child-Pugh Grade A, score five to six; moderate: Child-Pugh Grade B, score s nine; or severe: Child-Pugh Grade C, score ten to 15]) or healthy subjects

Table 1: Demography of subjects

Characteristics	Hepatic Impairment Cohort				
	Normal Function N = 8	Mild N = 8	Moderate N = 8	Severe N = 8	Overall N = 32
Gender, n (%)					
Male	5 (62.5)	7 (87.5)	6 (75.0)	4 (50.0)	22 (68.8)
Female	3 (37.5)	1 (12.5)	2 (25.0)	4 (50.0)	10 (31.3)
Age (years)					
Mean (SD)	54.0 (6.89)	56.6 (6.21)	54.9 (4.36)	56.4 (6.63)	55.5 (5.90)
Minimum, Maximum	44, 66	48, 66	47, 62	47, 65	44, 66
Median	52.5	57.0	55.0	56.0	55.5
Race, n (%)					
White	5 (62.5)	7 (87.5)	7 (87.5)	7 (87.5)	26 (81.3)
Black/African American	3 (37.5)	0	1 (12.5)	0	4 (12.5)
Other	0	1 (12.5)	0	1 (12.5)	2 (6.3)
Ethnicity, n (%)					
Hispanic/Latino	4 (50.0)	1 (12.5)	3 (37.5)	6 (75.0)	14 (43.8)
Not Hispanic/Latino	4 (50.0)	7 (87.5)	5 (62.5)	2 (25.0)	18 (56.3)
Height – Screening (cm)					
Mean (SD)	170.38 (12.919)	173.60 (5.888)	176.50 (13.360)	169.88 (9.746)	172.59 (10.707)
Minimum, Maximum	156.0, 198.0	165.0, 182.0	155.0, 199.5	153.0, 180.0	153.0, 199.5
Median	169.25	175.00	175.50	173.00	173.25
Body weight – Screening (kg)					
Mean (SD)	80.26 (20.668)	78.03 (9.464)	87.46 (21.493)	90.55 (16.655)	84.08 (17.624)
Minimum, Maximum	56.0, 121.5	63.3, 90.9	51.6, 124.6	65.5, 122.0	51.6, 124.6
Median	77.40	78.30	85.05	87.80	82.20
Body mass index – Screening (kg/m^2)					
Mean (SD)	27.20 (3.158)	25.91 (2.967)	27.74 (4.370)	31.36 (4.997)	28.05 (4.291)
Minimum, Maximum	23.0, 31.8	20.2, 30.0	21.5, 35.0	22.9, 38.5	20.2, 38.5
Median	27.00	26.00	27.60	32.50	27.35
Child-Pugh total score – Screening					
Mean (SD)	–	5.4 (0.52)	7.8 (0.71)	11.1 (0.83)	–
Minimum, Maximum	–	5, 6	7, 9	10, 12	–
Median	–	5.0	8.0	11.0	–

Abbreviations: SD = standard deviation
Source: Table 14.1.2; Table 14.1.3; Listing 16.2.4.2; Listing 16.2.4.4

Inclusion Crietria:

1. Males or females, between 18 and 75 years of age (inclusive), with a BMI range of 18 to 40 kg/m^2 (inclusive).
2. Negative test for selected drugs of abuse (excluding alcohol) at screening and at check-in. A positive test for approved prescriptions, such as opioids, was acceptable.
3. Females were to be nonpregnant, nonlactating, and either postmenopausal for at least 1 year (follicle-stimulating hormone levels were assessed at screening), surgically sterile (eg, tubal ligation, hysterectomy) for at least 90 days, or from the time of signing the informed consent or 10 days prior to check-in until 30 days after clinic discharge agreed to use one of the following forms of contraception: nonhormonal intrauterine device with spermicide; female condom with spermicide; contraceptive sponge with spermicide;

diaphragm with spermicide; cervical cap with spermicide; male sexual partner who agreed to use a male condom with spermicide; or sterile sexual partner. For all females of childbearing potential, the pregnancy test result must have been negative at screening and check-in.

4. Males were either sterile or from check-in until 90 days following clinic discharge agreed to use one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an intrauterine device with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system; a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects were to refrain from sperm donation from check-in until 90 days following clinic discharge.
5. Subjects were able to comprehend and willing to sign an ICF.
6. Subjects had venous access sufficient to allow for blood sampling as per the protocol.
7. Subjects were reliable and willing to make themselves available for the duration of the study and were willing to adhere to the prohibitions and restrictions specified in this protocol.

3.1.2. Subjects with Hepatic Impairment

8. The extent of hepatic impairment, as classified by Child-Pugh scoring criteria, was consistent with mild hepatic impairment (Child-Pugh Grade A, score five to six), moderate hepatic impairment (Child-Pugh Grade B, score seven to nine), or severe impairment (Child-Pugh Grade C, score ten to 15). Scoring was to be based on serum bilirubin and albumin levels, prolonged seconds over prothrombin time control or international normalized ratio, and the presence of ascites or hepatic encephalopathy, which are scored on a scale of one to three (mild to severe), with a possible maximum score of 15. Attempts were to be made to include subjects in each classification (mild, moderate, and severe) who were representative of the spectrum from the lower through upper boundary of Child-Pugh scores per cohort.
9. Subjects had a diagnosis of chronic hepatic impairment (>6 months) with no clinically significant changes within 90 days prior to study drug administration.
10. A stable medication regimen, defined as not starting a new drug(s), or not starting a clinically significant change in dosage(s) within 30 days prior to administration of the study drug, was required. Concomitant medications must have been approved by the Medical Monitor and/or Sage, and Investigator.
11. Abnormal laboratory values, judged by the Investigator and Medical Monitor to be compatible with the hepatic impairment of the subject, were acceptable; anemia secondary to hepatic disease was acceptable if hemoglobin was ≥9 g/dL and anemia symptoms were not clinically significant.

12. Subjects exhibited vital signs within the reference range for their age and level of hepatic impairment; subjects with vital signs outside the reference ranges may have been eligible for the study if the Investigator, Medical Monitor, and/or Sage felt that the results were not clinically significant, based on the age and hepatic impairment status of the subject, and would not impact study conduct.
13. Subjects had no clinical exacerbation of liver disease within the Screening Period or prior to study drug administration.
14. Subjects had not had a portal systemic shunt, which included portal systemic shunts and transjugular intrahepatic portosystemic shunt.
15. Subjects had shown no evidence of hepatorenal syndrome.
16. Subjects had not required hospitalization for an exclusionary reason, as determined by the Investigator, Medical Monitor, and/or Sage within 30 days prior to check-in (Day -1) or had a Grade 3 encephalopathy within the last 6 months.
17. Subjects had not exhibited evidence of acute viral hepatitis within the Screening Period or prior to study drug administration.
18. Subjects were in good general health, allowing for the concurrent illnesses associated with liver disease.

9.3.1.3. Healthy Subjects

19. Subjects had no clinically significant illness or disease as determined by medical history, physical examination, vital signs, and 12-lead ECG.
20. Subjects with stable, chronic medical conditions (eg, hypertension and hyperlipidemia) that, in the opinion of the Investigator, would not significantly alter the disposition of the drug, would not place the subject at increased risk by participating in the study, and would not interfere with interpretation of the data may have been permitted after discussion and agreement between the Investigator, Medical Monitor, and/or Sage.
21. Subjects had normal hepatic function.
22. Subjects were demographically comparable to the hepatically-impaired subjects.
23. The results of clinical laboratory evaluations (including clinical chemistry panel [after a minimum of 10 hours fasting], complete blood count, and urinalysis) were within the reference range for the test laboratory and for the population, or results with acceptable deviations were judged not to be clinically significant by the Investigator, Medical Monitor, and/or Sage. Clinically significant deviations associated with stable, chronic medical conditions permitted above may have been allowed after discussion and agreement between the Investigator, Medical Monitor, and Sage.

Exclusion Criteria:

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1. History of any active infection within 30 days prior to Day 1, if deemed clinically significant by the Investigator, Medical Monitor, and/or Sage.
2. Hospitalization within 30 days prior to check-in, unless the reason was considered not exclusionary by the Investigator, Medical Monitor, and/or Sage.
3. Received live vaccine(s) within 30 days of screening, or intend to during the study (Influenza vaccine was allowed, if administered >21 days prior to dosing).
4. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.
5. History or presence of an abnormal ECG, which, in the Investigator's opinion, was clinically significant.
6. History of alcoholism or drug addiction within 1 year prior to screening per the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition criteria.
7. Had an average weekly alcohol intake that exceeded 21 units per week (males up to age 65) and 14 units per week (males over 65 and females). One unit = 12 ounces or 360 mL of beer; 5 ounces or 150 mL of wine; 1.5 ounces or 45 mL of distilled spirits.
8. Was unwilling to stop alcohol consumption within 48 hours prior to check-in on Day -1 (as confirmed by alcohol breath test) and for the duration of the confinement period.
9. Current heavy users of nicotine (ie, smoking more than 20 cigarettes [eg, one pack] per day or equivalent) (eg, e-vapor cigarette, pipe, cigar, chewing tobacco, nicotine patch or nicotine gum).
10. Could not comply with the smoking restrictions of the study site during confined periods or were unable or unwilling to refrain from smoking and tobacco use for 2 hours prior to dosing and 4 hours following dose administration.
11. Regular use of known drugs of abuse and/or showed positive urine screen for drugs of abuse (excluding cotinine or approved prescriptions, such as opioids) at screening or Day -1; need for special dietary restrictions, unless the restrictions were approved by the Investigator, Medical Monitor, and/or Sage.
12. Participation in any other investigational study drug trial in which receipt of an investigational study drug occurred within five half-lives or 30 days, whichever was longer, prior to check-in (Day -1).
13. A positive test human immunodeficiency virus (HIV) antibody and/or showed evidence of HIV infection.
14. Hypersensitivity to the study drug.
15. Donation of blood from 30 days prior to screening through study discharge, inclusive, or of plasma from 14 days prior to screening through study discharge, inclusive.
16. Receipt of blood products within 60 days prior to check-in.

17. Evidence of any significant active disease other than that responsible for, or associated with, hepatic impairment that would significantly impact the ability of the subject to safely participate in the trial, in the opinion of the Investigator and Medical Monitor. This included gastroenterologic (other than hepatic cirrhosis), cardiac (eg, myocardial infarction in the past year, angina, or congestive heart failure), renal, respiratory, hematologic, neuropsychiatric, or neoplastic disease (basal or squamous cell skin cancer was acceptable).
18. Fulminant hepatitis.
19. History of transplant or currently on transplant list.
20. Variceal bleeding within 90 days of check-in (unless banded).
21. Severe ascites.
22. Presence of hepatocellular carcinoma.
23. Encephalopathy Grade ≥2.
24. Creatinine clearance <60 mL/min (calculated using the Cockroft-Gault equation) for Child-Pugh Grade C.
25. Acutely declining hepatic function.
26. A platelet count <30,000/μL or increased risk of bleeding.
27. Change in any clinical laboratory value from screening to Day -1 that was considered by the Investigator, Medical Monitor, and/or Sage to be clinically significant.

9.3.2.3. Healthy Subjects

28. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neoplastic (with the exception of basal or squamous cell cancer), neurological, or psychiatric disorder (as determined by the Investigator) capable of significantly altering the absorption of drugs; of constituting a risk when taking the study drug; or of interfering with the interpretation of the data.
29. History of bleeding or coagulopathy.
30. History of acute or chronic hepatic disease or significantly abnormal liver function tests (>1.5 times the upper limit of normal) at screening.
31. Creatinine clearance <80 mL/min.
32. Showed evidence of hepatitis B and/or positive hepatitis B surface antigen.
33. Showed evidence of hepatitis C and/or positive hepatitis C antibody.
34. Any subject determined by the Investigator to have a clinically significant comorbidity that could have affected the outcomes of the study.

35. Use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.
36. Use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17.
37. Use of any medications (prescription or over-the-counter), herbal tea, energy drinks, herbal products (eg, St. John's Wort, milk thistle), or supplement/supra-therapeutic doses of vitamins within 14 days prior to Day 1 and throughout the duration of the study, with the exception of those approved by the Investigator, Medical Monitor, and/or Sage. The exceptions, allowed as needed, were: prespecified medications (eg, antiviral, antihypertensives, diuretics, insulin, cholesterol-lowering agents, beta blockers, opioids) with a stable dose regimen established >30 days prior to study start and over-the-counter analgesics (other than antiplatelets) and stool softener.
38. Use of antiplatelets or anticoagulants within 30 days prior to study drug administration and throughout the study.
39. Use of benzodiazepines within 14 days, and antiepileptic medications within 30 days, prior to study drug administration and throughout the study.

New drugs were reviewed on a case-by-case basis by the Investigator, Medical Monitor, and/or Sage and were prohibited, unless deemed acceptable by the Investigator, Medical Monitor, and/or Sage.

PK Results

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PHARMACOKINETICS RESULTS:

Systemic exposure to total SAGE-547 generally decreased with increasing degree of hepatic impairment, geometric LS mean DN C_{max} values 5.2%, 20.6%, and 42.4% lower and geometric LS mean DN $AUC_{0-\infty}$ 10.8%, 8.0%, and 24.1% lower in the mild, moderate, and severe hepatic impairment cohorts, respectively compared with the normal hepatic function cohort.

Fraction of SAGE-547 unbound in plasma was 0.00625 in healthy subjects and increased by 10.4%, 52.8 85.6% in the mild, moderate, and severe hepatic impairment cohorts, respectively, compared to the norma hepatic function cohort.

Systemic exposure to unbound SAGE 547, based on geometric LS mean DN $AUC_{0-\infty,u}$ values, was simila between the mild hepatic impairment and normal hepatic function cohorts, while it increased by 35.9% ar 44.9% for moderate and severe hepatic impairment cohorts, respectively. There was no marked effect of impairment on DN $C_{max,u}$.

Decreasing exposure to total SAGE-547 and increasing exposure to unbound SAGE-547 concentrations a hepatic impairment increases based on AUC determinations is commensurate with the increase in free fra and can be associated directly to clearance processes. Mixed effects on total and unbound C_{max} highlight more complex relationship between this parameter, the volume of distribution, and clearance, but are also consistent with the observed differences in protein binding.

Table 8: Pharmacokinetic Parameters of Total SAGE-547 in Plasma Following Intravenous Infusion to Healthy Subjects and Subjects with Hepatic Impairment (Pharmacokinetic Population)

	Hepatic Impairment Cohort			
	Normal Function	Mild	Moderate	Severe
Parameter (units)	N = 8	N = 8	N = 8	N = 8
C_{max} (ng/mL)	85.2 (28.9)[a]	80.4 (35.0)	67.4 (23.6)[a]	49.1 (35.7)
DN C_{max} (ng/mL/mg/kg)	316 (29.2)[a]	300 (35.2)	251 (23.5)[a]	182 (35.1)
t_{max}[b] (h)	4.00 (3.92-4.00)[a]	3.97 (3.50-4.00)	3.92 (2.50-4.00)[a]	4.00 (2.50-4.50)
AUC_{0-t} (h*ng/mL)	286 (21.3)[a]	259 (25.5)	259 (24.7)[a]	225 (19.4)
DN AUC_{0-t} (h*ng/mL/mg/kg)	1080 (20.2)	965 (25.8)	963 (24.7)[a]	834 (19.1)
$AUC_{0-\infty}$ (h*ng/mL)	314 (17.7)[c]	282 (20.9)[a]	291 (28.9)[c]	241 (13.7)[c]
DN $AUC_{0-\infty}$ (h*ng/mL/mg/kg)	1180 (16.2)[a]	1050 (21.2)[a]	1080 (28.9)[c]	892 (13.5)[c]
λ_Z (1/h)	0.103 (56.6)[c]	0.174 (23.5)[a]	0.0612 (70.2)[c]	0.0540 (72.2)[c]
$t_{1/2}$[d] (h)	7.58 (4.11)[c]	4.07 (0.942)[a]	13.2 (7.09)[c]	14.8 (7.49)[c]
CL (L/h/kg)	0.856 (17.7)[c]	0.953 (21.2)[a]	0.924 (28.9)[c]	1.12 (13.5)[c]
V_z (L/kg)	8.33 (68.7)[c]	5.47 (21.6)[a]	15.1 (42.3)[c]	20.7 (63.8)[c]
V_{ss} (L/kg)	3.28 (74.9)[c]	2.21 (27.5)[a]	6.46 (45.3)[c]	10.9 (78.1)[c]

Abbreviations: λ_Z = apparent terminal elimination rate constant; AUC_{0-t} = area under the plasma concentration-time curve from time zero up to the time of the last quantifiable plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; CL = total clearance; C_{max} = time to maximum observed plasma concentration; CV = coefficient of variation; DN AUC_{0-t} = dose-normalized AUC_{0-t}; DN $AUC_{0-\infty}$ = dose-normalized $AUC_{0-\infty}$; DN C_{max} = dose-normalized C_{max}; SD = standard deviation; $t_{½}$ = apparent terminal elimination half-life; t_{max} = time to maximum observed plasma concentration; V_{ss} = volume of distribution at steady-state; V_z = volume of distribution during the terminal phase

[a] N = 7

[b] Median (min, max) presented for t_{max}.

[c] N = 6

[d] Arithmetic mean (SD) presented for $t_{½}$.

Note: Geometric mean (CV%) data are presented unless otherwise stated.

For Subject (b) (6) (normal), all pharmacokinetic parameters except for DN AUCs were excluded from descriptive statistics since the subject received 60 µg/kg/h instead of 90 µg/kg/h for the last 50 minutes of the 4-hour infusion.

For Subject (b) (6) (moderate), all pharmacokinetic parameters were excluded from descriptive statistics since the subject's plasma pharmacokinetic profile was considered anomalous.

$AUC_{0-\infty}$, DN $AUC_{0-\infty}$, λ_z, $t_{½}$, CL, V_z, and V_{ss} could not be calculated for Subjects (b) (6) (mild), (b) (6) (moderate), (b) (6) (severe), and (b) (6) (normal) since λ_z and $t_{½}$ could not be reliably estimated.

Source: Table 14.2.2.1

Table 9: Pharmacokinetic Parameters of Unbound SAGE-547 in Plasma Following Intravenous Infusion to Healthy Subjects and Subjects with Hepatic Impairment (Pharmacokinetic Population)

	Hepatic Impairment Cohort			
	Normal Function	Mild	Moderate	Severe
Parameter (units)	N = 8	N = 8	N = 8	N = 8
$C_{max,u}$ (ng/mL)	0.536 (28.9)[a]	0.554 (28.0)	0.636 (19.7)[a]	0.569 (34.6)
DN $C_{max,u}$ (ng/mL/mg/kg)	1.99 (29.2)[a]	2.07 (28.2)	2.36 (19.2)[a]	2.11 (34.1)
$AUC_{0-t,u}$ (h*ng/mL)	1.80 (21.7)[a]	1.79 (21.0)	2.44 (28.9)[a]	2.61 (21.3)
DN $AUC_{0-t,u}$ (h*ng/mL/mg/kg)	6.74 (20.3)	6.66 (21.2)	9.07 (28.5)[a]	9.66 (21.1)
$AUC_{0-\infty,u}$ (h*ng/mL)	1.97 (19.2)[b]	1.90 (19.7)[a]	2.67 (32.5)[b]	2.86 (21.1)[b]
DN $AUC_{0-\infty,u}$ (h*ng/mL/mg/kg)	7.32 (17.6)[a]	7.09 (19.8)[a]	9.94 (32.2)[b]	10.6 (21.2)[b]
CL_u (L/h/kg)	137 (19.3)[a]	141 (19.8)[a]	101 (32.2)[b]	94.4 (21.2)[b]
$V_{z,u}$ (L/kg)	1330 (69.6)[a]	810 (22.1)[a]	1640 (58.6)[b]	1750 (58.1)[b]
$V_{ss,u}$ (L/kg)	524 (76.7)[a]	327 (26.6)[a]	703 (57.3)[b]	918 (71.7)[b]
f_u	0.00625 (14.6)	0.00690 (11.7)	0.00955 (21.8)	0.0116 (14.1)

Abbreviations: AUC_{0-t} = area under the plasma concentration-time curve from time zero up to the time of the last quantifiable plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; CL = total clearance; C_{max} = time to maximum observed plasma concentration; CV = coefficient of variation; DN AUC_{0-t} = dose-normalized AUC_{0-t}; DN $AUC_{0-\infty}$ = dose-normalized $AUC_{0-\infty}$; DN C_{max} = dose-normalized C_{max}; f_u = fraction unbound; V_{ss} = volume of distribution at steady-state; V_z = volume of distribution during the terminal phase

[a] N = 7

[b] N = 6

Note: Geometric mean (CV%) data are presented unless otherwise stated.

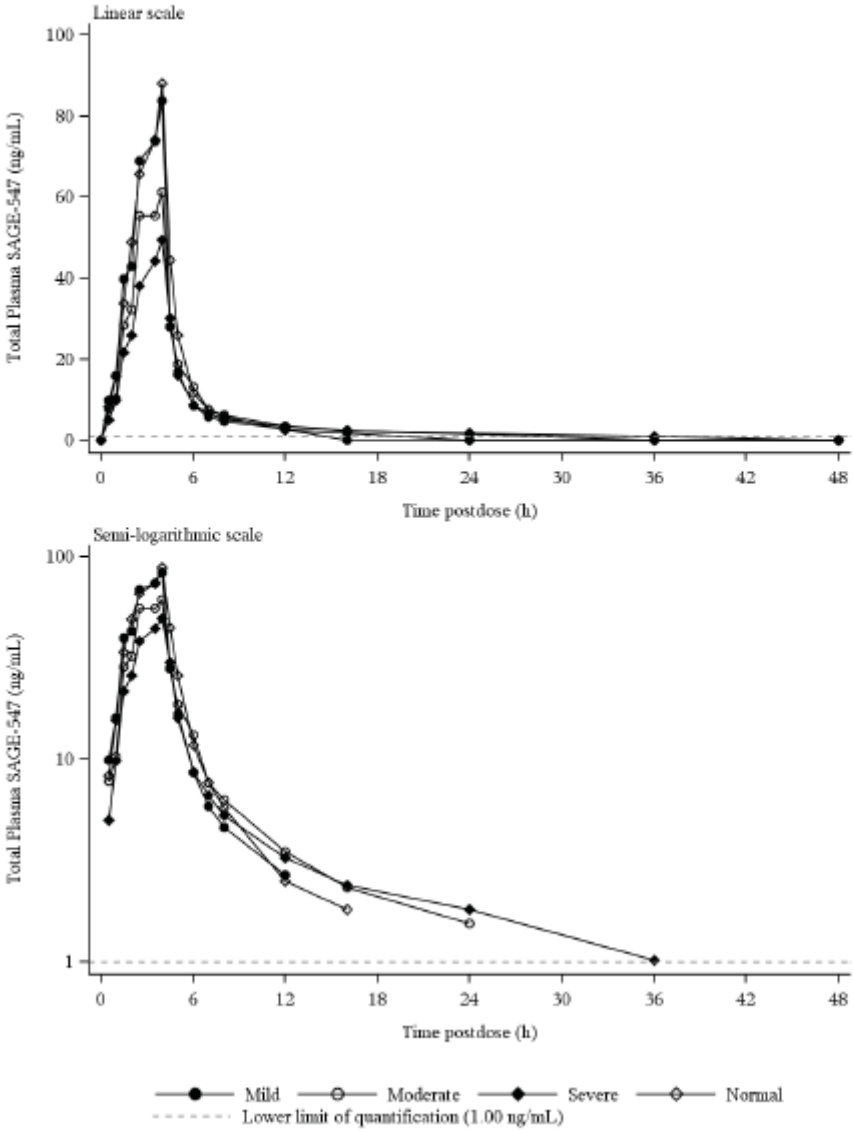
For Subject (b) (6) (normal), all pharmacokinetic parameters except for DN AUCs were excluded from descriptive statistics since the subject received 60 µg/kg/h instead of 90 µg/kg/h for the last 50 minutes of the 4-hour infusion.

For Subject (b) (6) (moderate), all pharmacokinetic parameters were excluded from descriptive statistics since the subject's plasma pharmacokinetic profile was considered anomalous.

$AUC_{0-\infty}$, DN $AUC_{0-\infty}$, λ_z, $t_{½}$, CL, V_z, and V_{ss} could not be calculated for Subjects (b) (6) (mild), (b) (6) (moderate), (b) (6) (severe), and (b) (6) (normal) since λ_z and $t_{½}$ could not be reliably estimated.

Source: Table 14.2.2.2

Figure 2: Arithmetic Mean Total Plasma Concentration-Time Profiles of SAGE-547 Following Intravenous Infusion to Healthy Subjects and Subjects with Hepatic Impairment (Linear and Semi-Logarithmic Scale)



Source: Table 14.2.1.1 and Figure 14.2.1.1

Based on the statistical analysis, geometric LS mean DN $AUC_{0-\infty,u}$ values were similar between the mild hepatic impairment cohort and normal hepatic function cohort, while the geometric LS mean DN $AUC_{0-\infty,u}$ values for the moderate and severe hepatic impairment cohorts were 35.9 and 44.9% higher, respectively, than the normal hepatic function cohort (Table 11). Geometric LS mean DN $C_{max,u}$ values were generally similar across all hepatic function cohorts, being approximately 3.9%, 18.9%, and 6.1% higher for the mild, moderate, and severe hepatic impairment cohorts, respectively, when compared with that of the normal hepatic function cohort. For each parameter and each comparison, the 90% CI was not entirely contained within the interval of 80 to 125%. It is notable that 90% CIs excluded unity only for DN $AUC_{0-\infty,u}$ for

the moderate and severe impairment cohorts.

Table 11: Statistical Analysis of the Unbound Plasma Pharmacokinetic Parameters of SAGE-547 (Pharmacokinetic Population)

Parameter (units)	Hepatic Function Cohort	N	Geometric LS Mean[a]	Comparison	Ratio of Geometric[b] LS Means (Test to Reference [%])	90% CI for Geometric LS Mean Ratio[c] of (Test to Reference)
DN $AUC_{0-\infty,u}$ (h*ng/mL/mg/kg)	Mild	7	7.09	Mild vs Normal	96.9	(78.7, 119.3)
	Moderate	6	9.94	Moderate vs Normal	135.9	(109.5, 168.7)
	Severe	6	10.6	Severe vs Normal	144.9	(116.7, 179.8)
	Normal	7	7.32	--	--	--
DN $C_{max,u}$ (ng/mL/mg/kg)	Mild	8	2.07	Mild vs Normal	103.9	(81.3, 132.8)
	Moderate	7	2.36	Moderate vs Normal	118.9	(92.3, 153.3)
	Severe	8	2.11	Severe vs Normal	106.1	(83.0, 135.7)
	Normal	7	1.99	--	--	--

Abbreviations: $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; CI = confidence interval; C_{max} = time to maximum observed plasma concentration; DN = dose normalized; LS = least squares
Subject (b) (6) (moderate hepatic impairment cohort) was excluded from statistical analysis for DN C_{max} since the subject's plasma pharmacokinetic profile was considered anomalous (DN $AUC_{0-\infty}$ could not be calculated for this subject). Subject (b) (6) (normal hepatic function cohort) was excluded from statistical analysis for DN C_{max} since the subject received 60 ug/kg/h instead of 90 ug/kg/h for the last 50 minutes of the 4-hour infusion. Since λ_z and $t_{½}$ could not be reliably estimated, DN $AUC_{0-\infty}$ could not be calculated for the following subjects: (b) (6) (mild), (b) (6) (moderate), (b) (6) (severe), and (b) (6) (normal).

[a] Least squares means from the analysis of variance model of natural log data were transformed back to the linear scale using the exponential function.

[b] LS Mean difference between test and reference of log transformed data were transformed back to the linear scale (expressed as a percent).

[c] 90% confidence interval for the LS mean difference of log transformed data were transformed back to the linear scale (expressed as a percent).

Source: Table 14.2.3.2; Listing 16.2.5.7

Safety Results

Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA

A total of six of the 32 subjects experienced treatment-emergent adverse events (TEAEs). No subject diec a serious or severe TEAE. Four subjects had TEAEs that were considered at least possibly related to treat with SAGE-547. There were no discontinuations due to TEAEs. The only TEAEs reported in more than subject across cohorts were headache (two subjects, one each in the mild and severe hepatic impairment c and somnolence (two subjects, one each in the normal and mild hepatic impairment cohorts).

One subject in the normal hepatic function cohort had the infusion rate reduced to 60 µg/kg/h due to mode somnolence that began 3 hours after the start of the infusion. The infusion was continued at the lower dos the subject completed the study.

Laboratory test results, vital signs, and ECG parameters were unremarkable over the course of the study. Generally, SSS scores were higher in the normal and mild hepatic impairment cohorts compared to the mc and severe hepatic impairment cohorts. Peak SSS scores were seen between 2 to 4 hours post start of infu normal, mild hepatic impairment, and moderate impairment cohorts; there was little change in the SSS rat the severe impairment cohort.

SAFETY RESULTS (continued): No post-infusion suicidal ideations or behaviors were reported via C- or TEAEs.

There were no apparent differences observed in the safety or tolerability of SAGE-547 associated with inc degrees of hepatic impairment.

Table 13: Summary of Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Population)

System Organ Class Preferred Term	Hepatic Impairment Cohort n (%)			
	Normal Function N = 8	Mild N = 8	Moderate N = 8	Severe N = 8
Subjects with any TEAE	2 (25.0)	2 (25.0)	0	2 (25.0)
Nervous System Disorders	1 (12.5)	2 (25.0)	0	1 (12.5)
Headache	0	1 (12.5)	0	1 (12.5)
Somnolence	1 (12.5)	1 (12.5)	0	0
Infections and Infestations	0	1 (12.5)	0	1 (12.5)
Hordeolum	0	0	0	1 (12.5)
Upper respiratory tract infection	0	1 (12.5)	0	0
Injury, Poisoning and Procedural Complications	1 (12.5)	0	0	0
Contusion	1 (12.5)	0	0	0

TEAE = treatment-emergent adverse event
Source: Table 14.3.1.2 and Table 14.3.1.3

Overall Sponsor Conslusions

In addition to changes in enzyme function and liver blood flow, hepatic impairment is known to cause changes in the amount and composition of plasma proteins. Increasing degrees of hepatic impairment result in changes in SAGE-547 pharmacokinetics that are characteristic of altered protein binding. Diminished plasma protein binding liberates SAGE-547, thus enabling its accelerated clearance from blood leading to the rank-ordered decrease in exposure to total (bound + unbound) SAGE-547 that was observed with increased degree of impairment.

Following correction for protein binding, an increase in exposure to unbound SAGE-547 and a corresponding decrease in clearance of unbound SAGE-547 was observed as impairment increased. Because SAGE-547 is a high clearance, high extraction compound, this change is most likely related to changes in hepatic clearance processes such as liver blood flow as opposed to decreased intrinsic enzyme activity.

The largest magnitude change in exposure to unbound SAGE-547 was observed in $AUC_{0-\infty}$ for the severe hepatic impairment cohort, which exhibited a 144.9% increase compared to the normal hepatic function cohort. In context, there is a 150% margin between 60 and 90 µg/kg/h, the doses currently under investigation for postpartum depression and a 167% margin between 90 and 150 µg/kg/h, the doses under study in super-refractory status epilepticus. While the present study was not powered to make formal inferences related to safety outcomes, it is important to note that changes in unbound concentrations of SAGE-547 were not associated with increased reports of somnolence or other safety findings in the hepatic impairment cohorts.

An IV infusion of SAGE-547 was generally well tolerated by subjects with mild, moderate, or severe hepatic impairment and healthy subjects with normal hepatic function.

In consideration of the observed pharmacokinetic changes that are of a similar magnitude to the range of studied doses, as well as the safety results that demonstrate SAGE-547 was well tolerated in this population, this study does not indicate the need for dose adjustment in subjects with hepatic impairment.

Reviewer Comments and Conclusions

7. *Study Design: This was an Open Label, non-randomized, Multi Center, Single Dose, Parallel group, safety, tolerability and PK study of SAGE-547 administered by IV infusion to healthy subjects and subjects with hepatic impairment. The overall study design was acceptable since:*
    - *It was a single dose study conducted at the target dose level (90 ug/kg/h).*
    - *IV dose was chosen because it is the intended route of administration.*
    - *Males and females, between the age of 18 and 75 years were included.*
    - *The subjects in control group were matched and balanced with subjects in hepatic impaired groups w.r.t., age, sex and BMI.*
    - *Adequate number of subjects (N= 32; with 8 subjects per cohort) were included in the study*

- *The final to-be-marketed formulation of SAGE 547 was used in this study.*

8. *Protocol deviation: No major protocol deviations were reported.*

9. *Data Analysis (i.e., any outliers etc.): Data from subject # (b) (6) (moderate hepatic impairment cohort) were not included in the PK analysis. Plasma concentrations were like other subject during the 4 hours of infusion but were anomalously high at 30 min after the infusion had been stopped. Though not documented by study personnel, it is likely that the PK sampling might have been performed from the same port used for the drug infusion leading to sample contamination.*

10. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

11. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *The control group in this study was the matched healthy subjects without hepatic impairment (i.e., comparable to at least one subject with impaired hepatic function with respect to age [±10 years], sex, and BMI [±20%]), which is a standard design element in clinical pharmacology studies designed to inform dosage recommendations for patients with hepatic dysfunction.*
- *The study excluded use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.*
- *The study excluded use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17. This is acceptable because it minimized the chance of any drug interactions.*

12. *Pharmacokinetic findings: We agree with the sponsor's PK analysis and the conclusions from the study.*

Overall Conclusion:
No dose adjustments are recommended for patients with hepatic impairment.

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CLINICAL PHARMACOLOGY STUDY REVIEW

PK Study in Renal Impaired

Study # 547-CLP-104	**Study Period:** 16-Feb-2016 to 22-June-2016
NDA 211371_Zulresso_Brexanolone IV infusion	
Title	Open-Label, Nonrandomized, Single-Dose, Parallel-Group, Safety, Tolerability, and Pharmacokinetic Study of SAGE-547 Administered by Intravenous Infusion to Healthy Subjects and Subjects with Renal Impairment
Objectives:	The objectives of this study were to evaluate: • PK profile of SAGE-547 in subjects with severe renal impairment compared to healthy subjects; • Safety and tolerability of SAGE-547 in subjects with severe renal impairment compared to healthy subjects; and • PK profile, safety, and tolerability of SAGE-547 in subjects with mild and moderate renal impairment (if enrolled) compared to healthy subjects
Study Design:	

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This was an open-label, nonrandomized, parallel-group study that investigated the safety, tolerability, and PK profile of SAGE-547 as a single intravenous (IV) infusion administered over a period of 4 hours to subjects with severe renal impairment (Cohort 1) and healthy subjects with normal renal function (Cohort 2; controls). Additional cohorts of subjects with mild and moderate renal impairment were to be added, if necessary, based on results of the interim analysis. The doses administered were 30 µg/kg/h for 1 hour, followed by 60 µg/kg/h for 1 hour, and followed by 90 µg/kg/h for 2 hours. For all subjects, renal cohort assignment was determined by estimated glomerular filtration rate (eGFR), as calculated using the Modification of Diet in Renal Disease (MDRD) equation.

Subjects were assigned to cohorts based on their level of renal impairment in accordance with the National Kidney Foundation guidelines (Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Chronic Kidney Disease 2002). Subjects in the severe renal impairment cohort were selected based on eGFR values that were represented by the range shown in Table 2. Each subject in the control cohort was to be demographically comparable to at least one subject with impaired renal function with respect to age (±10 years), sex, race, and body mass index (BMI; ±20%) (see Section 9.8). In order to more closely match demographics, control subjects were to be selected after subjects with severe renal impairment had been enrolled.

Table 2: Study Cohorts

Cohort	Number of Subjects[a]	Renal Impairment Cohort[b]	eGFR (mL/min/1.73 m^2)[c,d]
1	8	Severe	15 to 29
2	8	Normal (control)	≥90
Additional Cohorts[a]			
3	8	Mild	60 to 89
4	8	Moderate	30 to 59

eGFR = estimated glomerular filtration rate; MDRD = Modification of Diet in Renal Disease; min = minute

[a] A total of 16 subjects were to be enrolled (eight subjects per cohort). Depending on the results of the interim analysis, additional cohorts of subjects with mild and moderate renal impairment may have been enrolled (eight subjects per cohort).

[b] Stages of renal impairment were based on Kidney Disease Outcomes Quality Initiative Clinical Practice Guidelines for Chronic Kidney Disease from the National Kidney Foundation in 2002.

[c] eGFR: Estimate of GFR was based on the MDRD equation.

[d] For normal control subjects who were older than 70 years of age, eGFR values >75 mL/min/1.73m^2 were accepted.

Source: Guidance for Industry, Food and Drug Administration, Pharmacokinetics in Patients with Impaired Renal Function-Study Design, Data Analysis, and Impact on Dosing and Labeling, March 2010, Clinical Pharmacology, Draft Revision 1.

Following a Screening Period of up to 27 days, subjects who met eligibility criteria were admitted to the clinical research unit on Day -1 (check-in). On Day 1, subjects were administered SAGE-547, as a continuous IV infusion over a period of 4 hours; the dose regime was 30 µg/kg/h for 1 hour, 60 µg/kg/h for 1 hour, and 90 µg/kg/h for 2 hours. Subjects remain

in the clinic until Day 3 (Clinic Discharge) and returned for a Follow-Up Visit, which was to be scheduled on Day 8 (±1 day) postdose.

Serial blood samples for determination of plasma concentrations of SAGE-547 and sulphobutylether beta cyclodextrin (SBECD) were collected prior to dose administration, at various time points during the 4-hour infusions (including just prior to the end of infusion, while the pump was still on), and up to 44 hours after the end of the infusion. If the infusion duration was less than 240 minutes, then a PK sample was collected just prior to the end of the infusion, while the pump was still on, and then post-infusion samples were collected up to 44 hours after the end of the infusion. Urine samples were collected predose, approximately every 2 to 4 hours up to 12 hours postdose, and then from 12 to 24 and 24 to 48 hours postdose.

Safety-related assessments included physical examinations, 12-lead electrocardiograms (ECGs), vital signs, adverse events (AEs), Columbia-Suicide Severity Rating Scale (C-SSRS), and clinical laboratory evaluations. These assessments were performed at screening, at specified times during the study (Inpatient Confinement Period), and at the Follow-Up Visit (see Table 4). Sedation was assessed using the Stanford Sleepiness Scale (SSS) at specified times, or as deemed necessary, and was used to determine if it was safe to proceed with the dosing regimen or if dose adjustments needed to be made (Section 9.5.1.1.7). For this purpose, the SSS was to be performed after all other assessments so that subjects were as awake as possible, or subjects were to be awakened, if necessary, and instructed to report their level of sleepiness. All AEs, whether volunteered, elicited, or noted on physical examination, were to be recorded throughout the study (ie, from screening, Day -1 (check-in) through the remainder of the Inpatient Confinement Period [Days 1, 2, and 3 (Clinic Discharge)] and the Follow-Up Visit).

This study was a reduced design in subjects with severe renal impairment (Cohort 1) and subjects with normal renal function (Cohort 2 [controls]). Subjects with mild and/or moderate renal impairment were only to be enrolled after the severe renal impairment and control cohorts had completed Day 3 (Clinic Discharge) and the interim analysis had been performed. If a two-fold or greater increase in total exposure was observed in the severe renal impairment cohort compared to controls, then a full renal impairment study was to be conducted and additional subjects with mild and moderate renal impairment were to be enrolled. The interim analysis did not show a two-fold or greater increase in total exposure in the severely impaired cohort compared to controls, so the study was completed as a reduced study, with no additional subjects enrolled.

The study design schematic is shown in Figure 1.

Figure 1: Study Design Schematic

		Inpatient Confinement Period		
Screening	Check-in	Treatment (T) and Assessments	Assessments and Clinic Discharge	Follow-Up Visit
Days -28 to -2	Day -1	Day 1 (T), Day 2	Day 3	Day 8 (±1 day)

A copy of the clinical protocol is provided in Appendix 16.1.1. A sample CRF (unique pages only) is provided in Appendix 16.1.2.

Number of subject (planned and analyzed): Up to 32 subjects (eight per cohort) were planned and 17 (nine subjects in the severe renal impairment col and eight subjects in the normal renal function cohort) were enrolled and analyzed.
Diagnosis and main criteria for inclusion: Subjects with renal impairment (as classified by eGFR [seve moderate, or mild]) or healthy subjects
Test product, dose and mode of administration, batch number: SAGE-547 Injection 5 mg/mL, diluted with sterile water for injection. Continuous IV infusion of SAGE- increasing doses over 4 hours: 30 µg/kg/hr for 1 hour, 60 µg/kg/hr for 1 hour, and 90 µg/kg/hr for 2 hours The study drug lot number for SAGE-547 was B150493.

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Product Used:

Test product, dose and mode of administration, batch number: SAGE-547 Injection 5 mg/mL, diluted with sterile water for injection. Continuous IV infusion of SAGE-547 a increasing doses over 4 hours: 30 µg/kg/hr for 1 hour, 60 µg/kg/hr for 1 hour, and 90 µg/kg/hr for 2 hours The study drug lot number for SAGE-547 was B150493.

Route of Administration	IV infusion
PK Sampling Times and Parameters	For each subject, the following PK parameters were calculated based on the model independent approach and, whenever possible, based on the total plasma concentrations of SAGE-547 and SBECD: maximum observed plasma concentration (Cmax), dose normalized Cmax (DN Cmax), time to maximum observed plasma concentration (tmax), area under the serum concentration/time curve (AUC)0-t, DN AUC0-t, AUC0-∞, DN AUC0-∞, apparent terminal elimination rate constant (λz), apparent terminal elimination half-life ($t_{½}$), total clearance (CL), Vz, and Vss. Unbound PK parameters for SAGE-547 may have been calculated using

	fu. For each subject, the following urinary PK parameters were calculated, whenever possible, for SAGE-547 concentrations: amount of SAGE-547 or metabolite excreted into urine over a collection interval, cumulative amount of SAGE-547 or metabolite excreted into urine, percent of administered dose excreted as SAGE-547 into urine over a collection interval, cumulative percent of administered dose excreted as SAGE-547 into urine, and renal clearance calculated only for SAGE-547. Pharmacokinetic blood samples were collected on Day 1, Hour 0 (predose), 30 minutes, approximately 60 minutes (just prior to dose adjustment from 30 µg/kg/h to 60 µg/kg/h), 90 minutes, approximately 120 minutes (just prior to dose adjustment from 60 µg/kg/h to 90 µg/kg/h), 150 minutes, 210 minutes, approximately 240 minutes (just prior to end of infusion, while the pump was still on) after the start of infusion, and at 0.5, 1, 2, 3, 4, 8, 12, 20, 32, and 44 hours after the end of the infusion. Additionally, a PK sample was to be collected just prior to any unplanned dose adjustment (i.e., in the event that a subject became overly sedated or dosing of severe renal impairment subjects). If the infusion duration was less than 240 minutes, then a PK sample was collected just prior to the end of infusion, while the pump was still on, and then post-infusion samples were collected at the same time points after the end of the infusion as specified above up to 44 hours after the end of the infusion. Urine samples were collected predose and within 0 to 4, 4 to 6, 6 to 8, 8 to 12, 12 to 24, and 24 to 48 hours (Day 3 [Clinic Discharge]) postdose
Safety Parameters	Safety-related assessments included physical examinations, 12-lead electrocardiograms (ECGs), vital signs, adverse events (AEs), Columbia-Suicide Severity Rating Scale (C-SSRS), and clinical laboratory evaluations. These assessments were performed at screening, at specified times during the study (Inpatient Confinement Period), and at the Follow-Up Visit. Sedation was assessed using the Stanford Sleepiness Scale (SSS) at specified times, or as deemed necessary, and was used to determine if it was safe to proceed with the dosing regimen or if dose adjustments needed to be made. For this purpose, the SSS was to be performed after all other assessments so that subjects were as awake as possible, or

	subjects were to be awakened, if necessary, and instructed to report their level of sleepiness. All AEs, whether volunteered, elicited, or noted on physical examination, were to be recorded throughout the study (i.e., from screening, Day -1 (check-in) through the remainder of the Inpatient Confinement Period [Days 1, 2, and 3 (Clinic Discharge)] and the Follow-Up Visit).
PK Moieties	Sage-547
PD Endpoint(s)	None
Statistical Methods	All PK analyses were conducted using the PK Population, defined as all subjects who started the infusion of SAGE-547 and had at least one quantifiable PK concentration. Plasma concentrations and PK parameters for SAGE-547, SBECD, and any metabolite of SAGE-547 (if assayed) were summarized by renal impairment group using descriptive statistics and supporting figures were presented as appropriate. The effect of renal impairment in the severe cohort (test cohort in the reduced study design) was compared to the control cohort (reference cohort) using the PK parameters AUC∞ and Cmax for SAGE-547 and SBECD. The PK parameters AUC∞ and Cmax for SAGE-547 and SBECD were log-transformed (base e) prior to statistical analyses and analyzed using the Proc Mixed procedure in SAS®. The model included renal function group as a fixed effect. Mean differences were calculated between the subjects with severe renal impairment and subjects with normal renal function. The residual variance from the mixed model was used to calculate the 90% confidence interval (CI) for the difference between the test and reference cohorts. These values were back transformed to give the ratio of geometric least-squares means of the test cohorts relative to the reference cohort and the 90% CI for the ratio. No adjustment was made for multiplicity. For each renal impairment cohort, the cohort was concluded to be bioequivalent to the control cohort if the 90% CI for the ratio of geometric means for the respective renal impairment cohort were contained within the interval of 80% to 125% of the reference cohort for both Cmax and AUC∞. The Safety Population comprised all subjects who started the infusion of

	SAGE-547. Adverse events were classified by type, incidence, severity, and causality. The overall incidence of AEs was summarized using the Medical Dictionary for Regulatory Activities (MedDRA) Version 18.0 coding system and classified by system organ class and preferred term. Subjects were counted once per preferred term. Safety data from vital signs, clinical laboratory measures, ECG, SSS, and concomitant medication usage were also summarized.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547, metabolites were not assessed		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- N= 17 subjects (Health with normal renal function= 8; Severe renal impairment = 9)

Number of subject (planned and analyzed):
Up to 32 subjects (eight per cohort) were planned and 17 (nine subjects in the severe renal impairment coh and eight subjects in the normal renal function cohort) were enrolled and analyzed.

Table 1: Demography of subjects

Characteristics	Renal Impairment Cohort		
	Normal N = 8	Severe N = 9	Overall N = 17
Gender, n (%)			
Male	5 (62.5)	5 (55.6)	10 (58.8)
Female	3 (37.5)	4 (44.4)	7 (41.2)
Age (years)			
Mean (standard deviation)	58.5 (14.22)	59.3 (14.05)	58.9 (13.69)
Minimum, Maximum	32, 74	30, 78	30, 78
Median	59.5	63.0	63.0
Race, n (%)			
White	6 (75.0)	8 (88.9)	14 (82.4)
Black/African American	2 (25.0)	1 (11.1)	3 (17.6)
Ethnicity, n (%)			
Hispanic/Latino	3 (37.5)	2 (22.2)	5 (29.4)
Not Hispanic/Latino	5 (62.5)	7 (77.8)	12 (70.6)
Height – Screening (cm)			
Mean (standard deviation)	166.83 (6.807)	163.84 (10.736)	165.25 (8.958)
Minimum, Maximum	154.4, 179.2	147.0, 180.5	147.0, 180.5
Median	166.00	162.00	166.00
Body weight – Screening (kg)			
Mean (standard deviation)	81.00 (9.519)	84.69 (21.645)	82.95 (16.658)
Minimum, Maximum	64.0, 94.8	55.1, 115.9	55.1, 115.9
Median	80.60	85.30	81.20
Body weight – Check-in (kg)			
Mean (standard deviation)	81.11 (9.535)	84.61 (21.496)	82.96 (16.555)
Minimum, Maximum	64.3, 93.4	55.1, 115.3	55.1, 115.3
Median	80.90	84.90	81.50
Body mass index – Screening (kg/m^2)			
Mean (standard deviation)	29.08 (2.710)	31.06 (4.875)	30.12 (4.017)
Minimum, Maximum	23.2, 31.7	24.2, 38.3	23.2, 38.3
Median	29.50	30.70	29.50
Body mass index – Check-in (kg/m^2)			
Mean (standard deviation)	29.13 (2.839)	31.03 (4.814)	30.14 (4.008)
Minimum, Maximum	23.3, 32.0	24.2, 38.1	23.3, 38.1
Median	29.33	30.90	29.58

Source: Table 14.1.2; Listing 16.2.4.2; Listing 16.2.8.11

Inclusion Crietria:

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1. Able to comprehend and willing to sign an ICF.
2. Males or females, between 18 and 85 years of age (inclusive), with a BMI range of 18 to 40 kg/m^2 (inclusive).
3. Negative test for selected drugs of abuse at screening (excluding alcohol) and at check-in (including alcohol). A positive test for approved prescriptions, such as opioids, was acceptable.
4. Females were to be nonpregnant, nonlactating, and either postmenopausal for at least 1 year, surgically sterile (eg, tubal ligation, hysterectomy) for at least 90 days, or from the time of signing the informed consent or 10 days prior to check-in until 30 days after study discharge, agreed to use one of the following forms of contraception: nonhormonal intrauterine device with spermicide; female condom with spermicide; contraceptive sponge with spermicide; diaphragm with spermicide; cervical cap with spermicide; male sexual partner who agreed to use a male condom with spermicide; or sterile sexual partner. For all females of childbearing potential, the pregnancy test result must have been negative at screening and check-in.
5. Males were either sterile or from check-in until 90 days following study discharge agreed to use one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an intrauterine device with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system; a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects were to refrain from sperm donation from check-in until 90 days following study discharge.
6. Subjects had venous access sufficient to allow for blood sampling, as per the protocol.

7. Subjects were reliable and willing to make themselves available for the duration of the study and were willing to adhere to the prohibitions and restrictions specified in this protocol.
8. Subjects must have had severe renal impairment (eGFR: 15 to 29 mL/minute/1.73m^2) or normal renal function (eGFR: ≥90 mL/minute/1.73m^2 or >75 mL/minute/1.73m^2 in subjects who were older than 70 years of age), and, if applicable, mild renal impairment (eGFR: 60 to 89 mL/minute/1.73m^2) or moderate renal impairment (eGFR: 30 to 59 mL/minute/1.73m^2), at screening and confirmed at check-in (Day -1) based on eGFR, as determined by the MDRD formula. If renal function status for the subject changed from screening to Day -1, eGFR may have been repeated once within 24 to 48 hours. If the repeat eGFR value was consistent with the screening value (as determined by the Investigator), the subject was to be categorized per the Day -1 eGFR value, which must have been in the normal or renal impairment range, as applicable, to the initial classification of the subject.
9. Renal status must have remained stable within 90 days prior to check-in (Day -1).
10. Subject re-enrollment: This study permitted the re-enrollment of a subject who had discontinued the study for a medical or personal issue prior to dosing (Day 1). If the subject developed a medical or safety issue that resolved, the subject must have re-enrolled and met all safety and stability criteria prior to dosing. If the subject discontinued because of a personal or other non-medical reason, the subject may have proceeded with dosing, provided all screening activities occurred within the allowed time period.

9.3.1.2. Subjects with Renal Impairment

11. A stable medication regimen, defined as not starting a new medication(s), or not starting a clinically significant change in dosage(s) within 30 days prior to administration of the study drug, was required. Concomitant medications must have been approved by the Medical Monitor, Sage, and Investigator.
12. Abnormal laboratory values, judged by the Investigator and Medical Monitor to be compatible with the renal impairment of the subject, were acceptable; anemia secondary to renal disease was acceptable if hemoglobin was ≥9 g/dL and anemia symptoms were not clinically significant.
13. Subjects exhibited vital signs within the reference range for their age and level of renal impairment; subjects with vital signs outside the reference ranges may have been eligible for the study if the Investigator and Medical Monitor felt that the results were not clinically significant, based on the age and renal impairment status of the subject, and would not have impacted study conduct.
14. Subjects had no clinical exacerbation of renal disease within the Screening Period or prior to study drug administration.
15. Subjects were in good general health, allowing for the concurrent illnesses associated with renal disease.

9.3.1.3. Healthy Subjects

16. Subjects had no clinically significant illness or disease, as determined by medical history, physical examination, vital signs, and 12-lead ECG.
17. Subjects with stable, chronic medical conditions (eg, hypertension and hyperlipidemia) that, in the opinion of the Investigator, would not have significantly altered the disposition of the drug, would not have placed the subject at increased risk by participating in the study, and would not have interfered with interpretation of the data may have been permitted after discussion and agreement between the Investigator, Medical Monitor, and/or Sage.
18. Subjects had normal renal function (eGFR ≥90 mL/minute/1.73m^2); however, for healthy subjects who were older than 70 years of age, eGFR values >75 mL/minute/1.73m^2 were acceptable.
19. Subjects were demographically comparable to the subjects with renal impairment.
20. The results of clinical laboratory evaluations (including clinical chemistry panel [after a minimum of 10 hours fasting], hematology, and urinalysis) were within the reference range for the test laboratory and for the population, or results with acceptable deviations were judged not to be clinically significant by the Investigator, Medical Monitor, and/or Sage.

Exclusion Criteria:

9.3.2.1. All Subjects

1. History of any active infection within 30 days prior to Day 1, if deemed clinically significant by the Investigator, Medical Monitor, and Sage.
2. Hospitalization within 30 days prior to check-in, unless the reason was considered not exclusionary by the Investigator, Medical Monitor, and Sage.
3. Received live vaccine(s) within 30 days of screening, or intended to during the study (influenza vaccine was allowed if administered >21 days prior to dosing).
4. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.
5. History or presence of an abnormal ECG, which, in the Investigator's opinion, was clinically significant.
6. History of urinary incontinence.
7. History of alcoholism or drug addiction within 1 year prior to screening per the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition criteria.
8. Had an average weekly alcohol intake that exceeded 21 units per week (males up to age 65) and 14 units per week (males over 65 and females). One unit = 12 ounces or 360 mL of beer; 5 ounces or 150 mL of wine; 1.5 ounces or 45 mL of distilled spirits.

9. Subjects were unwilling to stop alcohol consumption within 48 hours prior to check-in on Day -1 (as confirmed by alcohol breath test) and for the duration of the confinement period.
10. Current heavy users of nicotine; ie, smoking more than 20 cigarettes (eg, 1 pack) per day or equivalent (eg, e-vapor cigarette, pipe, cigar, chewing tobacco, nicotine patch, or nicotine gum).
11. Could not comply with the smoking restrictions of the study site during confined periods or were unable or unwilling to refrain from smoking and tobacco use for 2 hours prior to dosing and 4 hours following dose administration.
12. Regular use of known drugs of abuse and/or showed positive urine screen for drugs of abuse (excluding cotinine or approved prescriptions, such as opioids) at screening or Day -1; needed special dietary restrictions, unless the restrictions were approved by the Investigator, Medical Monitor, and Sage or were indicated for subjects with renal disease.
13. Participation in any other investigational study drug trial in which dosing of an investigational study drug occurred within 5 half-lives or 30 days, whichever was longer.
14. A positive test for serum human immunodeficiency virus (HIV) antigen/antibody, hepatitis B surface antigen, or hepatitis C virus antibody.
15. Donation of blood from 30 days prior to screening through study discharge, inclusive, or of plasma from 14 days prior to screening through study discharge, inclusive.
16. Receipt of blood products within 60 days prior to check-in.
17. Any subject who required a repeat eGFR at check-in (Day -1; prior to dosing) was considered a pretreatment failure if the repeat value placed the subject into a renal function cohort different from that at screening.

9.3.2.2. Subjects with Renal Impairment

18. Subjects currently on dialysis were excluded, but subjects with severe renal impairment who had temporarily received dialysis more than 6 months prior to screening may have been enrolled based on the decision of Investigator, Medical Monitor and/or Sage.
19. Evidence of any significant active disease other than that responsible for, or associated with, renal impairment that would have significantly impacted the ability of the subject to safely participate in the trial, in the opinion of the Investigator and Medical Monitor. This included gastroenterologic, hepatic, cardiac (eg, myocardial infarction in the past year, angina, or congestive heart failure), respiratory, hematologic, neuropsychiatric, or neoplastic disease (basal or squamous cell skin cancer was acceptable).
20. History of renal transplant or currently on transplant list.
21. Acutely declining renal function.
22. Insufficient production of urine output to permit adequate urine collection (as determined by the Investigator).
23. Change in any clinical laboratory value from screening to Day -1 that was considered by the Investigator, Medical Monitor, and/or Sage to be clinically significant.

9.3.2.3. Healthy Subjects

24. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neoplastic (with the exception of basal or squamous cell cancer), neurological, or psychiatric disorder (as determined by the Investigator) capable of significantly altering the absorption of drugs; of constituting a risk when taking the study medication; or of interfering with the interpretation of the data.
25. eGFR values <90 mL/min/1.73m^2; for healthy subjects older than 70 years of age, exclusion of eGFR ≤75 mL/min/1.73m^2 applied.
26. Any subject determined by the Investigator to have had a clinically significant comorbidity that could have affected the outcomes of the study.

9.3.2.4. Excluded Concomitant Medications – All Subjects

27. Use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries), or juice (such as pineapple juice) primarily metabolized by cytochrome P (CYP)2C9, as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.
28. Use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase (UGT)2B7, and UGT2B17.
29. Use of any medications (prescription or over-the-counter), herbal tea, energy drinks, herbal products (eg, St. John's Wort, milk thistle), or supplement/supra-therapeutic doses of vitamins within 14 days prior to Day 1 and throughout the duration of the study, with the exception of those approved by the Investigator, Medical Monitor, and/or Sage. The exceptions, allowed as needed, were: prespecified medications (eg, antiviral, antihypertensives, diuretics, insulin, cholesterol-lowering agents, beta blockers, opioids) with a stable dose regimen established >30 days prior to study start and over-the-counter analgesics (nonantiplatelets) and stool softener.
30. Use of antiplatelets or anticoagulants within 30 days prior to study drug administration and throughout the study.
31. Use of benzodiazepines within 14 days, and antiepileptic medications within 30 days, prior to study drug administration and throughout the study.

New drugs were reviewed on a case-by-case basis by the Medical Monitor and/or Sage and were prohibited, unless deemed acceptable by the Investigator, Medical Monitor, and/or Sage.

PK Results

- Systemic exposure, based on C_{max} and DN $AUC_{0-\infty}$, to total SAGE-547 was 34.2% and 29.6% lower, respectively, in the severe renal impairment cohort compared with the normal renal function cohort; the 90% CIs for the geometric LS mean ratios were not contained entirely within the interval of 80% to 125%, indicating that equivalence between subjects with severe renal impairment and those with normal renal function could not be concluded.
- SAGE-547 was highly plasma protein bound, with geometric mean fraction unbound values of 0.00683 and 0.00661 for the normal renal function and severe impairment cohorts, respectively.
- Systemic exposure, based on $C_{max,u}$ and DN $AUC_{0-\infty,u}$, to unbound SAGE-547 was 34.0% and 30.6% lower, respectively, in the severe renal impairment cohort compared with the normal renal function cohort; the 90% CIs for the geometric LS mean ratios were not contained entirely within the interval of 80% to 125%, indicating that equivalence between subjects with severe renal impairment and those with normal renal function could not be concluded.
- Renal excretion of unchanged SAGE-547 was negligible, with less than 0.1% of the dose excreted in urine over the 48-hour collection period in all subjects.
- Systemic exposure, based on C_{max} and DN $AUC_{0-\infty}$, to the excipient SBECD was 1.72- and 5.51-fold higher, respectively, in the severe renal impairment cohort compared with the normal renal function cohort; the 90% CIs for the geometric LS mean ratios were entirely above the interval of 80% to 125%.

Figure: Arithmetic Mean Plasma Concentration-Time Profiles of Total SAGE-547 Following Intravenous Infusion of SAGE-547 Injection to Subjects with Normal Renal Function and Subjects with Severe Renal Impairment

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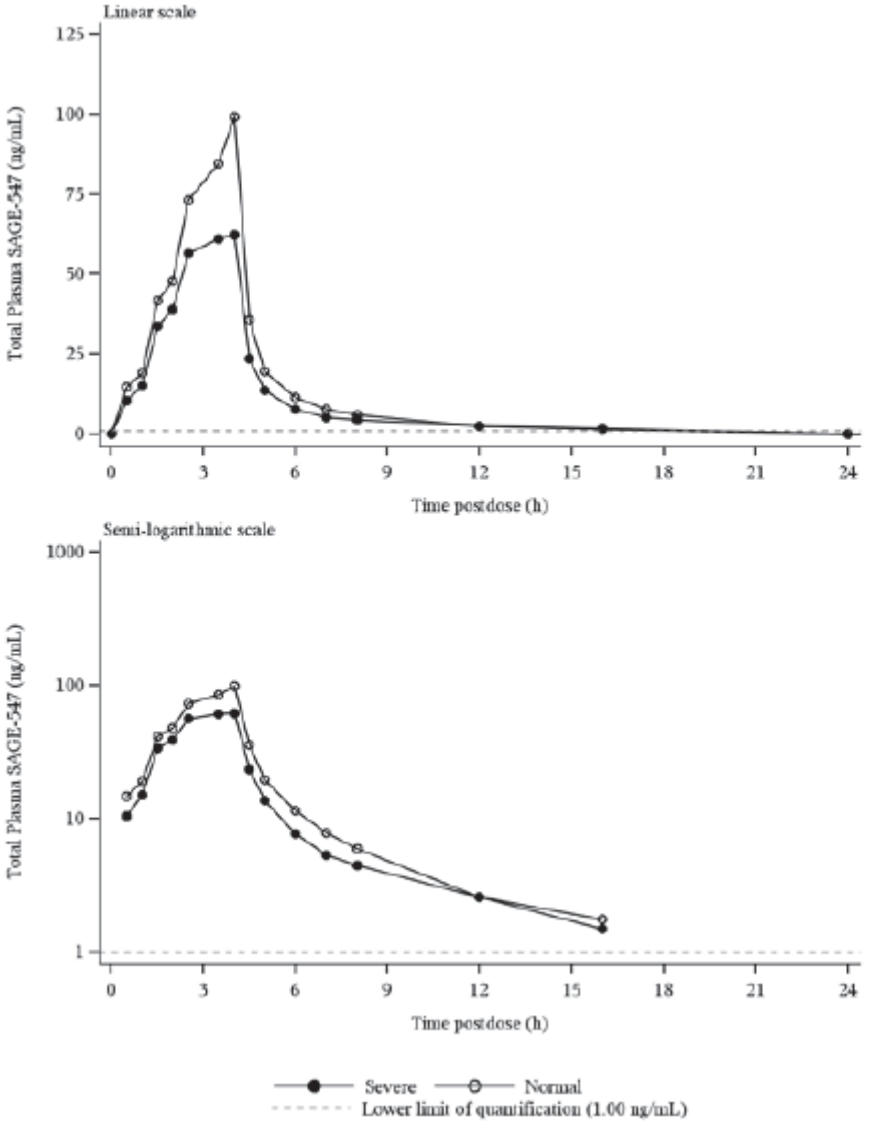
Linear scale
Total Plasma SAGE-547 (ng/mL)
125
100
75
50
25
0
0
3
6
9
12
15
18
21
24
Time postdose (h)
Semi-logarithmic scale
Total Plasma SAGE-547 (ng/mL)
1000
100
10
1
0
3
6
9
12
15
18
21
24
Time postdose (h)
Severe
Normal
Lower limit of quantification (1.00 ng/mL)

Table 8: Summary of Plasma and Cumulative Urinary Pharmacokinetic Parameters of Total SAGE-547 Following Intravenous Infusion of SAGE-547 Injection to Subjects with Normal Renal Function and Subjects with Severe Renal Impairment (Pharmacokinetic Population)

	Renal Impairment Cohort	
	Normal	Severe
Parameter (units)	N = 8	N = 9
C_{max} (ng/mL)	98.3 (17.9)	64.7 (24.3)[a]
t_{max} (h)[b]	3.98 (3.50, 4.00)	3.50 (2.50, 4.00)[a]
AUC_{0-t} (h*ng/mL)	312 (17.4)	233 (25.6)[a]
DN AUC_{0-t} (h*ng/mL/mg/kg)	1160 (17.0)	861 (21.0)
$AUC_{0-\infty}$ (h*ng/mL)	327 (17.1)	226 (26.2)[c]
DN $AUC_{0-\infty}$ (h*ng/mL/mg/kg)	1220 (16.6)	857 (20.6)[a]
λ_Z (1/h)	0.0981 (62.0)	0.117 (39.6)[c]
$t_{1/2}$ (h)[d]	8.12 (4.56)	6.27 (2.40)[c]
CL (L/h/kg)	0.821 (16.6)	1.18 (24.8)[c]
V_z (L/kg)	8.37 (60.9)	10.0 (28.6)[c]
V_{ss} (L/kg)	3.01 (55.8)	3.80 (28.2)[c]
Cum Ae_{0-48} (mg)[d]	0.00800 (0.00319)[a]	0.00176 (0.000700)[a]
Cum $\%Fe_{0-48}$ (%)[d]	0.0358 (0.0120)[a]	0.00829 (0.00353)[a]
CL_R (L/h/kg)[d]	0.000290 (0.0000881)[a]	0.0000877 (0.0000314)[a]

Abbreviations: AUC_{0-t} = area under the plasma concentration-time curve from time 0 (Hour 0) up to the time of the last quantifiable plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; CL = total clearance; CL_R = renal clearance; C_{max} = maximum observed plasma concentration; Cum Ae_{0-48} = cumulative amount of SAGE-547 excreted into urine up to 48 hours postdose; Cum $\%Fe_{0-48}$ = cumulative percent of administered dose excreted as SAGE-547 into urine up to 48 hours postdose; DN = dose normalized; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = apparent terminal elimination half-life; t_{max} = time to maximum observed plasma concentration; λ_Z = apparent terminal elimination rate constant; V_{ss} = volume of distribution at steady-state; V_z = volume of distribution during the terminal phase

Note: Geometric mean (CV%) data are presented unless otherwise stated.

For Subject [redacted] (b) (6) (severe), λ_z and λ_z–dependent parameters ($AUC_{0-\infty}$, DN $AUC_{0-\infty}$, $t_{½}$, CL, V_z, and V_{ss}) were excluded from descriptive statistics since $t_{½}$ exceeded half the total sampling interval (24 hours).

For Subject [redacted] (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics due to dose reduction to 60 µg/kg/h for the final 70 minutes of infusion.

For Subject [redacted] (b) (6) (normal), CL_R, Cum Ae, and Cum %Fe could not be calculated after 6 hours since urine void for the collection period of 6 to 8 hours was dropped by subject.

For Subject [redacted] (b) (6) (severe), λ_z and λ_z–dependent parameters ($AUC_{0-\infty}$, DN $AUC_{0-\infty}$, $t_{½}$, CL, V_z, and V_{ss}) could not be calculated since λ_z and $t_{½}$ could not be reliably estimated.

For Subject [redacted] (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics as subject had dose taper beginning at 1.5 hours and did not receive complete 4-hour infusion.

[a] N = 7.

[b] Median (min, max) presented for t_{max}.

[c] N = 5.

[d] Arithmetic mean (SD) presented for $t_{½}$, Cum Ae_{0-48}, Cum $\%Fe_{0-48}$, and CL_R.

Table 9: Summary of Plasma Pharmacokinetic Parameters of Unbound SAGE-547 Following Intravenous Infusion of SAGE-547 Injection to Subjects with Normal Renal Function and Subjects with Severe Renal Impairment (Pharmacokinetic Population)

	Renal Impairment Cohort	
	Normal	Severe
Parameter (units)	N = 8	N = 9
$C_{max,u}$ (ng/mL)	0.671 (23.6)	0.443 (33.5)[a]
$AUC_{0-t,u}$ (h*ng/mL)	2.13 (20.5)	1.59 (30.0)[a]
DN $AUC_{0-t,u}$ (h*ng/mL/mg/kg)	7.95 (20.3)	5.70 (27.9)
$AUC_{0-\infty,u}$ (h*ng/mL)	2.23 (20.4)	1.60 (35.9)[b]
DN $AUC_{0-\infty,u}$ (h*ng/mL/mg/kg)	8.31 (20.1)	5.76 (30.8)[a]
CL_u (L/h/kg)	120 (20.1)	166 (34.9)[b]
$V_{z,u}$ (L/kg)	1230 (62.7)	1410 (26.2)[b]
$V_{ss,u}$ (L/kg)	441 (58.6)	535 (20.6)[b]
f_u	0.00683 (16.4)	0.00661 (26.4)

Abbreviations: AUC = area under the plasma concentration-time curve; $AUC_{0-t,u}$ = unbound area under the plasma concentration-time curve from time 0 (Hour 0) up to the time of the last quantifiable plasma concentration $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; $AUC_{0-\infty,u}$ = unbound area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity CL_u = unbound total clearance; C_{max} = maximum observed plasma concentration; $C_{max,u}$ = maximum observed plasma concentration; DN = dose normalized; fu = fraction unbound; PK = pharmacokinetic; $t_{½}$ = apparent terminal elimination half-life; λ_Z = apparent terminal elimination rate constant; $V_{ss,u}$ = unbound volume of distribution at steady-state; $V_{z,u}$ = unbound volume of distribution during the terminal phase
Geometric mean (CV%) data are presented.
For Subject (b) (6) (severe), λ_z and λ_z–dependent parameters ($AUC_{0-\infty,u}$, DN $AUC_{0-\infty,u}$, CL_u, $V_{z,u}$, and $V_{ss,u}$) were excluded from descriptive statistics since $t_{½}$ exceeded half the total sampling interval (24 hours).
For Subject (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics due to dose reduction to 60 µg/kg/h for the final 70 minutes of infusion.
For Subject (b) (6) (severe), λ_z and λ_z–dependent parameters ($AUC_{0-\infty,u}$, DN $AUC_{0-\infty,u}$, CL_u, $V_{z,u}$, and $V_{ss,u}$) could not be calculated since λ_z and $t_{½}$ could not be reliably estimated.
For Subject (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics as subject had dose taper beginning at 1.5 hours and did not receive complete 4-hour infusion.
[a] N = 7.
[b] N = 5.

Table 10: Statistical Analyses of the Plasma Pharmacokinetic Parameters of Total and Unbound SAGE-547 (Pharmacokinetic Population)

Parameter (units)	Renal Impairment Cohort	N	Geometric LS Mean[a]	Comparison	Ratio of Geometric[b] LS Means (%)	90% CIs of the Ratio[c]
C_{max} (ng/mL)	Normal	8	98.3	Severe vs. Normal	65.8	(54.4, 79.7)
	Severe	7	64.7			
DN $AUC_{0-\infty}$ (h*ng/mL/mg/kg)	Normal	8	1220	Severe vs. Normal	70.4	(59.5, 83.3)
	Severe	7	857			
$C_{max,u}$ (ng/mL)	Normal	8	0.671	Severe vs. Normal	66.0	(51.1, 85.3)
	Severe	7	0.443			
DN $AUC_{0-\infty,u}$ (h*ng/mL/mg/kg)	Normal	8	8.31	Severe vs. Normal	69.4	(55.1, 87.3)
	Severe	7	5.76			

Abbreviations: $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; $AUC_{0-\infty,u}$ = unbound area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; CI = confidence interval; C_{max} = maximum observed plasma concentration; $C_{max,u}$ = maximum observed plasma concentration; DN = dose normalized; LS = least squares; $t_{½}$ = apparent terminal elimination half-life
The C_{max} and $C_{max,u}$ data for Subject (b) (6) (severe renal impairment cohort) were excluded from statistical analyses, as the subject had dose taper beginning at 1.5 hours and did not receive complete 4-hour infusion.
The C_{max} and $C_{max,u}$ data for Subject (b) (6) (severe renal impairment cohort) were excluded from statistical analyses due to dose reduction to 60 µg/kg/h for the final 70 minutes of the infusion.
The DN $AUC_{0-\infty}$ and DN $AUC_{0-\infty,u}$ data for Subject (b) (6) (severe renal impairment cohort) were excluded from statistical analyses since $t_{½}$ exceeded half the total sampling interval (24 hours).
[a] Least squares means from the analysis of variance model of natural log data were transformed back to the linear scale using the exponential function.
[b] Least squares mean difference between test and reference of log-transformed data were transformed back to the linear scale (expressed as a percent).
[c] 90% CIs for the LS mean difference of log-transformed data were transformed back to the linear scale (expressed as a percent).
Source: Table 14.2.3.1 and Table 14.2.3.2

Effect on Excipient (SBECD)

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Table 11: Summary Plasma Pharmacokinetic Parameters of SBECD Following Intravenous Infusion of SAGE-547 Injection to Subjects with Normal Renal Function and Subjects with Severe Renal Impairment (Pharmacokinetic Population)

	Renal Impairment Cohort	
Parameter (units)	Normal N = 8	Severe N = 9
C_{max} (µg/mL)	40.5 (21.3)	69.7 (13.0)[a]
t_{max} (h)[b]	3.98 (3.50, 4.00)	3.98 (2.50, 4.50)[a]
AUC_{0-t} (h*µg/mL)	133 (19.7)	769 (24.0)[a]
DN AUC_{0-t} (h*µg/mL/mg/kg)	9.96 (19.1)	56.4 (24.0)
$AUC_{0-\infty}$ (h*µg/mL)	146 (19.2)	799 (24.5)[a]
DN $AUC_{0-\infty}$ (h*µg/mL/mg/kg)	10.9 (18.5)	60.0 (22.1)
λ_Z (1/h)	0.440 (41.6)	0.0888 (33.3)[a]
$t_{1/2}$ (h)[c]	1.70 (0.820)	8.14 (2.39)[a]
CL (L/h/kg)	0.0920 (18.5)	0.0167 (23.0)[a]
V_z (L/kg)	0.209 (46.1)	0.188 (13.7)[a]
V_{ss} (L/kg)	0.218 (28.8)	0.187 (11.3)[a]

Abbreviations: AUC = area under the curve; AUC_{0-t} = area under the plasma concentration-time curve from time 0 (Hour 0) up to the time of the last quantifiable plasma concentration; $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; CL = total clearance; C_{max} = maximum observed plasma concentration; DN = dose normalized; PK = pharmacokinetic; SD = standard deviation; $t_{½}$ = apparent terminal elimination half-life; t_{max} = time to maximum observed plasma concentration; λ_Z = apparent terminal elimination rate constant; V_{ss} = volume of distribution at steady-state; V_z = volume of distribution during the terminal phase
Note: Geometric mean (CV%) data are presented unless otherwise stated.
For Subject (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics due to dose reduction to 60 µg/kg/h for the final 70 minutes of infusion.
For Subject (b) (6) (severe), all PK parameters except for DN AUCs were excluded from descriptive statistics as subject had dose taper beginning at 1.5 hours and did not receive complete 4-hour infusion.
[a] N = 7.
[b] Median (min, max) presented for t_{max}.
[c] Arithmetic mean (SD) presented for $t_{½}$.
Source: Table 14.2.2.1

Table 12: Statistical Analysis of the Plasma Pharmacokinetic Parameters of SBECD (Pharmacokinetic Population)

Parameter (units)	Renal Impairment Cohort	N	Geometric LS Mean[a]	Comparison	Ratio of Geometric [b]LS Means (%)	90% CIs of the Ratio[c]
C_{max} (µg/mL)	Normal	8	40.5	Severe vs. Normal	172.3	(146.4, 202.8)
	Severe	7	69.7			
DN $AUC_{0-\infty}$ (h*µg/mL/mg/kg)	Normal	8	10.9	Severe vs. Normal	551.6	(464.2, 655.4)
	Severe	9	60.0			

Abbreviations: $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time 0 (Hour 0) extrapolated to infinity; CI = confidence interval; C_{max} = maximum observed plasma concentration; DN = dose normalized; LS = least squares

The C_{max} data for Subject (b) (6) severe renal impairment cohort) were excluded from statistical analyses, as the subject had dose taper beginning at 1.5 hours and did not receive complete 4-hour infusion.

The C_{max} data for Subject (b) (6) severe renal impairment cohort) were excluded from statistical analyses due to dose reduction to 60 µg/kg/h for the final 70 minutes of the infusion.

[a] Least squares means from the analysis of variance model of natural log data were transformed back to the linear scale using the exponential function

[b] Least squares mean difference between test and reference of log-transformed data were transformed back to the linear scale (expressed as a percent).

[c] 90% CIs for the LS mean difference of log-transformed data were transformed back to the linear scale (expressed as a percent).

Source: Table 14.2.3.3

Safety Results

Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA

- No subject died or had a serious or severe TEAE.
- A total of two of the 17 subjects experienced a total of four TEAEs; both subjects were in the severe renal impairment cohort. All four TEAEs were mild and considered possibly related to study drug.
- There were no discontinuations or dose adjustments due to TEAEs; however, two subjects in the severe renal impairment cohort had infusion rate reductions, with subsequent early termination of infusion in one of the subjects, based on SSS findings, as specified in the protocol.
- Mean increases from baseline in SSS score were greatest between 1 and 3 hours after the start of the infusion, and were slightly greater for subjects in the severe renal impairment cohort versus the normal renal function cohort.
- Laboratory test results, vital signs, and ECG parameters were unremarkable over the course of the study.
- No post-infusion suicidal ideations or behaviors were reported via C-SSRS.

Overall Sponsor Conslusions

This open-label, nonrandomized, parallel-group study of subjects with severe renal impairment and healthy subjects with normal renal function demonstrated that administration of a 4-hour IV infusion of SAGE-547 resulted in modestly decreased exposure to SAGE-547 in subjects with severe renal impairment as compared to healthy controls. SAGE-547 was well tolerated in this study.

Reviewer Comments and Conclusions

13. *Study Design: This was an Open Label, non-randomized, Multi Center, Single Dose, Parallel group, safety, tolerability and PK study of SAGE-547 administered by IV infusion to healthy subjects and subjects with renal impairment. The overall study design was acceptable since:*
    - *It was a single dose study conducted at the target dose level (90 ug/kg/h).*
    - *IV dose was chosen because it is the intended route of administration.*
    - *Males and females, between the age of 18 and 75 years were included.*
    - *The subjects in control group were matched and balanced with subjects in renal impaired groups w.r.t., age, sex and BMI.*
    - *Adequate number of subjects (N= 17; with 8 subjects in normal and 9 subjects in severe cohort) were included in the study*
    - *The final to-be-marketed formulation of SAGE 547 was used in this study.*

14. *Protocol deviation: No major protocol deviations were reported.*

15. *Data Analysis (i.e., any outliers etc.): There were no outliers and the PK data from all subjects were included in the analysis.*

16. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

17. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *The control group in this study was the matched healthy subjects without renal impairment (i.e., comparable to at least one subject with impaired renal function with respect to age [±10 years], sex, and BMI [±20%]), which is a standard design element in clinical pharmacology studies designed to inform dosage recommendations for patients with renal dysfunction.*
- *The study excluded use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.*

- *The study excluded use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17. This is acceptable because it minimized the chance of any drug interactions.*

18. *Pharmacokinetic findings: We agree with the sponsor's PK analysis and the conclusions from the study.*

Overall Conclusion:

No dose adjustments are recommended for patients with renal impairment. However, due to the potential accumulation of SBECD, caution should be used in patients with moderate and severe renal impairment and use is not advised in patients with ESRD and eGFR < 15 mL/min/1.73 m2.

CLINICAL PHARMACOLOGY STUDY REVIEW

Drug Interaction Study- Effect of SAGE 547 on Phenytoin

Study # 547-CLP-105 **Study Period:** 04-Mar-2016 to 19-June-2016

NDA 211371_Zulresso_Brexanolone IV infusion

Title	A PHASE 1, OPEN-LABEL, TWO-PERIOD, SINGLE-SEQUENCE CROSSOVER STUDY TO EVALUATE THE EFFECT OF INTRAVENOUS SAGE-547 ON THE PHARMACOKINETIC PROFILE, SAFETY, AND TOLERABILITY OF ORAL PHENYTOIN IN HEALTHY SUBJECTS.
Objectives:	The objectives of this study were to examine the effect of SAGE-547 on the pharmacokinetic (PK) of a single oral dose of phenytoin in healthy subjects and to compare the safety and tolerability of a single dose of oral phenytoin alone and in the presence of SAGE-547 in healthy subjects.

Study Design:

9.1. Overall Study Design and Plan: Description

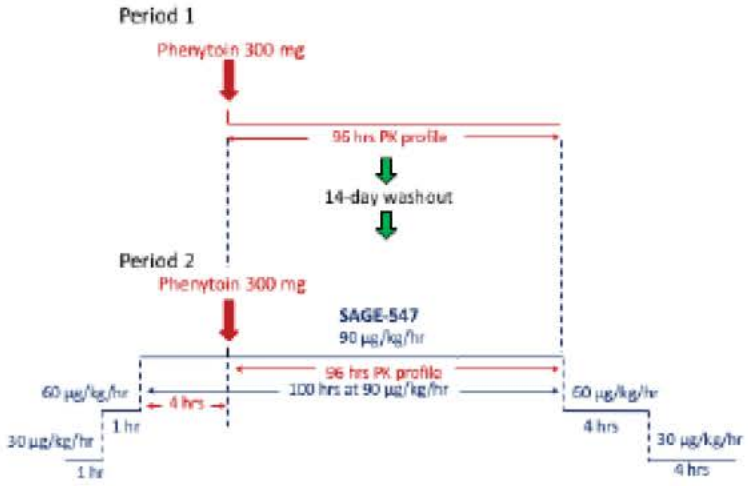
This was a single-center, open-label, two-period, single-sequence crossover study investigating the effect of SAGE-547 on the PK profile, safety, and tolerability of a single oral dose of phenytoin administered to healthy subjects. In Period 1, all subjects received phenytoin as an oral dose at 300 mg, administered as three 100-mg capsules. In Period 2, SAGE-547 was administered as an intravenous (IV) infusion for 110 hours (see Table 2 for details of dosing). At 6 hours after the start of the SAGE-547 infusion, a single oral dose of 300 mg phenytoin was administered as three 100-mg capsules (see Figure 1 for a schematic of the study design).

Table 2: SAGE-547 Infusion Type and Duration

Study Day (D)	Hour (H)[a]	Type and Duration of SAGE-547 Infusion	Description
D1		Titration infusion	
	H(-6) to H(-5)	1 hour	30 µg/kg/h
	H(-5) to H(-4)	1 hour	60 µg/kg/h
		Maintenance infusion	
D1 to D5	H(-4) to H96	100 hours	90 µg/kg/h
		Taper infusion	
	H96 to H100	4 hours	60 µg/kg/h
D5	H100 to H104[b]	4 hours	30 µg/kg/h

[a] Referenced to phenytoin administration.
[b] The infusion of SAGE-547 was complete at the end of Hour 104 (110 hours after the start of the SAGE-547 infusion). Subjects were discharged from the clinical research unit on Day 6.

Figure 1: Study Design



Note: Potential subjects were screened within 28 days prior to study entry (ie, prior to Check-in on Day -1 of Period 1) to assess their eligibility to enter the study.

Period 1: Eligible subjects were admitted to the clinical research unit for Check-in (Day -1 of Period 1 [P1D-1]). On Day 1 (P1D1), subjects received a single 300-mg oral dose of phenytoin, administered as three 100-mg capsules, at approximately the same time of day as scheduled in Period 2. Subjects remained at the clinical research unit for at least 96 hours post-dose for collection of serial blood samples for PK analysis and safety monitoring until clinic discharge on Day 5 (P1D5).

Period 2: After a washout of at least 14 days after the dose of phenytoin, subjects returned to the clinical research unit on Day -1 for Check-in (P2D-1). The following morning (P2D1), subjects were administered SAGE-547 by IV infusion as shown in Table 2. A single oral dose of phenytoin was administered 6 hours after the start of SAGE-547 infusion.

The decision to establish the 4-hour maintenance dose of 90 µg/kg/h before administration of phenytoin in Period 2 was based on the following requirements:

1. Ensure that the 90-µg/kg/h dose level was tolerated by the subjects prior to administration of the phenytoin dose; and
2. Ensure that SAGE-547 was close to steady state for a maximum drug-drug interaction effect.

Blood samples for PK analysis were collected at various time points from the start of the SAGE-547 IV infusion until 96 hours after the dose of phenytoin (102 hours after the start of the SAGE-547 infusion). Safety monitoring was ongoing from screening until discharge from the clinical research unit on Day 6 (P2D6). A Final Clinic Visit was scheduled on Day 13 (P2D13±1 day).

Phenytoin dosing occurred at approximately the same time of day in Period 1 and Period 2. The timing of meals in relation to phenytoin dosing was the same in Period 1 and Period 2. Fasting requirements were the same for Period 1 and Period 2 (at least a 6-hour fast prior to dosing and at least a 4-hour fast postdose of phenytoin).

Safety-related assessments included physical examinations, 12-lead electrocardiograms (ECGs), electroencephalograms, vital signs, adverse events (AEs), the Columbia Suicide Severity Rating Scale (C-SSRS), pulse oximetry (at any time during the drug infusion, as deemed necessary by the Investigator), and clinical laboratory evaluations. These assessments were performed at screening, at specified times during the study (Inpatient Confinement Periods), and at the Final Clinic Visit. Sleepiness was assessed using the Stanford Sleepiness Scale (SSS) at specified times, or as deemed necessary, and was used to determine whether it was safe to proceed with the dosing regimen or if it was necessary to adjust the dose. For this purpose, the SSS was performed after all other assessments so that subjects were as awake as possible, or subjects were awakened, if necessary, and instructed to report their level of sleepiness. Dose adjustments may have been necessary for SAGE-547 if the subjects became too sleepy, based on the SSS scores, or if there appeared to be a significant pattern of other drug-related AEs in addition to the SSS scores.

Phenytoin was administered at approximately the same time of day in Period 1 and Period 2. Fasting requirements were the same for Period 1 and Period 2 (at least a 6-hour fast prior to dosing and at least a 4-hour fast postdose of phenytoin). Except as part of dose administration, subjects restricted their consumption of water for 1 hour prior to dose and for 2 hours postdose; at all other times during the study, subjects may have consumed water on an ad libitum basis.

Reference therapy, dose and mode of administration, batch number: Not applicable
Duration of treatment: Period 1: Single oral dose of phenytoin Period 2: 5-day (102 hours) treatment period followed by an 8-hour dose-taper period

Table 3: Study Drugs

Study Drug	Dilantin® (Extended-Release Phenytoin Sodium Capsules, United States Pharmacopeia)	SAGE-547 Injection
Form[a]	oral capsule	solution
Strength	100 mg × 3 (300 mg)	5 mg/mL
Supplier	(b) (4)	(b) (4)
Manufacturer	Pfizer	

[a] Specific ingredients/purity were identified in the certificate of analysis (or equivalent) that was supplied with the study drug(s).

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Product Used:

Test product, dose and mode of administration, batch number:
Period 1: Phenytoin 300 mg oral
Period 2: Single IV infusion of SAGE-547 at increasing doses over 110 hours
Titration: 30 µg/kg/hr for 1 hour, 60 µg/kg/hr for 1 hour
Maintenance: 90 µg/kg/hr for 100 hours
Taper: 60 µg/kg/hr for 4 hours, 30 µg/kg/hr for 4 hours
Phenytoin 300 mg oral after 6 hours of SAGE-547 infusion

Route of Administration	IV infusion
PK Sampling Times and Parameters	**Pharmacokinetics:** For each subject, the following PK parameters were calculated, whenever possible, based on the total plasma concentrations of phenytoin, according to the model independent approach: Cmax, tmax, AUC0-t, AUC0-∞, λz, and t½. In addition, unbound PK parameters for phenytoin may have been calculated using fu. Total and unbound plasma concentrations and fu of SAGE-547 were reported
Safety Parameters	**Safety:** Safety-related assessments, including physical examinations, 12-lead electrocardiograms, electroencephalograms, vital signs, adverse events (AEs), the Columbia Suicide Severity Rating Scale (C-SSRS), pulse oximetry (at any time during the drug infusion, as deemed necessary by the Investigator), and clinical laboratory evaluations. These assessments were performed at screening, at specified times during the study (Inpatient Confinement Periods), and at the Final Clinic Visit.

	Sleepiness was assessed using the Stanford Sleepiness Scale (SSS) at specified times, or as deemed necessary, and was used to determine whether it was safe to proceed with the dosing regimen or if it was necessary to adjust the dose. For this purpose, the SSS was performed after all other assessments so that subjects were as awake as possible, or subjects were awakened, if necessary, and instructed to report their level of sleepiness. Dose adjustments may have been necessary for SAGE-547 if the subjects became too sleepy, based on the SSS scores, or if there appeared to be a significant pattern of other drug-related AEs in addition to the SSS scores.
PK Moieties	Sage-547
PD Endpoint(s)	None
Statistical Methods	

Statistical methods:

All PK analyses were conducted using the PK Population, defined as all subjects who received at least one dose of phenytoin, from whom at least one PK sample was obtained after dosing, and who had no protocol deviations that excluded them from the PK analysis. In addition, PK analyses for SAGE-547 were also conducted using this population.

The Safety Population consisted of all subjects who received any study drug (SAGE-547 or phenytoin). Adverse events were classified by type, incidence, severity, and causality. The overall incidence of AEs was summarized using the Medical Dictionary for Regulatory Activities coding system and classified by System Organ Class and preferred term. Subjects were counted once per preferred term.

Data from vital signs, clinical laboratory measures, ECG, SSS, and concomitant medication usage were also summarized.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547, Phenytoin		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- N= 29

Diagnosis and main criteria for inclusion: Healthy male and female subjects aged 18 to 55 years, inclusive with a body mass index of 20 to 30 kg/m^2, inclusive, and weight ≤100 kg

Table 1: Demography of subjects

Table 8: Demographics and Baseline Characteristics (Safety Population)

Characteristics	Overall N = 29
Gender, n (%)	
Male	16 (55.2)
Female	13 (44.8)
Age (years)	
Mean (SD)	35.9 (10.36)
Minimum, Maximum	22, 53
Median	35.0
Race, n (%)	
White	16 (55.2)
Black/African American	7 (24.1)
Asian	3 (10.3)
American Indian or Alaska Native	1 (3.4)
Other	2 (6.9)
Ethnicity, n (%)	
Hispanic/Latino	7 (24.1)
Not Hispanic/Latino	22 (75.9)
Height – Screening (cm)	
Mean (SD)	169.10 (10.700)
Minimum, Maximum	143.6, 187.5
Median	169.50
Body weight – Screening (kg)	
Mean (SD)	74.48 (12.759)
Minimum, Maximum	44.7, 97.8
Median	73.50
Body weight – Period 2 Check-in (kg)	(N = 28)
Mean (SD)	75.36 (12.828)
Minimum, Maximum	44.0, 97.6
Median	75.15
Body mass index – Screening (kg/m^2)	
Mean (SD)	25.88 (2.456)
Minimum, Maximum	20.4, 29.6
Median	26.30
Body mass index – Period 2 Check-in (kg/m^2)	(N = 28)
Mean (SD)	26.08 (2.464)
Minimum, Maximum	20.4, 29.5
Median	26.65

Source: Table 14.1.3; Table 14.1.4; Listing 16.2.4.1; Listing 16.2.4.2

Inclusion Crietria:

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1. Able to comprehend and willing to sign an ICF.
2. Males or females, aged 18 to 55 years (inclusive), with a body mass index range of 20 to 30 kg/m^2 (inclusive), and weight ≤100 kg.
3. Negative test for selected drugs of abuse at screening (including cotinine, but not including alcohol) and at Check-in on Day -1 of Period 1 (including cotinine and alcohol).
4. Negative hepatitis panel (including hepatitis B surface antigen [HBsAg] and hepatitis C virus [HCV] antibody) and negative human immunodeficiency virus (HIV) antibody screens.
5. Females were nonpregnant, nonlactating, and postmenopausal (defined as amenorrhea for at least 1 year and follicle-stimulating hormone levels ≥40 mIU/mL), surgically sterile (eg, tubal ligation, hysterectomy, oophorectomy) for at least 90 days, or agreed to use, from the time of signing the ICF or 10 days prior to check-in on Day -1 of Period 1 until 30 days after the Final Clinic Visit, one of the following forms of contraception: nonhormonal intrauterine device with spermicide, female condom with spermicide, contraceptive sponge with spermicide, diaphragm with spermicide, cervical cap with spermicide, male sexual partner who agreed to use a male condom with spermicide, or sterile sexual partner. For all women of child-bearing potential, the pregnancy test result must have been negative at screening and Check-in on Day -1 of both treatment periods.
6. Males were sterile or agreed to use, from check-in on Day -1 of Period 1 until 90 days after the Final Clinic Visit, one of the following approved methods of contraception: male condom with spermicide; sterile sexual partner; or use by female sexual partner of an intrauterine device with spermicide; a female condom with spermicide; a contraceptive sponge with spermicide; an intravaginal system (eg, NuvaRing®); a diaphragm with spermicide; a cervical cap with spermicide; or oral, implantable, transdermal, or injectable contraceptives. Subjects refrained from sperm donation from check-in on Day -1 of Period 1 until 90 days after the Final Clinic Visit.
7. Had venous access sufficient to allow for blood sampling as per the protocol.
8. Were reliable and willing to be available for the duration of the study and were willing to adhere to the prohibitions and restrictions specified in this protocol.
9. Had no clinically significant illness or disease, as determined by medical history, physical examination, vital signs, and 12-lead ECG.
10. The results of clinical laboratory evaluations (clinical chemistry panel [inclusive of glucose, alkaline phosphatase, and gamma glutamyl transpeptidase; fasted at least 10 hours], thyroid panel, hematology, and urinalysis) were within the reference range for the test laboratory and for the population, or results with acceptable deviations were judged not to be clinically significant by the Investigator.

Exclusion Criteria:

1. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, hematological, pulmonary, cardiovascular, gastrointestinal, neoplastic (with the exception of basal or squamous cell cancer), neurological, or psychiatric disorder (as determined by the Investigator) capable of significantly altering the absorption of drugs; of constituting a risk when taking the study medication; or of interfering with the interpretation of the data.
2. A history of any active infection within 30 days prior to Day 1, if deemed clinically significant by the Investigator, Medical Monitor, and Sage.
3. Any major surgical procedure or hospitalization within 6 months prior to check-in on Day -1 of Period 1 or during the study, unless deemed not clinically significant by the Investigator.
4. Had received live vaccine(s) within 30 days of screening, or intended to during the study (influenza vaccine was allowed, if administered >21 days prior to dosing or at the end of the study).
5. A history of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator.
6. History or presence of an abnormal ECG, which, in the Investigator's opinion, was clinically significant.
7. Use of oral, implantable, injectable, or transdermal contraceptives within 30 days prior to signing the ICF (females only).
8. History of alcoholism or drug addiction within 1 year prior to screening per the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition criteria.
9. History of depression or suicidal thoughts and/or behaviors within the past 1 year.
10. Had an average weekly alcohol intake that exceeded 21 units per week (males up to age 55) or 14 units per week (females). One unit=12 ounces (oz) or 360 mL of beer; 5 oz or 150 mL of wine; 1.5 oz or 45 mL of distilled spirits.
11. Were unwilling to stop alcohol consumption within 72 hours prior to check-in on Day -1 of Period 1 (as confirmed by urine alcohol screen) and for the duration of the study.
12. Used any tobacco- or nicotine-containing products (including, but not limited to, cigarettes, electronic cigarettes [of any kind], pipes, cigars, chewing tobacco, nicotine patches, nicotine lozenges, or nicotine gum) within 6 months prior to check-in on Day -1 of Period 1 and during the study.
13. Regular use of known drugs of abuse and/or positive urine screen for drugs of abuse at screening or check-in on Day -1 of both treatment periods.
14. Needed special dietary restrictions, unless the restrictions were approved by the Investigator, Medical Monitor, and Sage.

15. Dosing in any other investigational study drug trial in which receipt of an investigational study drug occurred within five half-lives of the investigational study drug or 30 days, whichever was longer, prior to check-in on Day -1 of Period 1 and during the study, or had a familial relationship with another study participant in the current study.
16. A positive test for HIV antigen/antibody, HBsAg, HCV antibody.
17. Hypersensitivity to the study drugs (SAGE-547, phenytoin sodium extended-release [ER], or other hydantoins).
18. Donation of blood from 30 days prior to screening through the Final Clinic Visit, inclusive. Donation of plasma from 14 days prior to screening through the Final Clinic Visit, inclusive.
19. Receipt of blood products within 60 days prior to check-in on Day -1 of Period 1, or during the study.
20. Any subject determined by the Investigator to have a clinically significant comorbidity that could have affected the outcome of the study.

9.3.2.2. Excluded Concomitant Medications

21. Use of any medications (prescription or over-the-counter) within 14 days prior to check-in on Day -1 of Period 1 and throughout the study, unless deemed acceptable by the Investigator.
22. Consumption of foods or juices rich in flavonoids, such as cranberries or pineapples, or foods (Seville oranges) or juices (grapefruit) that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase (UGT)2B7 and UGT2B17, or caffeine (xanthine-containing products) within 72 hours prior to check-in on Day -1 of Period 1 and throughout the study, unless deemed acceptable by the Investigator.
23. Consumption of herbal tea, energy drinks, herbal products (eg, St. John's wort, milk thistle), or supplement/supra-therapeutic doses of vitamins within 14 days prior to check-in on Day -1 of Period 1 and throughout the study, with the exception of those approved by the Investigator, Medical Monitor, and/or Sage. The exception, allowed as needed, was over-the-counter analgesics (acetaminophen).
24. Use of antiplatelets, anticoagulants, or antiepileptic medications within 30 days prior to Check-in on Day -1 of Period 1 and throughout the study.

PK Results

Table 9: Summary of Plasma Pharmacokinetic Parameters for Total and Unbound Phenytoin Following a Single Oral Dose of Phenytoin Alone or Co-Administered with Intravenous Infusion of SAGE-547 (Pharmacokinetic Population)

Parameter (units)	Phenytoin alone N = 28	SAGE-547 plus Phenytoin N = 26
Total		
C_{max} (ng/mL)	2768.55 (19.34)	2534.75 (31.12)
t_{max} (h)[a]	5.02 (2.00 - 12.00)	6.58 (2.00 - 12.00)
AUC_{0-t} (h*ng/mL)	100722.18 (28.70)	92377.53 (33.89)[b]
$AUC_{0-\infty}$ (h*ng/mL)	103207.00 (30.28)	93391.74 (33.86)[c]
$t_{1/2}$ (h)[d]	14.81 (4.27)	13.98 (2.81)[c]
Unbound		
$C_{max,u}$ (ng/mL)	339.48 (22.92)	324.22 (31.29)
$AUC_{0-t,u}$ (h*ng/mL)	12350.40 (29.89)	11731.49 (32.60)[b]
$AUC_{0-\infty,u}$ (h*ng/mL)	12655.08 (31.51)	11833.44 (32.85)[c]
f_u	0.12 (8.78)	0.13 (10.74)

Abbreviations: $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; $AUC_{0-\infty,u}$ = unbound area under the plasma concentration-time curve from time zero extrapolated to infinity; AUC_{0-t} = area under the plasma concentration-time curve from time zero up to the time of the last quantifiable plasma concentration; $AUC_{0-t,u}$ = unbound area under the plasma concentration-time curve from time zero up to the time of the last quantifiable plasma concentration; C_{max} = time to maximum observed plasma concentration; $C_{max,u}$ = unbound time to maximum observed plasma concentration; CV = coefficient of variation; f_u = fraction unbound; SD = standard deviation; $t_{½}$ = apparent terminal elimination half-life; t_{max} = time to maximum observed plasma concentration

Note: Geometric mean (CV%) data are presented unless otherwise stated.

[a] Median (minimum - maximum).

[b] N = 24.

[c] N = 25.

[d] Arithmetic mean (SD).

Figure 14.2.1 Mean Plasma Concentrations of Phenytoin over Time
Pharmacokinetic Population

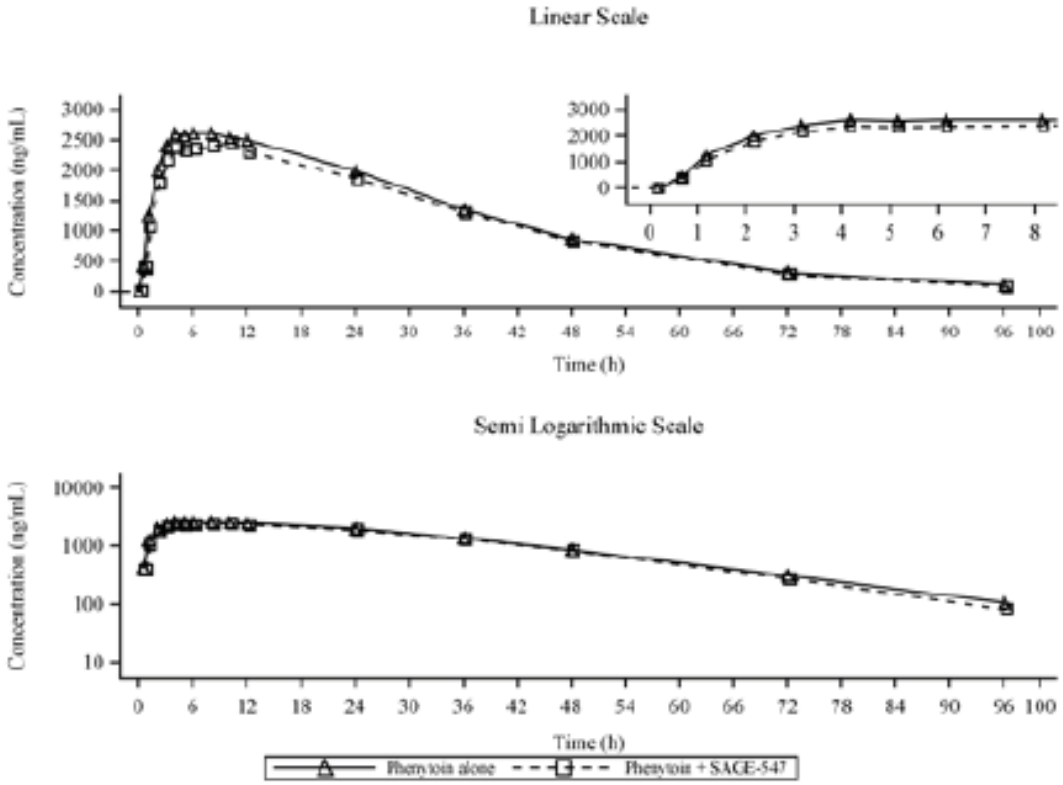

Table 10: Statistical Analysis of the Plasma Pharmacokinetic Parameters of Total and Unbound Phenytoin (Pharmacokinetic Population)

Parameter (units)	Comparison	Test	Reference	Test	Reference	Ratio of Geometric LS Means (Test to Reference)	90% CI for Geometric LS Mean Ratio of (Test to Reference)
Total							
C_{max} (ng/mL)	A (Test) vs B (ref)	26	28	2534.7	2768.6	0.916	(0.852, 0.983)
$AUC_{0-\infty}$ (h*ng/mL)	A (Test) vs B (ref)	25	28	95825.6	103207.0	0.928	(0.893, 0.966)
Unbound							
$C_{max,u}$ (ng/mL)	A (Test) vs B (ref)	26	28	325.2	339.5	0.958	(0.884, 1.038)
$AUC_{0-\infty,u}$ (h*ng/mL)	A (Test) vs B (ref)	25	28	12238.1	12655.1	0.967	(0.929, 1.007)
f_u	A (Test) vs B (ref)	26	28	0.1	0.1	1.046	(1.018, 1.076)

Abbreviations: $AUC_{0-\infty}$ = area under the plasma concentration-time curve from time zero extrapolated to infinity; $AUC_{0-\infty,u}$ = unbound area under the plasma concentration-time curve from time zero extrapolated to infinity; CI = confidence interval; C_{max} = time to maximum observed plasma concentration; $C_{max,u}$ = unbound time to maximum observed plasma concentration; f_u = fraction unbound; LS = least squares; PK = pharmacokinetic
Treatment A = phenytoin plus SAGE-547; Treatment B = phenytoin alone
The PK parameters were analyzed using a linear mixed model, with treatment as a fixed effect and subjects as a random effect.
Source: Table 14.2.1.5; Listing 16.2.6.1

PHARMACOKINETICS RESULTS:

SAGE-547 had no effect on the systemic exposure to phenytoin with the 90% CIs of the geometric LS me ratios for phenytoin $AUC_{0-\infty}$ and C_{max} fully contained within the 0.80 to 1.25 boundaries. The fraction of unbound phenytoin in the plasma was not affected when administered as SAGE-547 plus phenytoin. The of unbound SAGE-547 was similar when administered before and after phenytoin. These values are consi with prior SAGE-547 experience.

Safety Results

Was there any death or serious adverse events? ☐ Yes ☑ No ☐ NA

SAFETY RESULTS:

An IV infusion of SAGE-547, alone or with a single dose of phenytoin, was generally well tolerated by this group of healthy male and female subjects.

There were no serious adverse events.

Three subjects discontinued study drug and the study due to TEAEs. The TEAEs were considered to be not related to study drug in two subjects (pain in extremity [one subject] and alanine aminotransferase increased and aspartate aminotransferase increased [one subject]) and possibly related to study drug in one subject (somnolence).

All but two adverse events were mild in severity. One subject each reported moderate somnolence and pain in extremity, both of which resulted in study drug discontinuation.

The incidence of somnolence was greater during the SAGE-547 condition, both prior to (9/26 [34.6%]) and after administration of phenytoin (13/26 [50.0%]), than during the phenytoin alone condition (2/29 [6.9%]). While subjects were more likely to report sedative effects (eg, sleepiness, somnolence) during the administration of SAGE-547 plus phenytoin compared with SAGE-547 prior to phenytoin, the duration of the SAGE-547 infusion prior to phenytoin was considerably shorter (ie, 6 hours) than the duration of the SAGE-547 infusion after phenytoin was administered (ie, 104 hours).

Somnolence was considered to be related to study drug in all but one subject during the SAGE-547 prior to phenytoin condition.

Mean SSS scores were generally similar for both treatment conditions across all time points.

No clinically significant trends in clinical chemistry, hematology, urinalysis, vital signs, ECGs, or physical examinations were noted in this study. None of the subjects reported any suicidal ideation or behavior during the study.

Overall Sponsor Conslusions

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Systemic exposure to phenytoin was similar when administered alone and in combination w an IV infusion of SAGE-547, an in vitro inhibitor of CYP2C9. The 90% CIs of the geomet LS mean ratios for phenytoin $AUC_{0-\infty}$ and C_{max} were entirely contained within the 0.80 to 1. boundaries. Arithmetic mean $t_{½}$ was also comparable between the two treatment conditions values of 14.8 and 14.0 hours for phenytoin administered alone and in combination with SAGE-547, respectively. The unbound fraction of phenytoin in plasma was similar when administered alone (0.12) and in combination with SAGE-547 (0.13), indicating a minimal impact of SAGE-547 on the plasma protein binding of phenytoin. There was no significant difference in systemic exposure, based on $AUC_{0-\infty,u}$ and $C_{max,u}$, when phenytoin was admini with SAGE-547. The geometric mean total SAGE-547 concentration observed just prior to phenytoin dosing (81.10 ng/mL) was similar to those observed at selected time points follo phenytoin administration throughout the constant SAGE-547 infusion (geometric mean rang 70.42 to 94.14 ng/mL); these values are consistent with prior SAGE-547 experience. The fraction of SAGE-547 protein binding was also similar across time points (post-phenytoin d indicating that phenytoin did not impact SAGE-547 plasma protein binding.

SAGE-547 had no effect on the systemic exposure to phenytoin. The fraction of unbound phenytoin in plasm was not affected when SAGE-547 plus phenytoin was administered.

The fraction of unbound SAGE-547 was similar when administered before and after phenytoin. These values consistent with prior SAGE-547 experience.

An IV infusion of SAGE-547, alone or administered together with a single dose of phenytoin, was generally w tolerated by this group of healthy male and female subjects. There were no serious adverse events; three subje discontinued study drug and the study due to TEAEs. Somnolence was the most commonly reported TEAE a was reported by a greater percentage of subjects during the administration of SAGE-547 (prior to, or together with phenytoin) compared with phenytoin alone.

SAGE-547 was generally well tolerated and no clinically significant trends in the safety data collected were n in this study.

Reviewer Comments and Conclusions

19. *Study Design: This was an Open Label, 2 period, single sequence cross-over study to evaluate the effect of SAGE-547 administered as an IV infusion on the PK profile, safety and tolerability of oral phenytoin in healthy subjects:*
    - *The dose of SAGE-547 in this study (90 µg/kg/h) is the anticipated clinical dose, which has been previously administered in postpartum depression and found to be well-tolerated.*
    - *IV dose was chosen because it is the intended route of administration.*
    - *The dose of phenytoin was 300 mg, because the maintenance dose in patients with epilepsy is 300 mg/day or above. Also, the starting dose of phenytoin is up to 1000 mg*

in divided doses.
- *Phenytoin was dosed in fasted state to mitigate any interactions with food.*
- *Wash-out for 14 days is adequate because the T1/2 of phenytoin is around 22 hours.*
- *Males and females, between the age of 18 and 55 years were included.*
- *Adequate number of subjects (N= 29) were included in the study*
- *The final to-be-marketed formulation of SAGE 547 was used in this study.*

20. *Protocol deviation: No major protocol deviations were reported.*

21. *Data Analysis (i.e., any outliers etc.): There were no PK outliers. One subject was not used in PK data since there was no venous access. Additionally, 2 other subjects were dosed with phenytoin but could not be dosed with SAGE 547.*

22. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

23. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *The study excluded use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.*
- *The study excluded use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17. This is acceptable because it minimized the chance of any drug interactions.*

24. *Pharmacokinetic findings: We agree with the sponsor's PK analysis and the conclusions from the study.*

Overall Conclusion:

The present study demonstrates that SAGE-547 has no effect on the systemic exposure to phenytoin. Furthermore, the fraction of unbound phenytoin in plasma was not affected when SAGE-547 plus phenytoin was administered. Therefore, no dose adjustments are recommended for patients on Phenytoin who got concomitant SAGE 547.

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CLINICAL PHARMACOLOGY STUDY REVIEW

Oral BA of SAGE 547

Study # 547-CLP-107 **Study Period:** 14-July-2017 to 24-Aug-2017

NDA 211371_Zulresso_Brexanolone
IV infusion

Title	A Phase 1b Single Ascending Dose Study to Determine the Oral Bioavailability, Safety, Tolerability, Pharmacokinetics, and Food Effect of SAGE-547 in Healthy Adult Subjects
Objectives:	**Objectives:** The primary objective of the study was: • To determine the oral bioavailability and pharmacokinetics (PK) of SAGE-547 in healthy s aged 18 to 65 years. The secondary objectives of this study were: • To determine the effect of a high-fat meal on oral bioavailability of SAGE-547. • To assess the safety and tolerability of oral SAGE-547 in healthy subjects.

Study Design:

Methodology: SAGE-547 Injection is a proprietary formulation of allopregnanolone (scientific name), referred to as brexanolone (United States Adopted Name). Throughout this study report, when referring treatment group, SAGE-547 Injection is frequently shortened to SAGE-547. Further, "concentrations of SAGE-547" is synonymous with "concentrations of allopregnanolone" or "concentrations of brexanolon

This was a three-part, Phase 1b, open-label study to assess the oral bioavailability and PK of SAGE-547 Injection. The study also assessed the potential effect of food on the oral bioavailability following oral administration of SAGE-547 Injection and the safety and tolerability of orally administered SAGE-547 Injection.

For each part, eligible subjects were admitted to the unit on Day -1 and remained confined in the unit unt 48-hour postdose assessments were completed.

Subjects were screened on one or more visits between Day -28 and Day -1. On Day -1, eligible subjects admitted to the unit and began an overnight fast (≥8 hours). Clear liquids were permitted up to 2 hours b dosing.

Part 1 (Oral administration - fasted) was open-label, with a single ascending dose design to evaluate t bioavailability and PK of orally administered SAGE-547 Injection in healthy adult men and women. On each subject received a single oral dose of SAGE-547 after an 8-hour fast. Subjects were requested to sw the SAGE-547 dose all at once, followed by 240 mL of water. Aside from the water given during dosing subjects were not permitted food or liquids until they were given a meal approximately 4 hours after dosi

Methodology (continued): A maximum of four cohorts were planned in Part 1 of this study, with eight subjects in each cohort. After the overnight fast, Cohort 1 received SAGE-547 30 mg. If greater than four subjects had plasma concentrations below the limit of quantification of the assay for at least 50% of th sampling times in the first 24 hours postdose, dose escalation was to occur. However, dose escalation stop criteria were met following the first cohort and 30 mg was the selected dose for Part 2.

All available safety, tolerability, and plasma concentration data through 24 hours postdose for each comple cohort in Part 1 were reviewed by the Safety Review Committee (SRC). The SRC decided whether to initi the next cohort, and if initiated, determined the dose.

Part 2 (Oral administration - fed): The cohort of subjects in Part 1 who received the oral SAGE-547 do selected for Part 2 returned 11 days later for Part 2. After a ≥8-hour fast, subjects were given a high-fat m Thirty minutes later, they were administered the same oral dose that they received in Part 1 (30 mg).

Part 3 (Intravenous administration): Subjects who participated in Part 2 returned 8 days later and recei continuous intravenous (IV) infusion of SAGE-547 60 µg/kg/h administered over a period of 4 hours in P

For each part, subjects were discharged from the unit after completion of the 48-hour postdose assessment provided discharge was medically appropriate, in the opinion of the Investigator. Subjects returned for a Follow-up Visit on Day 7 (±1 day). Blood samples to measure plasma concentrations of allopregnanolon taken at various time points from predose until 24 hours postdose in each part of the study.

Number of subjects (planned and analyzed):

Up to 40 subjects (eight per cohort for up to four cohorts) were planned. Nine subjects (one cohort of eight subjects plus one replacement subject) were dosed and analyzed.

Diagnosis and main criteria for inclusion: Healthy male and female subjects aged ≥18 to ≤65 years at the time of screening, in good physical health with no clinically significant findings, as determined by the Investigator, on physical examination, 12-lead electrocardiogram (ECG), or clinical laboratory tests at screen or admission were eligible for enrollment.

Test product, dose and mode of administration, batch number:

30 mg SAGE-547 Injection 5 mg/mL administered as an oral solution when fasted (Part 1) or after a high-fat meal (fed, Part 2).

The SAGE-547 Injection lot number used for oral administration was B160556.

Reference therapy, dose and mode of administration, batch number:

IV (Part 3): Continuous IV infusion of SAGE-547 Injection 60 µg/kg/h administered for 4 hours. The SAGE-547 Injection lot number was B160556.

Duration of treatment: Eight subjects received two oral SAGE-547 doses and one IV SAGE-547 dose. Additionally, one subject was a replacement for Part 2 and Part 3 and received one oral dose in Part 2 and on IV dose in Part 3.

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Product Used:

Test product, dose and mode of administration, batch number:

30 mg SAGE-547 Injection 5 mg/mL administered as an oral solution when fasted (Part 1) or after a high-fa meal (fed, Part 2).

The SAGE-547 Injection lot number used for oral administration was B160556.

Route of Administration	Oral and IV infusion
PK Sampling Times and Parameters	**Pharmacokinetics**: Plasma samples were obtained from all cohorts for analysis for concentrations of allopregnanolone and, if needed, metabolites of allopregnanolone. Derived PK parameters were calculated, including area under the plasma concentration curve (AUC) from the time of dosing extrapolated to infinity (AUC0∞), AUC from the time of dosing to the last quantifiable concentration (AUC0-t), the apparent terminal elimination half-life (t½), maximum observed plasma concentration (Cmax), time to reach maximum plasma concentration (tmax), apparent total body clearance, and the absolute bioavailability for subjects receiving an IV dose. For the food-effect analysis, the log-transformed AUC and Cmax were compared across food conditions. For the IV infusion dosing, AUC0∞, AUC0-t, t½, Cmax, tmax, total body clearance, and steady-state volume of distribution were estimated.
Safety Parameters	**Safety:** Safety-related assessments included physical examinations, 12-lead ECGs, vital signs, AEs, Columbia Suicide Severity Rating Scale (C-SSRS), and clinical laboratory evaluations. These assessments were performed at specified times during the study. Subject's alertness levels, assessed using the Stanford Sleepiness Scale (SSS) at specified times, were used to determine if it was safe to proceed with the dosing regimen or if dose adjustments needed to be made.
PK Moieties	Sage-547

PD Endpoint(s)	None
Statistical Methods	APPEARS THIS WAY ON ORIG NAL

The Safety Population, defined as all subjects who were administered study drug, was used to provide descriptive statistics summaries of safety data. Adverse events (AEs) were coded using Medical Dictionary for Regulatory Activities version 19.1. The overall incidence of AEs was displayed by system organ class and by preferred term. The incidence of AEs was also presented by maximum severity and relationship to study drug. Vital signs, clinical laboratory measures, ECG, C-SSRS, and SSS data were summarized. Out-of-range safety endpoints were categorized as low or high, where applicable. Treatment-emergent AEs were summarized. All AEs (including those that occurred pretreatment) were listed.

The PK Population was defined as all subjects for whom at least one evaluable PK sample was available. The calculated PK parameters were summarized using descriptive statistics, including n, mean, standard deviation, median, minimum, and maximum values, and listed by subject. In addition, PK data collected in this study may have been combined with data from other studies for population-PK and exposure-response analyses.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- N= 8 subjects, healthy male and female, 18-65 years

Number of subjects (planned and analyzed):
Up to 40 subjects (eight per cohort for up to four cohorts) were planned. Nine subjects (one cohort of eigl subjects plus one replacement subject) were dosed and analyzed.

Table 1: Demography of subjects

Table 5: Demographic and Baseline Characteristics (Safety Population)

Characteristic	SAGE-547 30 mg Fasted State N = 8	SAGE-547 30 mg Fed State N = 8	SAGE-547 IV Administration N = 8
Gender, n (%)			
Male	3 (37.5)	3 (37.5)	3 (37.5)
Female	5 (62.5)	5 (62.5)	5 (62.5)
Age (years)			
Mean (SD)	49.1 (9.75)	46.6 (10.56)	46.6 (10.56)
Median	51.0	48.5	48.5
Minimum, maximum	34, 60	34, 60	34, 60
Race			
White	5 (62.5)	5 (62.5)	5 (62.5)
Black/African American	2 (25.0)	2 (25.0)	2 (25.0)
Native Hawaiian or Other Pacific Islander	1 (12.5)	1 (12.5)	1 (12.5)
Ethnicity, n (%)			
Not Hispanic or Latino	8 (100)	8 (100)	8 (100)
Height (cm)			
Mean (SD)	172.50 (7.507)	173.50 (7.806)	173.50 (7.806)
Median	171.75	173.00	173.00
Minimum, maximum	162.5, 188.0	162.5, 188.0	162.5, 188.0
Weight (kg)			
Mean (SD)	84.08 (11.163)	84.29 (11.184)	84.29 (11.184)
Median	83.20	84.05	84.05
Minimum, maximum	64.1, 100.7	64.1, 100.7	64.1, 100.7
Body mass index (kg/m^2)			
Mean (SD)	28.53 (3.690)	28.30 (3.714)	28.30 (3.714)
Median	27.63	27.20	27.20
Minimum, maximum	25.0, 37.0	25.0, 37.0	25.0, 37.0

SD = standard deviation
Source: Table 14.1.2.1 through Table 14.1.2.4

Inclusion Crietria:

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1. Subject was willing and able to participate in the study and had signed an ICF prio any study-specific procedures being performed.
2. Subject agreed to adhere to the study requirements and was willing and able to atte follow-up visits, including returning for Parts 2 and 3, if requested.
3. Subject was male or female and was ≥18 to ≤65 years of age at the time of screeni
4. Subject was in good physical health and had no clinically significant findings, as determined by the Investigator, on physical examination, 12-lead ECG, or clinical laboratory tests at screening or admission.
5. Subject agreed not to use any prescription or over-the-counter medication (other th vitamins or acetaminophen) during the study period.
6. If female, subject was postmenopausal (at least 12 months of spontaneous amenor with confirmatory follicle-stimulating hormone >40 mIU/mL), or had a bilateral tu ligation, or was surgically sterile (bilateral oophorectomy or hysterectomy).

Exclusion Criteria:

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1. Subject had a positive urine pregnancy test at the Screening or Admission Visits.
2. Subject had a known allergy to progesterone or allopregnanolone.
3. Subject had a clinically significant abnormal value for hematology, clinical chemis urinalysis at the Screening or Admission Visits. Any abnormal but non-clinically significant results required Sage medical approval prior to inclusion.
4. Subject had any clinically significant finding on physical examination at the Screer Admission Visits.
5. Subject had clinically significant abnormal vital signs. Any abnormal but non-clin significant results required Sage medical approval prior to inclusion.
6. Subject had any clinically significant finding on 12-lead ECG at the Screening or Admission Visits.
7. Subject had a recent history (within previous 2 years) of alcohol or drug abuse, as j by the Investigator.
8. Subject had a positive screening test for alcohol or drugs of abuse at the Screening Admission Visits.
9. Subject regularly consumed greater than two alcoholic drinks per day during the 3 months prior to screening (one alcoholic drink is approximately equivalent to: be [284 mL], wine [125 mL/4 ounces], or distilled spirits [25 mL/1 ounce]). Subjects consumed three alcoholic drinks per day but less than 14 per week may have been enrolled at the discretion of the Investigator.
10. Subject had used tobacco or tobacco-containing products (eg, cigarettes, electronic cigarettes, chewing tobacco, pipes) in the 6 months prior to the Screening Visit.
11. Subject had a positive urine cotinine test (>400 ng/mL) at the Screening or Admiss Visits.
12. Subject had received treatment with an investigational drug or device during the 30 prior to the Screening Visit.
13. Subject had used any known strong inhibitors and/or inducers of cytochrome P450 within 14 days or five half-lives (whichever was longer) or consumed grapefruit ju grapefruit, Seville oranges, or St. John's Wort or products containing these within 3 prior to receiving the first dose of study drug.

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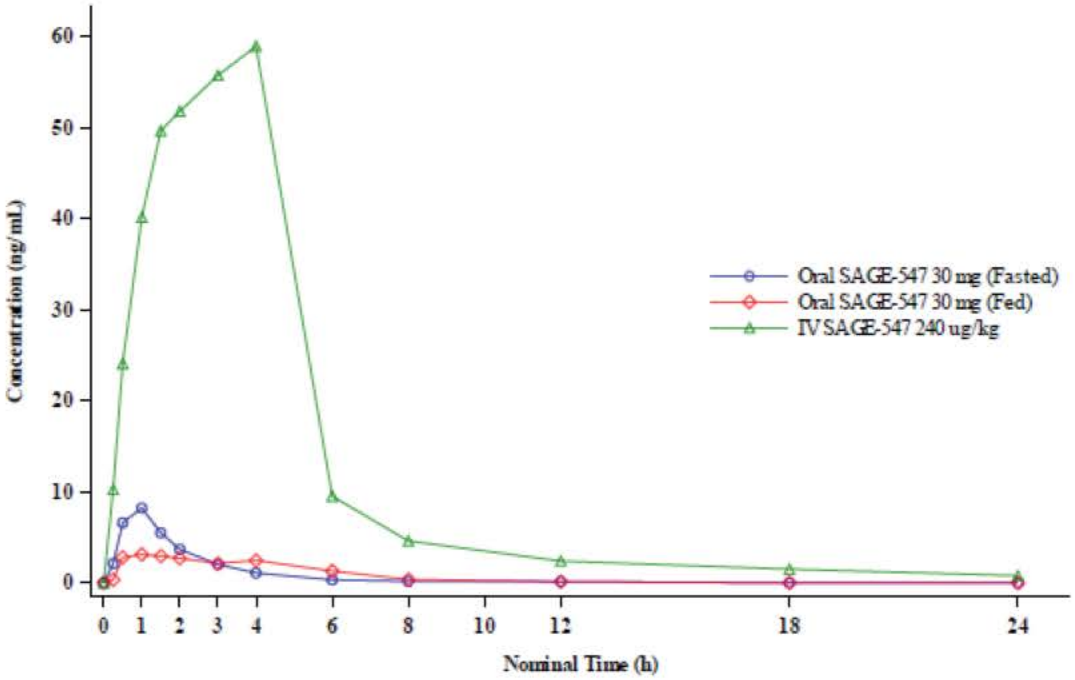
PK Results

Blood samples were taken following oral (30 mg) or IV infusion (60 µg/kg/h for 4 hours) administration in order to derive PK parameters of SAGE-547. Pharmacokinetic parameters of SAGE-547 are presented in Tables below.

The t_{max} following oral administration of SAGE-547 30 mg was generally 1 hour. Following IV infusion, t_{max} was generally at the end of the infusion (4 hours). Quantifiable plasma concentrations of SAGE-547 were observed for up to 2 to 12 hours following oral administration of SAGE-547 30 mg to fasted subjects and up to 3 to 12 hours following a high-fat meal.

The mean bioavailability (F) of SAGE-547, calculated from AUC_{0-t} values, was low (< 5%) when administered to subjects either in the fasted state or in fed state. A high-fat meal reduced the Cmax by ~50% and AUC by ~10% compared to fasted state PK in oral dosing.

Mean Plasma Concentration Profile of Allopregnanolone Following Administration of SAGE-547
(All Parts) – Linear Scale
(Pharmacokinetic Population)



Summary PK Parameter of Allopregnanolone Following Administration of SAGE-547
IV Part 3
(Pharmacokinetic Population)

Part	Treatment	Subject ID	AUC0-t (hr*ng/mL)	AUC0-inf (hr*ng/mL)	Cmax (ng/mL)	tmax (hr)	t1/2 (hr)	Vss (mL
3	IV SAGE-547 240 ug/kg	(b) (6)	245	254a	52.9	4	6.8a	273
			382	404a	85.9	4	11.5a	254
			256	279a	46.3	3	10.9a	494
			314	328a	59.1	4	8.2a	286
			307	317	62.1	4	4.9	181
			265	291a	53.3	3	13.5a	488
			265	276a	55.7	4	6.6a	244
			305	313a	72.8	2	3.1a	115
	N		8	1	8	8	1	1
	Mean		292.4	317.0	61.0	3.5	4.9	1810
	SD		44.69	NA	12.72	0.76	NA	NA
	Min		245	317	46	2	5	181
	Median		285.0	317.0	57.4	4.0	4.9	1810
	Max		382	317	86	4	5	181
	CV%		15.3	NA	20.9	21.6	NA	NA
	Geometric Mean		289.6	317.0	60.0			1810
	CV% Geometric Mean		14.6	NA	19.8			NA

Summary of Allopregnanolone PK Parameter Following Oral Administration of 30 mg SAGE-547
Fasted State Part 1
(Pharmacokinetic Population)

Part	Treatment	Subject ID	AUC0-t (hr*ng/mL)	AUC0-inf (hr*ng/mL)	Cmax (ng/mL)	tmax (hr)	t1/2 (hr)	CL/F (L/h/kg)	F (%
1	Oral SAGE-547 30 mg (Fasted)	(b) (6)	7.73	9.06a	5.32	1	0.9a	39.3a	
			17.4	19.3	11.1	0.5	1.2	20.1	4.
			44	67.4a	19	1	14.3a	5.47a	7.
			23.4	25.9a	16.1	0.5	1.3a	18.1a	4.
			20.6	22.9	7.22	1.5	1.5	13.7	5.
			11.1	13.2a	5.01	1.5	1.2a	27.7a	2.
			7.49	9.35a	6.16	1	1.1a	31.9a	2.
			3.12		2.83	1			0.
	N		8	2	8	8	2	2	7
	Mean		16.855	21.1	9.095	1.0	1.4	16.9	3.8
	SD		13.0206	2.55	5.7743	0.38	0.21	4.53	2.2
	Min		3.12	19	2.83	1	1	14	0.
	Median		14.250	21.1	6.700	1.0	1.4	16.9	4.3
	Max		44.00	23	19.00	2	2	20	7.
	CV%		77.3	12.1	63.5	37.8	15.7	26.8	58
	Geometric Mean		12.843	21.0	7.625			16.6	
	CV% Geometric Mean		98.7	12.1	71.2			27.6	

Summary PK Parameter of Allopregnanolone Following Oral Administration of SAGE-547
Fed State Part 2
(Pharmacokinetic Population)

Part	Treatment	Subject ID	AUC0-t (hr*ng/mL)	AUC0-inf (hr*ng/mL)	Cmax (ng/mL)	tmax (hr)	t1/2 (hr)	CL/F (L/h/kg)	
2	Oral SAGE-547 30 mg (Fed)	(b) (6)	9.16	11.9a	3.8	1.033	1.7a	33.2a	2
			40.8	44.7a	7.32	4	2.9a	8.24a	7
			24	27.1	6.35	1	2	17.5	4
			10.5	226a	2.36	1	81.6a	1.40a	2
			15.2	23.7a	3.91	1	3.8a	15.4a	3
			5.39		1.89	2			1
			4.85		2.45	1.5			1
			4.32	15.1a	3.36	0.5	5.7a	23.2a	0
	N		8	1	8	8	1	1	
	Mean		14.278	27.1	3.930	1.5	2.0	17.5	2.
	SD		12.5715	NA	1.9460	1.10	NA	NA	2.
	Min		4.32	27	1.89	1	2	18	0
	Median		9.830	27.1	3.580	1.0	2.0	17.5	2.
	Max		40.80	27	7.32	4	2	18	7
	CV%		88.1	NA	49.5	73.1	NA	NA	6
	Geometric Mean		10.619	27.1	3.552			17.5	
	CV% Geometric Mean		95.1	NA	50.3			NA	

Summary of the Food Effect Statistical Analysis
(Pharmacokinetic Population)

Parameter	N	Intra-CV	Geometric LSmeans		Ratio (%)	90% Confidence Interval	
			Fed	Vs Fasted		Lower Bound	Upper Bo
Cmax (ug/mL)	7	31.0	3.581	8.027	44.6	32.58	61.08
AUC0-t (ng*h/mL)	7	31.7	12.075	13.809	87.4	63.42	120.5

PHARMACOKINETICS RESULTS:

- The t_{max} following oral administration of SAGE-547 30 mg was generally 1 hour. Follow infusion, t_{max} was generally at the end of the infusion (4 hours).
- The oral bioavailability of SAGE-547, calculated from AUC_{0-t} values, was low when adm to fasted subjects and was similar when administered after a high-fat meal.
- Following oral administration of SAGE-547 30 mg after a high-fat meal, the C_{max} values average less than half those following administration in the fasted state. Overall, the AU values were broadly similar.

Safety Results			
Was there any death or serious adverse events?	☐ Yes	☑ No	☐ NA

SAFETY RESULTS:

- SAGE-547 (both oral and IV) was generally well tolerated across all parts of the study.
- There were no deaths, other serious AEs (SAEs), or discontinuations from the study or st due to treatment-emergent AEs (TEAEs).
- Three of nine subjects (33.3%) reported TEAEs in at least one part of the study, with sim incidences across each part.
- All TEAEs were considered mild in severity, resolved, and most were considered by the Investigator to be related to study drug.
- There was no evidence of clinically important changes in vital signs, ECG, or clinical che hematology, or urinalysis parameters.

Overall Sponsor Conslusions

This was a three-part, Phase 1b, open-label study to assess the oral bioavailability and PK of SAGE-547. The study also assessed the potential effect of food on the oral bioavailability of allopregnanolone following oral administration of SAGE-547 and the safety and tolerability of oral SAGE-547. Three cohorts were followed in the study, which included a fasted state, fed state, and IV administration. A total of nine subjects received SAGE-547 and were followed for PK and safety evaluation.

Consistent with preclinical findings in the rat, in which the bioavailability for SAGE-547 is low (<5%), the mean bioavailability of SAGE-547 30 mg in this study was low, when orally administered to fasted or fed subjects. Oral administration following a high-fat meal resulted in a diminution of C_{max}, with no marked change in AUC_{0-t}.

SAGE-547 Injection, administered orally in the fasted and fed states and administered intravenously, was generally well tolerated. Three of nine subjects (33.3%) across all parts of the study reported a total of four TEAEs. All TEAEs were reported 1 to 8 hours postdosing, were considered mild in severity, and resolved by the end of the study. There were no deaths, other SAEs, or discontinuations from the study or study drug due to TEAEs and no clinically important changes in vital signs, ECG, or clinical chemistry, hematology, or urinalysis parameters occurred.

Reviewer Comments and Conclusions

25. *Study Design: This was a cross-over study design to evaluate the oral bioavailability of SAGE-547 administered as an oral dose vs. IV infusion in healthy adult subjects:*
    - *The oral dose of SAGE-547 was 30 mg. This was similar to a total IV dose when infused at the rate of 90 µg/kg/h to a 70 kg subject over 4 hours.*
    - *IV dose was chosen because it is the intended route of administration.*
    - *Males and females, between the age of 18 and 65 years were included.*
    - *Adequate number of subjects (N= 8) were included in the study*
    - *The final to-be-marketed formulation of SAGE 547 was used in this study.*

26. *Protocol deviation: No major protocol deviations were reported.*

27. *Data Analysis (i.e., any outliers etc.): There were no PK outliers.*

28. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

29. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *The study excluded use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.*
- *The study excluded use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17. This is acceptable because it minimized the chance of any drug interactions.*

30. *Pharmacokinetic findings: We agree with the sponsor's PK analysis and the conclusions from the study.*

Overall Conclusion:
We agree with the sponsor's analysis and conclusions.

APPEARS THIS WAY ON ORIGINAL

CLINICAL PHARMACOLOGY STUDY REVIEW

Transfer of SAGE 547 in Breast Milk of Lactating Women

Study # 547-CLP-108	**Study Period:** 19-June-2017 to 22-July-2017
NDA 211371_Zulresso_Brexanolone IV infusion	
Title	An Open-Label Study Evaluating Concentrations of Allopregnanolone Following Administration of SAGE-547 Injection in the Breast Milk of Adult Lactating Women
Objectives:	The primary objective of the study was to assess the concentration of allopregnanolone in breast milk samples after a 60-hour intravenous (IV) infusion of SAGE-547 Injection in lactating women. The secondary objectives of the study were to: • Assess the concentration of allopregnanolone in plasma after a 60-hour infusion of IV infusion of SAGE-547 Injection in lactating women; and • Assess the safety and tolerability of a 60-hour IV infusion of SAGE-547 Injection in lactating women.
Study Design:	

APPEARS THIS WAY ON ORIGINAL

This was a Phase 1b, open-label study designed to evaluate the concentration of allopregnanolone in breast milk of adult lactating women following a 60-hour infusion of SAGE-547 Injection.

Subjects underwent screening procedures at the Screening Visit to determine eligibility. Subjects continued breastfeeding or maximally pumping breast milk from Day -7 to predose Day 1 of the study. Subjects were required to temporarily cease giving breast milk to their infant(s) from just prior to receiving study drug infusion (predose on Day 1) until the completion of the Day 7 Visit. Subjects maximally pumped all breast milk predose Day 1 through the Day 7 Visit.

Subjects were confined to the study center from predose on Day 1 until after the 72-hour assessments on Day 3. Subjects began a continuous IV infusion of SAGE-547 Injection on Day 1. The dosing regimen was as follows: 30 µg/kg/hr (0 to 4 hours), then 60 µg/kg/hr (4 to 24 hours), then 90 µg/kg/hr (24 to 52 hours), followed by 60 µg/kg/hr (52 to 56 hours), and 30 µg/kg/hr (56 to 60 hours). The protocol allowed for dose adjustment in the case of intolerable adverse events. Breast milk was pumped and collected predose and at least every 12 hours throughout the Treatment Period and thereafter through the Day 7 Visit. The date, time, and volume of each expressed sample was recorded, and the entire sample was frozen for analysis. After discharge, subjects continued to pump and collect breast milk and brought the frozen samples to the study site at the Day 7 visit. Blood samples were collected at pre-defined timepoints and blood and breast milk were analyzed for concentrations of allopregnanolone.

Study-specific assessments for safety and pharmacokinetics (PK) outcome measures were completed at prespecified times over a 72-hour period during the Treatment Period (Table 3).

A follow-up visit was conducted on Day 7 on an outpatient basis. Subjects were allowed to resume breastfeeding after the Day 7 study visit.

Number of subjects (planned and analyzed): Enrollment continued until eight subjects had provided analyzable breast milk samples for at least six days out of the seven days of collection. Twelve subjects were enrolled to achieve this goal.
Diagnosis and main criteria for inclusion: Healthy women between 18 and 45 years of age, inclusive, and ≤6 months postpartum at screening who were lactating and maximally pumping breast milk or actively breastfeeding from Day -7 to screening.
Test product, dose and mode of administration, batch number: SAGE-547 Injection was administered as 60-hour IV infusion (with increasing doses to 52 hours followed by tapering doses to 60 hours). The study drug lot number for SAGE-547 Injection was B160267.
Duration of treatment: Single 60-hour infusion

The specific infusion dose of SAGE-547 Injection was calculated based on weight for each subject at Screening. SAGE-547 Injection was administered as a 60-hour IV infusion, according to the schedule in Table 2. Infusion bags and lines were changed every 24 hours.

Table 2: Infusion Rates

Time point	Day 1 0 to 4 hours	Day 1 4 to 24 hours	Day 2–3 24 to 52 hours	Day 3 52 to 56 hours	Day 3 56 to 60 hours
Dose	30 µg/kg/hr	60 µg/kg/hr	90 µg/kg/hr	60 µg/kg/hr	30 µg/kg/hr

The study drug (lot number B1600267) was provided to the clinical site by the supplier/manufacturer.

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Product Used:

Test product, dose and mode of administration, batch number: SAGE-547 Injection was administered as a 60-hour IV infusion (with increasing doses to 52 hours followed by tapering doses to 60 hours).
The study drug lot number for SAGE-547 Injection was B160267.

Route of Administration	IV infusion
PK Sampling Times and Parameters	**Pharmacokinetics:** Blood samples for analysis of plasma concentrations of allopregnanolone were collected at regular time points throughout the three days of the Treatment Period and at Follow-up.

	Subjects were instructed to express/pump and retain all breast milk whenever they felt the need to express breast milk, at least every 12 hours, from Day 1 to the Day 7 Visit. Blood samples for analysis of plasma concentrations of allopregnanolone were collected pre-infusion and at 12, 24 (before infusion rate change), 36, 48, 56, 60 (before infusion end), 61, 62, 64, and 72 hours after the start of infusion. Samples were also collected on Day 7. Pharmacokinetic blood draws during the Treatment Period had a window of ±10 minutes. Breast milk was collected pre-infusion, at least every 12 hours between Hour 0 and Hour 72, and on Days 4, 5, 6, and 7. Subjects were instructed to express/pump and retain all breast milk whenever they felt the need to express breast milk (at least every 12 hours) from Day 1 to the Day 7 Visit. The date, time, and volume of each expressed sample were recorded, and the entire sample was frozen for analysis; it was possible that not all samples were analyzed as ideal and sample timing may have been refined with cumulative knowledge from previous samples. When possible, expression of breast milk samples occurred within ±1 hour of the collection of blood samples for pharmacokinetic analysis, including the predose sample. A key time point for collection was 48 hours after the start of the infusion, which covered the period 36 hours and 48 hours after the start of the infusion, when the maximum dose was being administered.
Safety Parameters	**Safety:** Safety was assessed by adverse events (AEs), vital signs, clinical laboratory measures, electrocardiograms (ECGs), and suicidal ideation and behavior was evaluated with the Columbia Suicide Severity Rating Scale (C-SSRS).
PK Moieties	Sage-547
PD Endpoint(s)	None
Statistical Methods	

Statistical methods:

The safety set consisted of all subjects who were administered SAGE-547 Injection. The PK set consisted of all subjects for whom at least one evaluable PK sample was available. The breast milk set consisted of all subjects who began the infusion with SAGE-547 Injection and had at least one breast milk sample collected.

For the PK set, the PK parameters were summarized by descriptive statistics, including n, mean, standard deviation, minimum, and maximum values.

Individual subject breast milk concentrations were summarized by visit (24-hour pooled data) for the breast milk set. The breast milk concentration of allopregnanolone, volume, and amount secreted were summarized by descriptive summary statistics.

The safety set was used to provide descriptive summaries of safety parameters including assessment of AEs, vital signs, clinical laboratory measures, ECGs, and C-SSRS responses. The overall incidence of AEs was displayed by system organ class and by preferred term. The incidence of AEs was presented by maximum severity and relationship to study drug. Out-of-range safety endpoints were categorized as low or high, where applicable.

Continuous hematology and chemistry results were summarized at each scheduled time point. The number and percentage of subjects with shift changes from baseline based on the laboratory normal ranges were tabulated.

In addition, a listing containing individual subject hematology and chemistry values outside the reference ranges was provided. Vital sign changes from baseline were summarized using descriptive statistics. Electrocardiogram raw values and changes from baseline values were provided, and ECG results were tabulated as normal, abnormal not clinically significant (NCS), and abnormal clinically significant (CS). Individual subject physical examinations were also listed.

The number and percentage of subjects with any suicidal ideation or behavior item on the C-SSRS were summarized at each scheduled time point.

Analytical Method

Method Type	LC/MS/MS	Matrix	Plasma
Analytes	Sage-547, Phenytoin		

/alidation	▪ Method validated prior to use	☑ Yes ☐ No
	▪ Method validation acceptable	☑ Yes ☐ No
tudy ample nalysis	▪ Samples analyzed within the established stability period	☑ Yes ☐ No
	▪ Quality control samples range acceptable	☑ Yes ☐ No
	▪ Chromatograms provided	☑ Yes ☐ No
	▪ Accuracy and precision of the calibration curve acceptable	☑ Yes ☐ No
	▪ Accuracy and precision of the quality control samples acceptable	☑ Yes ☐ No
	▪ Overall performance acceptable	☑ Yes ☐ No

Study Population:

- N= 12 (Healthy adult females, 22-42 years)

Table 1: Demography of subjects

Variable/ Status	Safety Set (N=12)
Sex	
Female	12 (100.0 %)
Age (years)	
Mean (SD)	28.8 (5.74)
Median (min, max)	28.5 (22, 42)
Ethnicity	
Hispanic or Latino	1 (8.3 %)
Not Hispanic or Latino	11 (91.7 %)
Race	
Black or African American	1 (8.3 %)
Native Hawaiian or other Pacific Islander	1 (8.3 %)
White	9 (75.0 %)
American Indian or Alaskan Native	1 (8.3 %)
Height (cm)	
Mean (SD)	167.4 (6.49)
Median (min, max)	169.5 (157, 176)
Weight (kg)	
Mean (SD)	82.97 (19.345)
Median (min, max)	79.60 (60.4, 127.5)
BMI (kg/m^2)	
Mean (SD)	29.49 (5.852)
Median (min, max)	28.20 (20.9, 41.6)

Source: Table 14.1.2

Inclusion Crietria:

APPEARS THIS WAY ON ORIGINAL

1. Subject had signed an ICF prior to any study-specific procedures being performed.
2. Subject was generally healthy, non-smoking, ambulatory, between 18 and 45 years of age, inclusive, and ≤6 months postpartum at screening.
3. Subject agreed to adhere to the study requirements.
4. Subject was lactating and maximally pumping breast milk or actively breastfeeding from Day -7 to screening.
5. Subject agreed to continue to maximally pump or breastfeed to predose Day 1 and agreed to thereafter pump breast milk through the Day 7 visit.
6. Subject agreed to temporarily cease giving breast milk to their infant(s) from just prior to receiving study drug (Day 1) through the Day 7 Visit.
7. Subject agreed to use one of the following methods of birth control during participation in the study and for 60 days following the end of the study (or longer if required by local regulations): bilateral tubal ligation or surgically sterile; total abstinence (no sexual intercourse); hormonal contraceptives (birth control) including birth control pills, or implantable or injectable contraceptives (Norplant® or Depo-Provera®); a barrier form of contraception such as a condom or occlusive cap with a spermicide; or an intrauterine device.
8. Subject was willing to delay the start of any new pharmacotherapy regimens from screening until after Day 7 of the study.

Exclusion Criteria:

Any of the following was regarded as a criterion for exclusion from the study:

1. Subject had a positive pregnancy test at screening or Day 1 (prior to initiation of the SAGE-547 infusion).
2. Subject was in renal failure requiring dialysis or fulminant hepatic failure or was anemic (hemoglobin ≤10 g/dL).
3. Subject had a known allergy to progesterone or allopregnanolone.
4. Subject had taken any medication (other than vitamins, acetaminophen, or oral contraceptives) within seven days prior to screening.
5. Subject had current/active alcohol or drug abuse (including benzodiazepines) within the 30 days prior to screening, as assessed by the Investigator.
6. Subject had exposure to another investigational study drug or device within 30 days prior to screening.
7. Subject had previously participated in any other study employing SAGE-547.
8. Subject had any medical, psychiatric, social, or other circumstances that may have interfered with study compliance, completion, or accurate assessment of study outcomes, as assessed by the Investigator.

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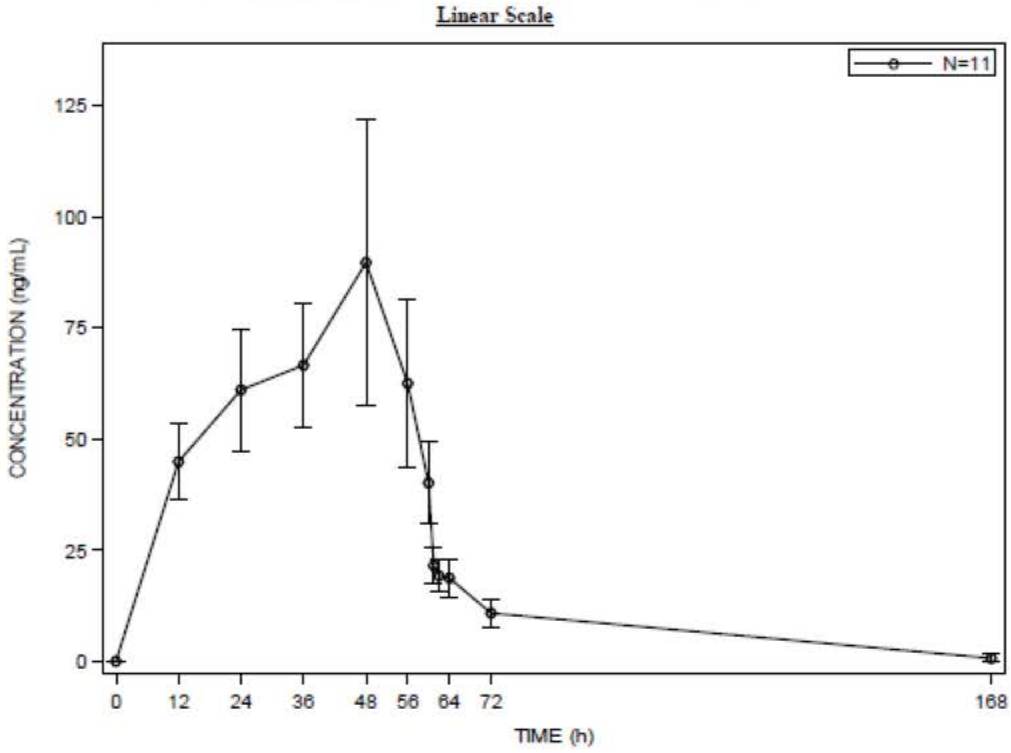
PK Results

All predose plasma samples were BQL (i.e., ≤1 ng/mL) for allopregnanolone. The mean plasma concentration increased with dose titration and declined with the dose tapering as shown in figure.

Figure 1: Mean Plasma Concentration-Time Profiles (Arithmetic Mean and Standard Deviation) of Allopregnanolone (Pharmacokinetic Set)



Note: The limit of quantification for plasma was 1 ng/ml. The pre-dose concentrations of allopregnanolone for all subjects were BQL, therefore, no baseline correction was performed.

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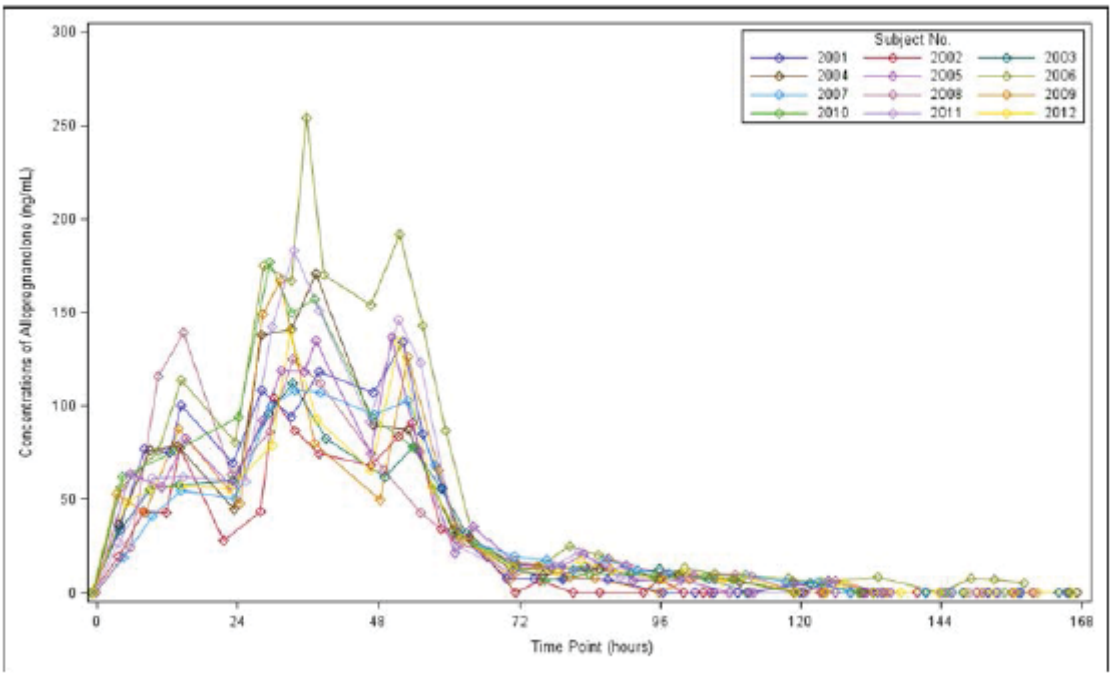
Table 6: Summary (Geometric Mean and 95% Confidence Interval of GM) of Plasma Pharmacokinetic Parameters of Allopregnanolone (Pharmacokinetics Set)

PK Parameters	N[a]	GM	95% CI
C_{max}, ng/mL	11	89.67	(74.19, 108.39)
T_{max}, h[b]	11	47.83	(47.83, 55.83)
C_{avg}, ng/mL	11	70.56	(61.86, 80.48)
C_{ss}, ng/mL	11	80.07	(70.91, 90.41)
AUC_{0-60}, ng•h/mL	11	3358.38	(2998.58, 3761.35)
AUC_{24-56}, ng•h/mL	11	2257.91	(1979.49, 2575.50)
AUC_{0-t}, ng•h/mL	11	3557.76	(3177.60, 3983.40)
$AUC_{0-\infty}$, ng•h/mL	11	3736.51	(3309.13, 4219.09)
T_{last}, h	11	72.00	(72.00, 72.00)
R^2 adjusted	11	0.93	(0.90, 0.97)
$t_{1/2}$, h	11	11.34	(9.79, 13.14)
λ_z, 1/h	11	0.06	(0.05, 0.07)
CL, L/h	11	87.48	(78.76, 97.18)
V_z, L	11	1431.16	(1224.80, 1672.30)

[a] The concentration data for Subject (b) (6) were listed but excluded from the statistical analysis due to discontinuation of infusion
[b] Median (min, max) reported for T_{max}
Source: Table 14.2.2

Individual subjects' concentrations of allopregnanolone in breast milk over time are shown in Figure 2. Because breast milk collection times varied by subject, mean concentration of allopregnanolone over time could not be directly compared with the mean plasma concentration over time, so the data are summarized individually.

Figure 2: Individual Concentration of Allopregnanolone in Breast Milk (Linear Scale)



Note: The limit of quantification was 5ng/mL for breast milk
Source: Figure 14.2.1-1

PHARMACOKINETICS RESULTS:

- There was no apparent accumulation of allopregnanolone in plasma or breast milk.
- All predose plasma samples were below the quantifiable limit (BQL) for allopregnanolone. Over the course of the 60-hour infusion, the mean plasma concentration increased with dose titration and declined during dose taper. By 72 hours, plasma allopregnanolone concentrations were approaching the lowest quantifiable limit (1 ng/mL) for all subjects. All subjects were near or below the quantifiable limit by Day 7.
- Plasma PK parameters show a mean (95% CI) C_{max} of 89.67 (74.19 to 108.39) ng/mL in the 11 subjects analyzed, with a median time at maximum plasma concentration (T_{max}) of 47.83 hours, and half-life ($t_{½}$) of 11.34 hours. The area under the concentration versus time curve from time 0 to infinity (AUC_{∞}) was 3736.51 ng•h/mL and the area under the concentration versus time curve from time 24 hours to 56 hours (AUC_{24-56}) (during the maximum dose infusion) was 2257.91 ng•h/mL.
- At Hour 60, the concentrations of allopregnanolone in breast milk were rapidly declining in parallel with plasma levels. By Hour 72, all subjects were approaching the lowest quantifiable limit (of 5 ng/mL), and by Hour 96, BLQ levels were observed. All but one subject (11 of 12 subjects) were below the limit of quantification at Hour 144 with values of <5 ng/mL. The remaining subject's levels were <10 ng/mL and remained there through Hour 168.

Milk-to-Plasma Ratio: Results of a study to examine the amount of brexanolone in breast milk, CLP-108, demonstrated that the milk concentrations were found to be 1.36-fold greater than plasma.

In order to compute the relative infant dose of brexanolone, the highest observed maternal range of AUC values from 24 to 48 hours was used, when the maximum 90 µg/kg/h infusion was given (PPD-202C Pharmacokinetic Report GF30KT). Using these data, the relative infant dose (RID) was

calculated, as shown in Table below.

Table 7: Calculation of Maximum Brexanolone Relative Infant Dose (RID)

	Plasma AUC_{24-48}, ng.h/mL	Milk AUC_{24-48}[a], ng.h/mL	Average Brexanolone in Milk[b], ng/mL	Daily Dose in Milk[c], ng/kg/day	RID[d],%
Minimum	462	628	26.18	3927	0.1818
Median	1760	2394	99.73	14960	0.6926
Maximum	3410	4638	193.2	28980	1.342

[a] Computed as 1.36*Milk AUC
[b] Milk AUC divided by 24 hours
[c] Based on feeding rate of 150 mL/kg/day
[d] Computed as infant dose divided by maternal dose (90 µg/kg/h*24 h) *100% (Bennett 1996)

Based on Literature (British Journal of Clinical Pharmacology, 42, 673-4, 1996), any drug with a Relative Infant Dose (RID) of <10% constitutes a low risk to an infant who is breast fed during administration of the drug. The maximum RID for brexanolone, calculated for the 90 µg/kg/h dose for Hour 24 to Hour 48 of the infusion, is 1.3%. Additionally, the oral bioavailability of brexanolone is known to be <5%. Taken together, this suggests that the risk to an infant who is breast fed during the 60-hour infusion is low.

Safety Results

Was there any death or serious adverse events?

SAFETY RESULTS:

- No subjects died or had a serious or severe treatment-emergent adverse event (TEAE). One subject discontinued dosing due to TEAEs (oedema, pain, and redness at the infusion site).
- A total of seven of the 12 subjects experienced TEAEs (58.3%). The most frequently reported TEAEs were in the General Disorders and Administration Site Conditions System Organ Class (SOC): infusi site pain and infusion site swelling in six subjects (50%) each and infusion site erythema in four subje (33.3%).
- Two subjects (16.7%) experienced moderate TEAEs; both subjects reporting moderate infusion site pa All other TEAEs were reported as mild.
- Two subjects (16.7%) experienced TEAEs considered by the investigator to be related to study drug (c with nausea and one with abnormal dreams).
- Laboratory test results, vital signs, and ECG parameters were unremarkable over the course of the stud
- There was no evidence suggesting that administration of SAGE-547 Injection was associated with an increase in suicidal ideation or behavior in this study as assessed by C-SSRS.

☐ Yes
☑ No
☐ NA

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Adverse events were reported for 58.3% (7/12) of subjects. Two subjects experienced TEAEs

that were moderate in intensity: One subject experienced moderate infusion site edema, infusion site erythema, and infusion site pain, and one subject experienced moderate infusion site pain and infusion site swelling. The rest of the reported TEAEs were mild

Table 9 Treatment-Emergent Adverse Events by System Organ Class and Preferred Term (Safety Set)

System Organ Class Preferred Term	N = 12 n (%)
Subjects with any AE	7 (58.3)
Gastrointestinal Disorders	
Nausea	1 (8.3)
General Disorders and Administration Site Conditions	
Infusion site pain	6 (50.0)
Infusion site swelling	6 (50.0)
Infusion site erythema	4 (33.3)
Infusion site bruising	1 (8.3)
Infusion site irritation	1 (8.3)
Infusion site oedema	1 (8.3)
Infusion site rash	1 (8.3)
Nervous System Disorders	
Headache	2 (16.7)
Psychiatric Disorders	
Abnormal dreams	1 (8.3)

Source: Table 14.3.1.1 and Table 14.3.1.2

Overall Sponsor Conclusions

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This open-label study was designed to evaluate the concentration of allopregnanolone in breast milk of adult lactating women after a 60-hour IV infusion of SAGE-547 Injection. SAGE 547 was well-tolerated in this population of adult lactating women. There were no deaths, SAEs, severe AEs, or clinically significant laboratory evaluations. One subject discontinued study drug at H47 due to moderate infusion site reactions.

Over the course of the 60-hour infusion, the mean plasma concentration increased during dose titration and declined during dose taper. For all subjects, the concentration of allopregnanolone in plasma was approaching the lowest quantifiable limit (1 ng/mL) by Day 3 and all subjects' plasma concentrations of allopregnanolone were near or below the quantifiable limit by Day 7. In general, changes in allopregnanolone concentrations in breast milk followed a pattern similar to that seen for changes in allopregnanolone concentrations in plasma. At Hour 60, the concentrations of allopregnanolone in breast milk were rapidly declining in parallel with plasma levels. By Hour 72, all subjects were approaching the lowest quantifiable limit (5 ng/mL), and by Hour 96, BLQ levels were observed. All but one subject (11 of 12 subjects) were below the limit of quantification at Hour 144 with values of <5 ng/mL. The remaining subject's levels were <10 ng/mL and remained there through Hour 168. There was no apparent accumulation of allopregnanolone in plasma or breast milk.

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Reviewer Comments and Conclusions

31. *Study Design: This was an Open Label study evaluating the concentrations of allopregnanolone in breast milk of lactating women following the administered of SAGE-547 as an IV infusion at the proposed clinical dose and duration.*
    - *The dose of SAGE-547 in this study (90 µg/kg/h) is the anticipated clinical dose, which has been previously administered in postpartum depression and found to be well-tolerated.*
    - *IV dose was chosen because it is the intended route of administration.*
    - *Healthy females, between the age of 18 and 42 years were included.*
    - *Adequate number of subjects (N= 12) were included in the study*
    - *The final to-be-marketed formulation of SAGE 547 was used in this study.*

32. *Protocol deviation: No major protocol deviations were reported.*

33. *Data Analysis (i.e., any outliers etc.): There were no PK outliers. PK parameters were calculated excluding 1 subject due to the discontinuation of infusion in this subject.*

34. *Bioanalytical Method: A validated bio-analytical methodology was used which was acceptable.*

35. *Inclusion and Exclusion Criteria: The subject inclusion and exclusion criteria were acceptable since:*

- *The study excluded use of any medications (prescription or over-the-counter), or foods rich in flavonoids (such as cranberries) or juice (such as pineapple juice) primarily metabolized by cytochrome P450 (CYP) 2C9 (CYP2C9), as in vitro studies indicate SAGE-547 has the potential to alter the metabolism of CYP2C9 substrates when administered concomitantly.*
- *The study excluded use of any medications (prescription or over-the-counter), foods, or juices that are strong inhibitors and/or inducers of CYP2C8, CYP2C9, CYP2C19, CYP3A4, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) and UGT2B17. This is acceptable because it minimized the chance of any drug interactions.*

36. *Pharmacokinetic findings: We agree with the sponsor's PK analysis and the conclusions from the study.*

Overall Conclusion:

Based on concentrating of SAGE 547 in mothers plasma vs. milk, it is observed that the milk concentrations were 1.36-fold greater than plasma. However, the comparison of the effective dosage (in ug/kg/day) for the child vs. mother clearly demonstrates that maximal dosage in child is ~29 ug/kg/day vs. ~ 2160 ug/kg/day in mother. Therefore, in the worst-case scenario, only ~ 1% of the dose (normalized by weight) is orally delivered to the child. Additionally, the oral bioavailability of brexanolone is known to very poor <5%. Therefore, eventually only ~ 0.05% of the dose (normalized by weight) is available systemically in the child. Thus, we agree that the risk to an infant who is breast fed during the 60-hour infusion is low.

Thus, we recommend that it is acceptable for nursing mothers to continue to breast-feed during the infusion.

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22.4.4. **Summary of In Vitro Studies**

In Vitro Studies

Solubility and LogD

Study # SSN-409
Title: Tier 1 (Solubility and LogD) Analysis of Selected Compounds
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4223-distrib\ssn-408\

- **Objective:**

The objective of this Tier 1 study was to conduct the following experiments: solubility, and distribution coefficient (LogD)

- **Methods:**

Solubility

Compound stocks (10mM in DMSO) were diluted to 10 µM in phosphate buffered saline (PBS) with and without 0.02% Cremophor in glass vials: final DMSO concentration was 0.1%. The solutions were sonicated for 1 minute and shaken for 1 hour at room temperature (typically 24°C). Samples were taken for both direct analysis (precentrifugation) and solubility determination.

LogD

Standards consisting of steroids with known LogD were run for HPLC/MS and their retention times were used to construct a linear standard curve with LogD versus RT. Compounds were typically injected at 20 µM in MeOH and run under the same conditions as the standards. LogD was assessed by comparison of RT with the standard curve.

- **Analytical Method:** HPLC/MS

- **Results and Conclusions:**

Sample	LogD	Solubility (µM) in 0.1% DMSO	
		Without Cremophor	With 0.02% Cremophor
SAGE-547	4.87	3.3	8.8

- **Reviewer's Comments:**
- *Sponsor's conclusions for solubility and LogD seem to be acceptable.*

2. Plasma Protein Binding

Study # SSN-01423
Title: In Vitro Protein Binding of SAGE-547 in Human Plasma, Isolated Human Serum Albumin, and Isolated Human α1-Acid Glycoprotein
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4223-distrib\ssn-01423\

- **Objective:** The objectives of this study were to determine concentration dependence of protein binding to human plasma

proteins, and to examine the relative affinities to serum albumin (HSA) or α1-acid glycoprotein (AAG).

- **Methods:**

Protein binding was determined by equilibrium dialysis at 37°C under an atmosphere of 5% CO2 and saturated relative humidity for 6 hours at 750, 1000, 1500, 2000, and 4000 ng/mL (2.5, 3.1, 4.7, 6.3, 12.6 µM) SAGE-547 in human plasma, HSA (45 mg/mL) and AAG (1.0 mg/mL). The dialysis time was established based on data from the time-to-equilibrium experiment.

Equations:

1. Protein Binding by Equilibrium Dialysis

Percent Drug Bound = $[(C_m - C_d) / C_m] \times 100$
Percent Drug Unbound = 100 – Percent Bound
where:
C_d Concentration of test article in dialysate (ng/mL)
C_m Concentration of test article (ng/mL) in matrix (plasma, HSA, or AAG)

2. Recovery for Equilibrium Dialysis

$$\text{Recovery}(\%) = \frac{[(C_m \times V_m) + (C_d \times V_d)]}{C_o \times V_o} \times 100$$

where:
C_m Concentration of test article (ng/mL) in matrix (plasma, HSA, or AAG)
C_d Concentration of test article in dialysate (ng/mL)
C_o Original concentration of test article (ng/mL) in matrix (plasma, HSA, or AAG) prior to loading the dialysis device
V_m Nominal volume (mL) of the matrix (donor side)
V_d Nominal volume (mL) of the dialysate (receiver side)
V_o Nominal volume (mL) of the original matrix added to the dialysis device

- **Analytical Method:** LC-MS/MS

Limit of quantitation 1 ng/mL
Curve Range: 1 – 500 ng/mL

- **Results:**

1. Time to Equilibrium

Table 1. Percent bound and unbound SAGE-547 in human plasma (4000 ng/mL) after dialysis at 37°C for 3, 4, 5, 6, and 7 hours.

Dialysis Time (Hour)	Concentration		Percentage of SAGE-547							
	Donor (ng/mL)	Receiver (ng/mL)	Bound			Unbound		Percent Recovery		
			Rep	Mean	SD[a]	Rep	Mean	Rep	Mean	SD
3	4070	7.50	99.8	99.8	0.0	0.2	0.2	99.0	98.5	4.77
	3840	6.72	99.8			0.2		93.4		
	4230	7.59	99.8			0.2		103		
	DNU	8.61	NA			NA		NA		
4	3910	6.79	99.8	99.8	0.0	0.2	0.2	95.1	98.5	3.34
	4020	7.99	99.8			0.2		97.8		
	4240	6.55	99.8			0.2		103		
	4020	9.35	99.8			0.2		97.9		
5	3840	6.52	99.8	99.8	0.0	0.2	0.2	93.4	100	4.78
	4220	7.66	99.8			0.2		103		
	4100	8.40	99.8			0.2		99.8		
	4280	9.85	99.8			0.2		104		
6	3830	7.95	99.8	99.8	0.0	0.2	0.2	93.2	95.2	4.45
	4050	7.22	99.8			0.2		98.5		
	3690	8.48	99.8			0.2		89.9		
	4070	10.9	99.7			0.3		99.2		
7	3770	8.07	99.8	99.8	0.0	0.2	0.2	91.8	94.9	4.61
	3830	8.34	99.8			0.2		93.2		
	4180	9.47	99.8			0.2		102		
	3810	11.7	99.7			0.3		92.9		

DNU Data not used due to sample preparation error.
NA Not applicable.
Rep Replicate.
SD Standard deviation.
a Standard deviation applies to both bound and unbound percentages.

Mean unbound SAGE-547 was 0.2% among all time points. Mean percent recovery ranged from 94.9 to 100% among all incubation time points. A 6-hour incubation time was chosen as the equilibration time for all subsequent experiments.

Table 2. Percent bound and unbound SAGE-547 in human plasma at various concentrations after dialysis at 37°C for 6 hours.

Species	Concentration (ng/mL)	Concentration		Percentage of SAGE-547							
		Donor (ng/mL)	Receiver (ng/mL)	Bound			Unbound		Percent Recovery		
				Rep	Mean	SD[a]	Rep	Mean	Rep	Mean	SD
Human	750	795	2.31	99.7	99.8	0.1	0.3	0.2	94.1	95.0	4.98
		858	BLQ	99.8			0.2		101		
		755	1.81	99.8			0.2		89.3		
		807	BLQ	99.9			0.1		95.2		
	1000	1040	BLQ	99.9	99.9	0.0	0.1	0.1	94.4	95.9	3.39
		1110	BLQ	99.8			0.2		101		
		1030	BLQ	99.9			0.1		93.6		
		1040	1.88	99.8			0.2		94.6		
	1500	1370	3.07	99.8	99.7	0.1	0.2	0.3	86.2	94.6	17.1
		1330	2.86	99.8			0.2		83.7		
		1400	6.19	99.6			0.4		88.4		
		1910	3.88	99.8			0.2		120		
	2000	1950	2.85	99.9	99.8	0.0	0.1	0.2	89.4	102	14.5
		2220	3.50	99.8			0.2		102		
		2660	3.73	99.9			0.1		122		
		2030	4.08	99.8			0.2		93.1		
	4000	4100	13.0	99.7	99.8	0.1	0.3	0.2	98.2	99.7	6.09
		4290	7.44	99.8			0.2		102		
		4440	7.78	99.8			0.2		106		
		3840	11.4	99.7			0.3		91.9		
Grand Mean and SD					99.8	0.1		0.2		97.3	3.08

BLQ Below limit of quantification (1.00 ng/mL).
Rep Replicate.
SD Standard deviation.
a Standard deviation applies to both bound and unbound percentages.

Table 3. Percent bound and unbound SAGE-547 in HSA solution (45 mg/mL) at various concentrations after dialysis at 37°C for 6 hours.

Species	Concentration (ng/mL)	Concentration Donor (ng/mL)	Concentration Receiver (ng/mL)	Percentage of SAGE-547 Bound Rep	Bound Mean	Bound SD[a]	Unbound Rep	Unbound Mean	Percent Recovery Rep	Percent Recovery Mean	Percent Recovery SD
Human	750	605	1.19	99.8	99.8	0.1	0.2	0.2	103	102	7.95
		544	1.48	99.7			0.3		92.5		
		595	1.77	99.7			0.3		101		
		659	1.24	99.8			0.2		112		
	1000	1030	1.70	99.8	99.8	0.0	0.2	0.2	109	97.1	8.96
		826	2.12	99.7			0.3		87.7		
		892	2.08	99.8			0.2		94.7		
		910	2.54	99.7			0.3		96.7		
	1500	1510	2.88	99.8	99.8	0.1	0.2	0.2	106	107	6.58
		1660	5.13	99.7			0.3		116		
		1440	3.08	99.8			0.2		101		
		1510	4.24	99.7			0.3		106		
	2000	1680	6.15	99.6	99.7	0.1	0.4	0.3	87.9	97.4	9.10
		2000	4.60	99.8			0.2		104		
		1750	4.34	99.8			0.2		91.4		
		2030	4.20	99.8			0.2		106		
	4000	3500	7.61	99.8	99.7	0.0	0.2	0.3	84.7	86.6	3.59
		3440	10.1	99.7			0.3		83.4		
		3570	8.92	99.8			0.2		86.5		
		3780	9.77	99.7			0.3		91.6		
Grand Mean and SD					99.8	0.0		0.2		98.1	7.66

Rep Replicate.
SD Standard deviation.
a Standard deviation applies to both bound and unbound percentages.

Table 4. Percent bound and unbound SAGE-547 in AAG solution (1.0 mg/mL) at various concentrations after dialysis at 37°C for 6 hours.

Species	Concentration (ng/mL)	Concentration Donor (ng/mL)	Concentration Receiver (ng/mL)	Percentage of SAGE-547 Bound Rep	Bound Mean	Bound SD[a]	Unbound Rep	Unbound Mean	Percent Recovery Rep	Percent Recovery Mean	Percent Recovery SD
Human	750	75.3	41.0	45.6	50.4	11.4	54.4	49.6	24.0	28.3	3.56
		80.3	47.8	40.5			59.5		26.8		
		101	51.7	48.8			51.2		31.3		
		120	39.9	66.8			33.3		31.2		
	1000	183	53.5	70.8	59.4	8.5	29.2	40.6	36.7	33.7	2.72
		154	59.9	61.1			38.9		34.2		
		126	58.3	53.7			46.3		30.1		
		139	66.6	52.1			47.9		33.7		
	1500	185	94.2	49.1	50.3	6.6	50.9	49.7	27.8	29.0	4.23
		193	81.1	58.0			42.0		26.7		
		241	116	51.9			48.1		35.3		
		165	95.5	42.1			57.9		26.4		
	2000	218	108	50.5	54.9	12.4	49.5	45.1	27.5	32.6	7.62
		192	113	41.1			58.9		26.3		
		416	123	70.4			29.6		43.0		
		286	121	57.7			42.3		33.7		
	4000	408	201	50.7	55.1	9.5	49.3	44.9	24.8	29.3	4.48
		702	217	69.1			30.9		35.5		
		477	226	52.6			47.4		28.5		
		455	237	47.9			52.1		28.4		
Grand Mean and SD					54.0	3.8		46.0		30.6	2.40

Rep Replicate.
SD Standard deviation.
a Standard deviation applies to both bound and unbound percentages.

- **Sponsor's conclusions:**

1. SAGE-547 was highly protein bound to human plasma and HSA (fraction unbound of 0.2% for both matrices).
2. SAGE-547 was poorly bound to AAG (fraction unbound of 46.0%).
3. There was no evidence of concentration dependence in any of the matrices over the

range of 750 to 4000 ng/mL (2.5 to 12.6 µM) for SAGE-547.

- ***Reviewer's Comments:***

1. *The test concentrations of SAGE-547 (2.5 to 12.6 µM) were higher than the clinical relevant exposures (< 120 ng/mL or 0.36 µM), possibility due to the analytical challenges associated with determining the plasma protein binding of highly bound compound.*
2. *Positive and negative controls were missing in this study.*
3. *The mean percent recovery of plasma protein binding for AAG among all concentrations ranged from 28.3 to 33.7%, possibility due to compound solubility or nonspecific binding issues to the dialysis device. Poor recovery in the plasma protein binding assay may lead to inaccurate measurement of plasma protein binding. Thus, it is inconclusive to say SAGE-547 was poorly bound to AAG.*
4. *A high plasma protein binding of > 99% for SAGE-547 was confirmed in both studies SSN-01423 and SSN-408. Thus, despite the issues discussed above, the sponsor's conclusion for plasma protein binding of SAGE-547 seems to be reasonable.*

Study # SSN-408
Title: Plasma Protein Binding Analysis of Selected Compounds
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4223-distrib\ssn-408\

- **Objective:** The objectives of this study were to determine the plasma protein binding of SAGE-547 in mouse, rat, dog and human plasma.

- **Methods:** Plasma was incubated at 37°C for 6 hours in wells with at a nominal concentration of 10 µM of SAGE-547, SGE-565, SGE-708, SGE-746 and SGE-808 using equilibrium dialysis. Aliquots of the incubated sample were removed, extracted, and analyzed using an LC-MS/MS system. The free and bound concentrations for each compound was determined. Positive control for high plasma protein binding [warfarin (10 µM)] were tested in parallel.

- **Analytical Method:** LC-MS/MS method (LLOQ = 0.0061 µM, 2 ng/mL). However, this method was not validated, and SAGE-547 was quantified by using the fit against the calibration curve.

- **Results:**

Table 1. Protein Binding of SAGE-547 in Mouse, Rat, Dog, and Human Plasma

	Plasma Protein Binding					
	SAGE-547	SGE-565	SGE-708	SGE-746	SGE-808	Warfarin
Mouse	99.7%	98.2%	95.6%	95.8%	96.1%	77.0%
Rat	99.6%	96.2%	97.3%	93.1%	93.4%	99.2%
Dog	99.7%	95.4%	97.0%	89.7%	94.5%	95.1%
Human	99.2%	98.9%	> 98.5%	97.4%	98.8%	99.0%

- **Sponsor's conclusions:** In all species, plasma protein binding for SAGE-547, SGE-565, SGE-708, SGE-746 and SGE-808 were >99% at 10 μM.
-
- ***Reviewer's Comments:***

1. *The test concentration of SAGE-547 (10 μM) were higher than its clinical relevant exposures (< 0.38 μM), possibility due to the difficulty of determining the plasma protein binding of for highly bound compound.*
2. *The recovery for the equilibrium dialysis assay was not reported.*
3. *A high plasma proteibin binding of > 99% for SAGE-547 was confirmed in both studies SSN-01423 and SSN-408. Thus, despite the issues discussed above, the sponsor's conclusion for SAGE-547 seems to be reasonable.*

Study # SSN-02178
Title: Plasma protein binding determination with SGE-03211 and SGE-03212 in human plasma by rapid equilibrium dialysis
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4223-distrib\ssn-02178\

- **Objective:** The purpose of this in vitro study was to determine plasma protein binding of test articles SGE-03211 and SGE-03212 in human plasma.

- **Methods:** Human adult and pediatric plasma were incubations at 37˚C for 3 hours in wells with SGE-03211 and SGE-03212 using equilibrium dialysis. Aliquots of the incubated sample were removed, extracted, and analyzed using an LC-MS/MS system. The free and bound concentrations for each compound was determined. Positive controls for high plasma protein binding [warfarin (10 μM)] and low plasma protein binding [linezolid (10 μM)] were tested in parallel.

- **Analytical Method:** LC-MS/MS method.

- **Results:**

Table 1. Results of Plasma Protein Binding and Recovery of SGE-03211, SGE-03212, Warfarin and Linezolid in Human Plasma.

Compound	Conc. (µM)	Species	% Bound					% Recovered				
			A	B	C	Mean	SD	A	B	C	Mean	SD
SGE-03211	0.3	Human (adult)	99.4	99.3	99.4	99.4	0.028	106	165	93	121	38
SGE-03211	3	Human (adult)	99.9	100	100	100	0.045	160	151	162	158	5.7
SGE-03212	0.3	Human (adult)	98.1	98.8	98.8	98.6	0.38	99	87	95	94	5.9
SGE-03212	3	Human (adult)	99.7	99.7	99.7	99.7	0.014	108	122	104	111	9.5
Warfarin	10	Human (adult)	99.4	99.2	99.5	99.4	0.12	102	69	82	84	16
Linezolid	10	Human (adult)	47.2	48.8	54.1	50.0	3.6	62	67	59	63	3.6

- **Sponsor's conclusions:**

1. Plasma protein binding of SGE-03211 was high, with percent binding values ranging from 99.3 to 100% across the two concentrations tested (at 0.3 µM and 3 µM).
2. Plasma protein binding of SGE-03212 was high, with percent binding values ranging from 98.1 to 99.7% across the two concentrations tested (at 0.3 µM and 3 µM).
3. Relative recovery of SGE-03211 and SGE-03212 (compared to the non-dialyzed spiked plasma standards) ranged from 93-165% for SGE-03211 and 87-122% for SGE-03212 over the two concentrations tested. High % recovered values (>100%) resulted in some of the dialyzed donor samples relative to test article concentration in the non-dialyzed spiked plasma sample, which indicated potential non-specific binding of the test article in the non-dialyzed spiked plasma condition. Since percent plasma protein binding was calculated without the data from the non-dialyzed spiked plasma condition, the high relative recovery values had no impact on the plasma protein binding results of the test articles.

- ***Reviewer's Comments:***

The plasma protein binding for SGE-03227 (M137), which is the 3rd major circulating metabolite, was not determined. Since SGE-03227 is an inactive metabolite of SAGE-547, the determination of plasma protein binding for M137 is recommended but not required.

3. In Vitro Drug Metabolism Studies

Study # SSN-594
Title: Reaction Phenotyping of SAGE-547
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-594\

- **Objective:**

To determine the role of human cytochrome P450 (CYP), UDP-glucuronosyltransferases (UGT) and flavin-containing monooxygenase (FMO) enzymes in the metabolism of

SAGE-547.

- **Methods:**

SAGE-547 (1 μM) was incubated with recombinant human CYP enzymes (rCYP1A2, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C19, rCYP2D6 and rCYP3A4, 50 pmol CYP per incubation), UGT enzymes (rUGT1A1, 1A4, 1A8, 1A9, 2B7, 2B15 and 2B17) and human FMO enzymes (FMO1, FMO3 and FMO5, 0.25 mg/mL) for 60 min. After specific incubation time, reaction was terminated by stop reagent. And the remaining amount of SAGE-547 was quantified.

- **Analytical Method:** LC-MS/MS

- **Results:**

1. Disappearance of SAGE-547 was observed following a 60-minute incubation with recombinant CYP2C8 (34%), CYP2C9 (72%), CYP2C19 (99%), and CYP3A4 (100%). Incubations with the remaining recombinant human CYP enzymes evaluated resulted in less than 16% substrate disappearance.

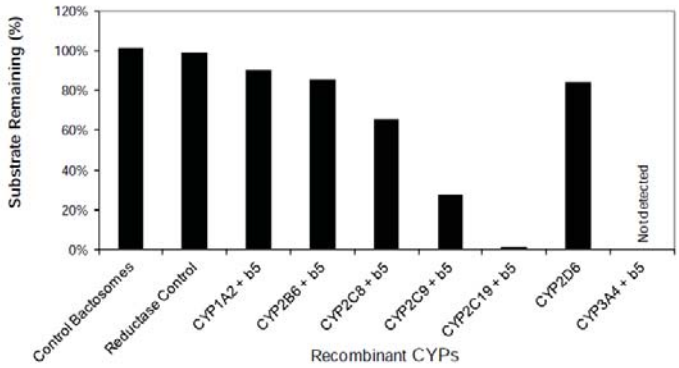

Figure 1. Metabolism of SAGE−547 (1 μM) after 60 min incubation by recombinant human UGT enzymes (0.25 mg protein/mL) 1 μM SAGE−547.

2. When incubated with recombinant UGT enzymes, disappearance of SAGE-547 was observed with UGT2B7 (63%) and UGT2B17 (51%). Incubations with the other recombinant human UGT enzymes evaluated resulted in less than 13% substrate disappearance.

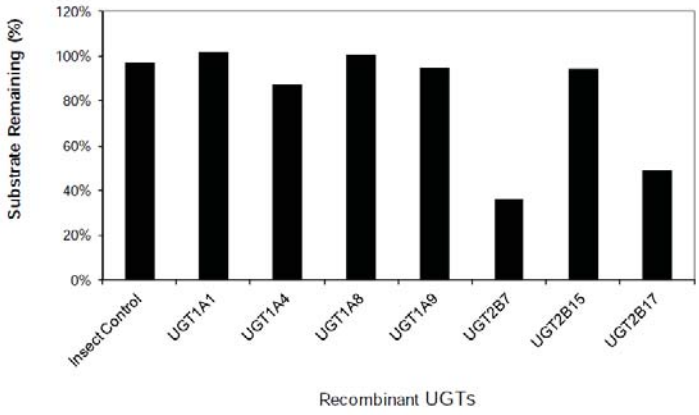

Figure 2. Metabolism of SAGE−547 (1 μM) after 60 min incubation by a panel of recombinant human FMO enzymes (0.25 mg/mL) 1 μM SAGE−547.

3. FMO enzymes did not metabolize SAGE-547.

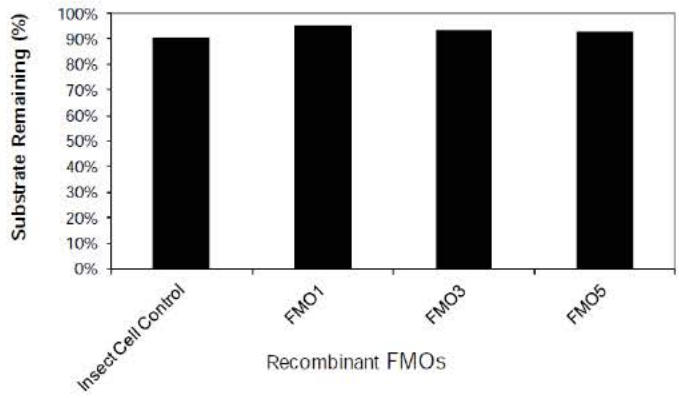

Figure 3. Metabolism of SAGE−547 (1 μM) after 60 min incubation by a panel of recombinant human FMO enzymes (0.25 mg/mL) 1 μM SAGE−547.

- **Sponsor's conclusions:**

SAGE-547 was metabolized by CYP2C8, CYP2C9, CYP2C19, CYP3A4, UGT2B7 and UGT2B17 *in vitro* and these enzymes may be involved in the biotransformation of SAGE-547 *in vivo*.

- ***Reviewer's Comments:***

Though in vitro studies suggested that CYPs and UGTs may be involved in the metabolism of SAGE-547, the in vivo mass-balance and metabolite ID study clearly demonstrate that the metabolites of SAGE-547 were mainly formed via non-CYP based pathways. SAGE-547 undergoes biotransformation via the following main pathways: 1) reduction of the C-20 keto moiety, presumably by an enzyme of the aldo-keto reductases (AKR) family, 2) epimerization at the C-3 hydroxy position, and 3) sulfonation or glucuronidation of SAGE-547 and the corresponding 5α-pregnan-3,20 diol metabolites.

Based on in vitro data, the sponsor's conclusion that SAGE-547 is a substrate of CYPs and UGTs is reasonable, although it may have overestimated the contribution of CYPs and UGTs to overall systemic clearance for SAGE-547 due to the use of a recombinant system (which over-expresses a single enzyme to artifically high levels) and the absence of competing enzymes and reactions in the in vitro recombinant system. Based on the in vivo data, it is reasonable to conclude that the non CYP based pathways namely- UGTs, AKRs and sulfotransferases (SULTs) contribute extensively to the biotransformation of SAGE-547.

4. In Vitro Drug Interaction Studies

Study # SSN-01924
Title: In Vitro Evaluation of SAGE-547 as an Inhibitor of Cytochrome P450 (CYP) and UDP-Glucuronosyltransferase (UGT) Enzymes in Human Liver Microsomes
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-01924\

- **Objective**

To evaluate the ability of SAGE-547 to inhibit, in vitro, select CYP and UGT enzymes in human liver microsomes (namely CYP1A2, CYP2C19, CYP2D6, CYP3A4/5, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, UGT2B15 and UGT2B17) with the aim of ascertaining the potential of SAGE-547 to inhibit the metabolism of concomitantly administered drugs.

- **Methods**
- Direct inhibition potential for CYP and UGT enzymes in human liver microsomes (HLM)

HLM from a pool of 200 individuals were incubated with marker substrates (typically at approximately K_m) for CYP1A2, CYP2C19, CYP2D6, CYP3A4/5, UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, UGT2B7, UGT2B15 and UGT2B17 in the presence (0.03 to 30 µM) or absence of SAGE-547. Residual enzyme activities were measured following adding aliquot of co-factors for CYP assays (NADPH-generating system) and UGT assays (UDPGA). The assays were automatically terminated at the appropriate time by the addition of the appropriate internal standard and stop reagent.

- Time-dependent and metabolism-dependent inhibition potential for CYP enzymes in HLM:

To distinguish between time-dependent and metabolism-dependent inhibition for CYP1A2, CYP2C19, CYP2D6 and CYP2A4/5, SAGE-547 was preincubated with HLM for 30 min without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrates. Following the 30-min preincubation period, marker substrates (0.03 to 30 µM) were automatically added, and the incubations were continued as described previously to measure residual enzyme activity. Known direct and metabolism-dependent inhibitors of CYP enzymes and known director inhibitors of UGT enzymes were included as positive controls in all experiments.

Table 1. Summary of assay conditions to measure microsomal CYP and UGT enzyme activity

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Enzyme	Substrate	[Substrate] (µM)	Incubation volume (µL)	Protein [a] (mg/mL)	Incubation time (min)	Preincubation time (min)	Stop reagent	SAGE-547	
								Target concentrations (µM)	Solvent volume [b] (µL)
CYP1A2	Phenacetin	90	200	0.1	5	30	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
CYP2C19	*S*-Mephenytoin	60	200	0.1	5	30	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
CYP2D6	Dextromethorphan	10	200	0.1	5	30	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
CYP3A4/5	Midazolam	3	200	0.05	5	30	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
CYP3A4/5	Testosterone	60	200	0.1	5	30	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT1A1	17β-Estradiol	12	200	0.1	5	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT1A3	Chenodeoxycholic acid	160	400	0.1	10	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	4
UGT1A4	Trifluoperazine	20	400	0.1	5	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	4
UGT1A6	1-Naphthol	2	200	0.005	5	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT1A9	Propofol	16	200	0.1	5	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT2B7	Morphine	400	200	0.1	5	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT2B15	Oxazepam	100	200	0.1	10	NA	Acetonitrile	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2
UGT2B17	Testosterone	5	200	0.1	10	NA	Acetonitrile + 2% formic acid	0, 0.03, 0.1, 0.3, 1, 3, 10, 30	2

NA Not applicable. UGT enzymes were not evaluated for time- or metabolism-dependent inhibition.
a The human liver microsomal sample used for these experiments was a pool of 200 individuals (catalog number: H2600, lot number: 1210347).
b Methanol was the vehicle used to dissolve the test article.

Kinetic constants for enzyme activities in human liver microsomes

Enzyme	Marker Substrate	Kinetic Constants	
		K_m (µM)	V_{max} (pmol/mg protein/min)
CYP1A2	Phenacetin	**96.0 ± 12.7**	**913 ± 6**
CYP2A6	Coumarin	**0.603 ± 0.110**	**1180 ± 100**
CYP2B6	Bupropion	**95.6 ± 4.3**	**660 ± 10**
CYP2B6	Efavirenz	**5.45 ± 0.98**	**154 ± 8**
CYP2C8	Amodiaquine	**2.44 ± 0.19**	**2820 ± 90**
CYP2C8	Paclitaxel	**16.0 ± 2.1**	**386 ± 70**
CYP2C9	Diclofenac	**12.4 ± 1.1**	**2640 ± 300**
CYP2C19	*S*-Mephenytoin	**64.5 ± 2.7**	**67.6 ± 3.6**
CYP2D6	Dextromethorphan	**10.0 ± 1.0**	**261 ± 16**
CYP2E1	Chlorzoxazone	**45.1 ± 3.4**	**2420 ± 300**
CYP3A4/5	Testosterone	**61.0 ± 2.9** [1]	**4080 ± 780**
CYP3A4/5	Midazolam	**2.72 ± 0.25**	**1240 ± 90**
CYP3A4/5	Nifedipine	**13.6 ± 1.9**	**2840 ± 20**
CYP3A4/5	Atorvastatin	**81.8 ± 2.0**	**816 ± 6**
CYP4A11	Lauric acid	**5.04 ± 0.53**	**1130 ± 100**
UGT1A1	17β-Estradiol	**12.6 ± 0.4** [2]	**886 ± 75**
UGT1A3	Chenodeoxycholic acid	**169 ± 34**	**110 ± 10**
UGT1A4	Trifluoperazine	**19.1 ± 0.1**	**906 ± 78**
UGT1A6	1-Naphthol	**1.90 ± 0.20**	**1640 ± 60**
UGT1A9	Propofol	**16.8 ± 0.7**	**6470 ± 110**
UGT2B7	Morphine	**519 ± 43**	**3990 ± 450**
UGT2B15	Oxazepam [3]	**96.6 ± 13.9**	**187 ± 42**
UGT2B17	Testosterone	**4.70 ± 0.41**	**733 ± 8**

[1] For this assay, the K_m column represents S_{50} (Hill coefficient = 1.4).
[2] For this assay, the K_m column represents S_{50} (Hill coefficient = 1.7).
[3] *S*-Oxazepam glucuronidation was measured as representative of UGT2B15 activity.
Kinetic constants are mean ± standard deviation of two or more determinations

- **Analytical Method:** LC-MS/MS

- **Results**

Table 2. Summary of results: In vitro evaluation of SAGE-547 as an inhibitor of human CYP and UGT enzymes in HLM

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (μM) [a]	Maximum inhibition (%) [b]	IC_{50} (μM) [a]	Maximum inhibition (%) [b]	IC_{50} (μM) [a]	Maximum inhibition (%) [b]	
CYP1A2	Phenacetin	> 30	21	> 30	11	> 30	14	No
CYP2C19	*S*-Mephenytoin	29 ± 6	47	> 30	46	21 ± 3	54	No
CYP2D6	Dextromethorphan	> 30	9.9	> 30	7.3	> 30	11	No
CYP3A4/5	Midazolam	> 30	34	> 30	28	> 30	41	No
CYP3A4/5	Testosterone	> 30	38	> 30	32	> 30	31	No
UGT1A1	17β-Estradiol	> 30	18	ND	ND	ND	ND	ND
UGT1A3	Chenodeoxycholic acid	21 ± 5	52	ND	ND	ND	ND	ND
UGT1A4	Trifluoperazine	26 ± 8	47	ND	ND	ND	ND	ND
UGT1A6	1-Naphthol	> 30	7.5	ND	ND	ND	ND	ND
UGT1A9	Propofol	> 30	23	ND	ND	ND	ND	ND
UGT2B7	Morphine	23 ± 5	50	ND	ND	ND	ND	ND
UGT2B15	Oxazepam	6.3 ± 1.3	67	ND	ND	ND	ND	ND
UGT2B17	Testosterone	1.7 ± 0.2	86	ND	ND	ND	ND	ND

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.
b Maximum inhibition observed (%) is calculated with the following formula for the concentration of inhibitor demonstrating the most inhibition (results are rounded to two significant figures):
Maximum inhibition observed (%) = 100% − Percent solvent control.
c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.
ND Not determined. UGT enzymes were not evaluated for time- or metabolism-dependent inhibition.

Table 3. Positive control inhibition data for IC_{50} determinations

Enzyme	Substrate	Type of inhibitor	Inhibitor	Inhibition after zero-min preincubation (%)	Inhibition after 30 min preincubation plus NADPH (%)	Difference after preincubation (%)
CYP1A2	Phenacetin	Direct	0.5 μM α-Naphthoflavone	82.9	NA	NA
		MDI	2 μM Furafylline	10.6	68.9	58.3
CYP2C19	*S*-Mephenytoin	Direct	400 μM Modafinil	79.1	NA	NA
		MDI	10 μM Esomeprazole	25.2	73.2	48.0
CYP2D6	Dextromethorphan	Direct	5 μM Quinidine	92.0	NA	NA
		MDI	1 μM Paroxetine	42.3	84.5	42.2
CYP3A4/5	Midazolam	Direct	0.075 μM Ketoconazole	83.8	NA	NA
		MDI	7.5 μM Troleandomycin	54.3	79.6	25.3
CYP3A4/5	Testosterone	Direct	0.075 μM Ketoconazole	83.7	NA	NA
		MDI	7.5 μM Troleandomycin	17.7	72.0	54.3
UGT1A1	17β-Estradiol	Direct	12 μM Troglitazone	80.6	NA	NA
UGT1A3	Chenodeoxycholic acid	Direct	100 μM Troglitazone	81.5	NA	NA
UGT1A4	Trifluoperazine	Direct	3 μM Troglitazone	77.8	NA	NA
UGT1A6	1-Naphthol	Direct	20 μM Troglitazone	47.3	NA	NA
UGT1A9	Propofol	Direct	20 μM Troglitazone	87.0	NA	NA
UGT2B7	Morphine	Direct	100 μM Troglitazone	78.4	NA	NA
UGT2B15	Oxazepam	Direct	50 μM Troglitazone	58.0	NA	NA
UGT2B17	Testosterone	Direct	50 μM Troglitazone	67.6	NA	NA

NA Not applicable
MDI Metabolism-dependent inhibition

- **Sponsor's conclusions**
- SAGE-547 is not a direct or time-dependent inhibitor for major CYP enzymes.
- SAGE-547 is a direct inhibitor of UGT1A3, UGT1A4, UGT2B7, UGT2B15, UGT2B17 and CYP2C19 with IC_{50} values ranging from 1.7 to 29 μM.
- Greater than 20% direct inhibition of UGT1A9, CYP1A2 and CYP3A4/5-mediated midazolam 1′-hydroxylation and testosterone 6β-hydroxylation activity was observed at 30 μM SAGE-547; however, the inhibition was insufficient to calculate an IC_{50} value.
- There was little or no evidence of direct inhibition of UGT1A1, UGT1A6 or CYP2D6 by SAGE-547, and there was little or no evidence of time- or metabolism-dependent

inhibition of any of the CYP enzymes evaluated by SAGE-547.

- ***Reviewer's Comments:***
-
- *We agree with the sponsor's conclusion that SAGE-547 is not a direct or time-dependent inhibitor of clinical relevance for the major CYP enzymes.*

According to the draft drug interaction guidance, the predicated ratio of victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition is calculated as following.

Table 3. Predicted ratio of victim drug's AUC in the presence and absence of an inhibitor

CYP & UGT isoform	Basic Models of Reversible Inhibition			
	IC_{50} (μM)	K_i (μM)	$I_{max,u}$ (μM) *	R_1
CYP2C19	29 ± 6	14.5	0.038	1.003
UGT1A3	21 ± 5	10.5	0.038	1.004
UGT1A4	26 ± 8	13	0.038	1.003
UGT2B7	23 ± 5	11.5	0.038	1.003
UGT2B15	6.3 ± 1.3	3.1	0.038	1.01
UGT2B17	1.7 ± 0.2	0.9	0.038	1.04
	Estimated C_{max} values at the steady state in humans at 90 μg/kg/h dose were 0.38 μM (120 ng/mL). Thus $I_{max,u}$ = 0.38 * 1% μM = 0.038 μM. Assuming competitive mode of inhibition, $K_i = 0.5 \times IC_{50}$ * According to the draft drug interaction guidance, considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.			

The calculated R_1 values were smaller than the recommended cutoff value of 1.02 for CYP2C19, UGT1A3, UGT1A4, UGT2B7, and UGT2B15. Per Guidance recommendation, in vivo studies to evaluate the inhibition potential for CYP2C19, UGT1A3, UGT1A4, UGT2B7, and UGT2B15 are not required.

SAGE-547 was identified to be a potent inhibitor for UGT2B17 (IC_{50} = 1.7 μM), indicating it may have the potential to inhibit the metabolism of a UGT2B17 substrate drug. However, there is no specific citations of clinically relevant DDIs ascribed to this enzyme, and there is limited information on its role in the clinical ADME of drugs. Thus, an additional in vivo study to evaluate the inhibition potential for UGT2B17 is not required.

Study # SSN-412
Title: In Vitro Evaluation of SAGE-547 as an Inhibitor of CYP2C9 in HLM
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-412\

- **Objective:**

To evaluate the ability of SAGE-547 to inhibit CYP2C9 in HLM.

Methods:

Determination of IC_{50} values

To evaluate SAGE-547 as a direct, time-dependent and metabolism-dependent inhibitor of CYP2C9 activity, HLM from a pool of sixteen individuals were incubated with marker substrate (diclofenac, K_m 6.5 ± 0.3 µM, 6 µM) in the presence or absence of SAGE-547 (0.03 – 30 µM). To distinguish between time-dependent and metabolism-dependent inhibition, SAGE-547 was preincubated with HLM for 30 minutes without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrate.

Determination of a K_i value

Further evaluation of direct inhibition of CYP2C9 by SAGE-547 was also conducted with pooled HLM and marker substrate (diclofenac, 1.8 – 60 µM), at multiple concentrations, in the presence and absence of SAGE-547 (0.1 – 4 µM) to determine the K_i value and mechanism for direct inhibition. Known direct (sulfaphenazole) and metabolism-dependent inhibitors (tienilic acid) of CYP2C9 were included as positive controls in the experiments.

Analytical Method: LC-MS/MS

- **Results:**

Under the experimental conditions examined, SAGE-547 directly inhibited CYP2C9 with an IC_{50} value of 0.41 µM. After further evaluation of direct inhibition, SAGE-547 was found to be a mixed inhibitor of CYP2C9 with a K_i value of 0.256 µM. There was no evidence of time-dependent inhibition or metabolism-dependent inhibition of CYP2C9 by SAGE-547.

Table 1. In vitro evaluation of SAGE-547 as an inhibitor of human CYP2C9

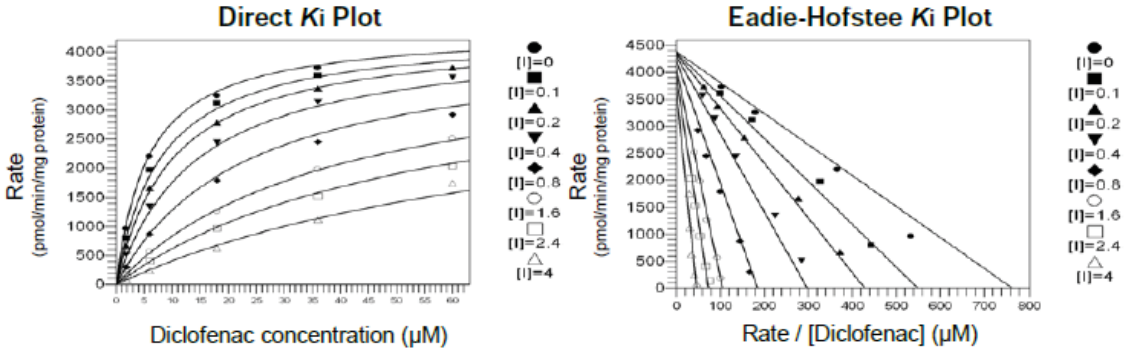
Enzyme	Enzyme reaction	Direct inhibition				Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-minute preincubation				30-minute preincubation without NADPH		30-minute preincubation with NADPH		Potential for time-dependent and/or metabolism-dependent inhibition [c]
		IC_{50} (µM) [a]	Inhibition observed at 30 µM (%) [b]	K_i (µM)	Type of inhibition	IC_{50} (µM) [a]	Inhibition observed at 30 µM (%) [b]	IC_{50} (µM) [a]	Inhibition observed at 30 µM (%) [b]	
CYP2C9	Diclofenac 4'-hydroxylation	0.41	95	0.256	Mixed	0.44	94	1.0	94	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.

b Inhibition observed (%) is calculated with the following formula:
Inhibition observed (%) = 100% − Percent solvent control.

c Time-dependent inhibition and metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

Figure 1. Inhibition of CYP2C9 by SAGE-547: K_i determination



Mechanism: Mixed Inhibition	Value ± standard error
Ki (μM)	0.256 ± 0.020
Km (μM)	5.73 ± 0.31
Vmax (pmol/min/mg protein)	4380 ± 60
Alpha	83.8 ± 54.9

- **Sponsor's conclusions:**

1. SAGE-547 directly inhibited CYP2C9 with an IC_{50} value of 0.41 μM. There was no evidence of time-dependent inhibition or metabolism-dependent inhibition of CYP2C9 by SAGE-547.
2. After further evaluation of direct inhibition, SAGE-547 was found to be a mixed inhibitor of CYP2C9 with a K_i value of 0.256 μM.

- **Reviewer's Comments:**

1. According to the draft drug interaction guidance, the predicated ratio of victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition is calculated as following.

Table 2: Predicted ratio of victim drug's AUC in the presence and absence of an inhibitor

CYP isoform	Basic Models of Reversible Inhibition			
	IC_{50} (μM)	K_i (μM)	$I_{max,u}$ (μM)*	R_1
2C9	0.41	0.256	0.038 μM	1.15
	Estimated C_{max} values at the steady state in humans at 90 μg/kg/h dose were 0.38 μM (120 ng/mL). Thus $I_{max,u}$ 0.38 × 1% μM 0.038 μM. $R_1 = 1 + (I_{max,u} / K_i)$ * According to the draft drug interaction guidance, considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma ($f_{u,p}$) = 0.01) if experimentally determined to be < 1%.			

The calculated value of R_1 = 1.15 is larger than the recommended cutoff value of 1.02 for CYP2C9. Per Guidance recommendation, an in vivo study to evaluate the inhibition potential for CYP2C9 is required.

The potential for CYP2C9 inhibition has been assessed in study CLP-105 using oral phenytoin as a CYP2C9 substrate. Phenytoin PK were not significantly different in the

presence of SAGE-547 over a 110-hour infusion; therefore, meaningful PK interaction of SAGE-547 with drugs metabolized via CYP2C9 is unlikely.

2. *Since alpha value is determined to be 83.5, the inhibition type is more likely to be competitive rather than mixed.*

Study # SSN-01539
Title: In Vitro Evaluation of SAGE-547 as an Inhibitor of CYP2B6 and CYP2C8 in HLM
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-01539\

- **Objective:**

To evaluate the ability of SAGE-547 to inhibit CYP2B6 and CYP2C8 in vitro in HLM.

- **Methods:**

To evaluate SAGE-547 as a direct, time-dependent and metabolism-dependent inhibitor of CYP2B6 and CYP2C8 activity, HLM from a pool of 200 individuals were incubated with marker substrates in the presence or absence of SAGE-547. To distinguish between time-dependent and metabolism-dependent inhibition, SAGE-547 was preincubated with HLM for 30 min without and with an NADPH generating system, respectively, prior to the incubation with the marker substrates. Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments.

Table 1. Summary of assay conditions to measure microsomal CYP2B6 and CYP2C8 enzyme activities

	Marker Substrate	K_m (μM)	Substrate conc. (μM)	SAGE-547 concentration (μM)
CYP2B6	Efavirenz	5.45 ± 0.98	5	0.03, 0.1, 0.3, 1, 3, 10, 30
CYP2C8	Amodiaquine	2.44 ± 0.19	2	0.03, 0.1, 0.3, 1, 3, 10, 30

- **Analytical Method:** LC-MS/MS
- **Results:**

Table 2. In vitro evaluation of SAGE-547 as an inhibitor of human CYP2B6 and CYP2C8

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} (μM) [a]	Maximum inhibition observed (%) [b]	IC_{50} (μM) [a]	Maximum inhibition observed (%) [b]	IC_{50} (μM) [a]	Maximum inhibition observed (%) [b]	
CYP2B6	Efavirenz	> 30	30	> 30	26	> 30	37	Little or no
CYP2C8	Amodiaquine	23 ± 7	49	17 ± 5	53	20 ± 4	52	Little or no

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.
b Maximum inhibition observed (%) is calculated with the following formula: Maximum inhibition observed (%) = 100% – the minimum percent solvent control for any test article concentration.
c Metabolism-dependent inhibition was determined by comparison of IC_{50} values for samples preincubated with and without NADPH-generating system, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

Table 3. Positive control inhibition data

Enzyme	Substrate	Type of inhibition	Inhibitor	Zero-min preincubation (% inhibition)	30-Min preincubation plus NADPH (% inhibition)	Increase in inhibition after preincubation (%)
CYP2B6	Efavirenz	Direct	750 µM Orphenadrine	73.6	NA	NA
		MDI	30 µM Phencyclidine	0	53.2	53.2
CYP2C8	Amodiaquine	Direct	0.05 µM Montelukast	79.8	NA	NA
		MDI	5 µM Gemfibrozil glucuronide	0	56.9	56.9

NA Not applicable
In cases where inhibition is not observed, the percent inhibition is reported as "zero".

- **Sponsor's conclusions:**

1. SAGE-547 is a direct inhibitor of CYP2C8 with an IC_{50} value of 23 µM.
2. SAGE-547 is a weak inhibitor for CYP2B6 with approximately 30% inhibition observed at 30 µM.
3. There was little or no evidence of time- or metabolism-dependent inhibition of CYP2B6 or CYP2C8 by SAGE-547.

- **Reviewer's Comments:**

According to the draft drug interaction guidance, the predicated ratio of victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition is calculated as following.

Table 4: Predicted ratio of victim drug's AUC in the presence and absence of an inhibitor

CYP isoform	Basic Models of Reversible Inhibition			
	IC_{50} (µM)	K_i (µM) [1]	$I_{max,u}$ (µM) [2]	R_1
2C8	23	11.5	0.038 µM	1.003
	Estimated C_{max} values at the steady state in humans at 90 µg/kg/h dose were 0.38 µM (120 ng/mL). Thus $I_{max,u}$ = 0.38 * 1% µM = 0.038 µM. $R_1 = 1 + (I_{max,u} / K_i)$ [1]K_i is calculated assuming competitive mode of inhibition. [2]According to the draft drug interaction guidance, considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma (fu,p) = 0.01) if experimentally determined to be < 1%.			

The calculated value of R_1 = 1.003 turned out to be smaller than the recommended cutoff value of 1.02 for CYP2C8. Per Guidance recommendation, an in vivo study to evaluate the inhibition potential for CYP2C8 is not warranted.

Study # SSN-02080
Title: In Vitro Evaluation of SGE-03211 (M133), SGE-03212 (M136) and SGE-03227 (M137) as Inhibitors of Cytochrome P450 (CYP) Enzymes in HLM
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-02080\

- **Objective:**

To evaluate the ability of SGE-03211, SGE-03212 and SGE-03227 to each inhibit cytochrome P450 (CYP) enzymes in HLM (namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 [using two different substrates]).

- **Methods:**

HLM from a pool of 200 individuals were incubated with marker substrates (based on

internal K_m or S_{50} data) in the presence or absence of test article. To distinguish between time- and metabolism-dependent inhibition, each test article was preincubated with HLM for 30 min without and with an NADPH-generating system, respectively, prior to the incubation with the marker substrates. Known direct and metabolism-dependent inhibitors of CYP enzymes were included as positive controls in all experiments.

Table 1. Summary of assay conditions to measure microsomal CYP enzyme activity – direct, time- and metabolism-dependent inhibition of enzymes by SGE-03211, SGE-03212 and SGE-03227

Enzyme	Substrate	Substrate concentration (µM)	Incubation volume (µL)	Protein [a] (mg/mL)	Incubation time (min)	Preincubation time (min)	Target test article concentrations (µM)	Solvent volume (µL) [b]
CYP1A2	Phenacetin	90	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP2B6	Efavirenz	5	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP2C8	Amodiaquine	2	200	0.0125	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP2C9	Diclofenac	12	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP2C19	*S*-Mephenytoin	60	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP2D6	Dextromethorphan	10	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP3A4/5	Midazolam	3	200	0.05	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2
CYP3A4/5	Testosterone	60	200	0.1	5	30	0, 0.1, 0.3, 1, 3, 10, 30, 100	2

a The human liver microsomal sample used for these experiments was a pool of 200 individuals (catalog number: H2600, lot number: 1210347).
b Methanol was the solvent used to dissolve the test articles.

- **Analytical Method:** LC-MS/MS

- **Results:**

Table 2. Summary of results: In vitro evaluation of SGE-03211 as an inhibitor of human CYP enzymes in HLM

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} ± SE (µM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (µM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (µM) [a]	Maximum inhibition (%) [b]	
CYP1A2	Phenacetin	> 100	51	56 ± 2	68	49 ± 4	78	No
CYP2B6	Efavirenz	49 ± 4	80	27 ± 5	100	27 ± 3	100	No
CYP2C8	Amodiaquine	17 ± 1	100	18 ± 1	100	13 ± 1	100	No
CYP2C9	Diclofenac	40 ± 5	80	68 ± 11	62	52 ± 7	72	No
CYP2C19	*S*-Mephenytoin	> 100	39	> 100	35	> 100	43	No
CYP2D6	Dextromethorphan	> 100	32	> 100	37	> 100	37	No
CYP3A4/5	Midazolam	91 ± 10	55	55 ± 9	65	36 ± 3	77	No
CYP3A4/5	Testosterone	> 100	36	> 100	44	73 ± 9	64	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.
b Maximum inhibition observed (%) is calculated with the following formula: Maximum inhibition observed (%) = 100% − the minimum percent solvent control for any test article concentration.
c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

Table 3.In vitro evaluation of SGE-03212 as an inhibitor of human CYP and UGT enzymes in HLM

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	
CYP1A2	Phenacetin	84 ± 6	56	40 ± 1	82	45 ± 4	85	No
CYP2B6	Efavirenz	42 ± 3	81	31 ± 4	100	30 ± 4	100	No
CYP2C8	Amodiaquine	12 ± 1	100	12 ± 1	100	8.3 ± 0.9	100	No
CYP2C9	Diclofenac	85 ± 19	56	> 100	49	82 ± 9	60	No
CYP2C19	*S*-Mephenytoin	> 100	25	> 100	16	> 100	28	No
CYP2D6	Dextromethorphan	> 100	30	> 100	38	> 100	39	No
CYP3A4/5	Midazolam	13 ± 3	89	13 ± 2	91	12 ± 2	93	No
CYP3A4/5	Testosterone	57 ± 6	64	82 ± 10	59	42 ± 6	74	Yes

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.

b Maximum inhibition observed (%) is calculated with the following formula: Maximum inhibition observed (%) = 100% − the minimum percent solvent control for any test article concentration.

c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

Table 4. In vitro evaluation of SGE-03227 as an inhibitor of human CYP enzymes in HLM

Enzyme	Substrate	Direct inhibition		Time-dependent inhibition		Metabolism-dependent inhibition		
		Zero-min preincubation		30-min preincubation without NADPH		30-min preincubation with NADPH		Potential for metabolism-dependent inhibition [c]
		IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	IC_{50} ± SE (μM) [a]	Maximum inhibition (%) [b]	
CYP1A2	Phenacetin	> 100	6.9	> 100	2.7	> 100	5.5	No
CYP2B6	Efavirenz	> 100	26	> 100	16	> 100	17	No
CYP2C8	Amodiaquine	> 100	23	> 100	18	> 100	31	No
CYP2C9	Diclofenac	> 100	6.0	> 100	3.3	> 100	9.4	No
CYP2C19	*S*-Mephenytoin	> 100	13	> 100	7.9	> 100	13	No
CYP2D6	Dextromethorphan	> 100	9.7	> 100	4.4	> 100	7.8	No
CYP3A4/5	Midazolam	> 100	22	> 100	18	> 100	27	No
CYP3A4/5	Testosterone	> 100	21	> 100	17	> 100	28	No

a Average data (i.e., percent of control activity) obtained from duplicate samples for each test article concentration were used to calculate IC_{50} values.

b Maximum inhibition observed (%) is calculated with the following formula: Maximum inhibition observed (%) = 100% − the minimum percent solvent control for any test article concentration.

c Metabolism-dependent inhibition was determined by comparison of IC_{50} values both with and without preincubation and with and without NADPH-generating system present in the preincubation, by comparison of the observed inhibition (%) for all preincubation conditions and by visual inspection of the IC_{50} plots.

Sponsor's conclusions

1. SGE-03211 directly inhibited CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5 mediated metabolism with IC_{50} values of 49, 17, 40 and 91 μM, respectively. A maximum of 51, 39, 32 and 36% direct inhibition of CYP1A2, CYP2C19, CYP2D6 -mediated metabolism, respectively, were observed in the presence of SGE-03211 concentrations up to 100 μM, and the associated IC_{50} values were reported as > 100 μM.

   There was no evidence of metabolism-dependent inhibition of any of the CYP enzymes evaluated by SGE-03211.

2. SGE-03212 directly inhibited CYP1A2, CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5 (as measured by midazolam 1′-hydroxylation and testosterone 6β-hydroxylation) activities with IC_{50} values of 84, 42, 12, 85, 13 and 57 μM, respectively. A maximum of 25 and 30% direct inhibition of CYP2C19 and CYP2D6 activities, respectively, was observed in the presence of SGE-03212 concentrations up to 100 μM, and the associated IC_{50} values were reported as > 100 μM.

   After a 30-min preincubation with pooled HLM in the presence of NADPH cofactor, SGE-03212 caused metabolism-dependent (i.e., time- and NADPH-dependent) inhibition of CYP3A4/5-mediated testosterone 6β-hydroxylation.

There was no evidence of metabolism-dependent inhibition of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 or CYP3A4/5-mediated midazolam 1′-hydroxylation by SGE-03212.

3. SGE-03227 directly inhibited CYP2B6, CYP2C8 and CYP3A4/5 (as measured by midazolam 1′-hydroxylation and testosterone 6β-hydroxylation) activities with a maximum of 26, 23, 22 and 21% inhibition observed, respectively, in the presence of SGE-03227 concentrations up to 100 µM, and the associated IC_{50} values were reported as > 100 µM. There was no direct inhibition of CYP1A2, CYP2C9, CYP2C19 or CYP2D6 activities by SGE-03227.

   There was no evidence of metabolism-dependent inhibition of any of the CYP enzymes evaluated by SGE-03227.

- **Reviewer's Comments**

1. *Direct inhibition*
   Though SGE-03211 is a direct inhibitor for CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5, theIC_{50} values ranging from 17 -91 µM, that are 100's of fold higher than clinical concentrations. SGE-03212 is a direct inhibitor for CYP1A2, CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5 with IC_{50} values ranging from 13 – 85 µM (that are 100's of fold higher than clinical concentrations). SGE-03227 is not a direct inhibitor for tested CYP enzymes.
2. *Time-dependent inhibition*
   A IC_{50} fold shift of greater than 1.5 is considered a significant shift and the compound is classed as a time dependent inhibitor. The IC_{50} fold shift values for SGE-03211 and SGE-03212 are calculated as following.

	Enzyme	*Zero-min preincubation (µM)*	*30-min preincubation without NADPH(µM)*	*30-min preincubation with NADPH (µM)*	*Fold-shift 1*	*Fold-shift 2*	*Fold-shift 3*
SGE-03211	*CYP1A2*	*> 100*	*56 ± 2*	*49 ± 4*	*> 1.8*	*1.1*	*>2.0*
	CYP2B6	*49 ± 4*	*27 ± 5*	*27 ± 3*	*1.8*	*1.0*	*1.8*
	CYP2C8	*17 ± 1*	*18 ± 1*	*13 ± 1*	*0.9*	*1.4*	*1.3*
	CYP2C9	*40 ± 5*	*68 ± 11*	*63 ± 7*	*0.6*	*1.1*	*0.6*
	CYP3A4/5 (midazolam)	*91 ± 10*	*55 ± 9*	*36 ± 3*	*1.7*	*1.5*	*2.5*
	CYP3A4/5 (Testosterone)	*> 100*	*> 100*	*73 ± 9*	*NA*	*>1.4*	*>1.4*
SGE-03212	*CYP1A2*	*84 ± 6*	*40 ± 1*	*45 ± 4*	*2.1*	*0.9*	*1.8*
	CYP2B6	*42 ± 3*	*31 ± 4*	*30 ± 4*	*1.4*	*1.0*	*1.4*
	CYP2C8	*12 ± 1*	*12 ± 1*	*8.3 ± 0.9*	*1.0*	*1.4*	*1.4*

	CYP2C9	*85 ± 19*	*> 100*	*82 ± 9*	*< 1*	*1.2*	*1.0*
	CYP3A4/5 (midazolam)	*13 ± 3*	*13 ± 2*	*12 ± 2*	*1.0*	*1.1*	*1.1*
	CYP3A4/5 (Testosterone)	*57 ± 6*	*81 ± 10*	*42 ± 6*	*0.7*	*1.9*	*1.4*

Fold-shift 1 = IC_{50} (Zero-min preincubation) / IC_{50} (30-min preincubation without NADPH)
Fold-shift 2 = IC_{50} (30-min preincubation without NADPH) / IC_{50} (30-min preincubation with NADPH)
Fold-shift 2 = IC_{50} (Zero-min preincubation) / IC_{50} (30-min preincubation with NADPH)

For SGE-03211, fold-shift 2 and fold-shift 3 were greater than 1.5 for CYP3A4/5 using midazolam as the substrate, indicating both reversible and time dependent inhibition. Fold-shift 2 and fold-shift 3 were also greater than 1.4 for CYP3A4/5 using testosterone as the substrate.

For SGE-03212, fold-shift 2 was great than 1.5 for CYP3A4/5 using testosterone as the CYP3A4/5 substrate, indicating a potential for time-dependent inhibition of CYP3A4/5. However, this observation was not repeatable using midazolam as the substrate. In addition, increased IC_{50} when preincubated with NADPH as comparing to zero-minute incubation is a rare case with unclear reasons. Thus, considering data from both midazolam and testosterone, it is likely there is no time-dependent inhibition for CYP3A4/5 by SGE-03211.

Large fold-shift 1 (>1.5 fold) was observed for CYP1A2 and CYP2B6 for SGE-03211 and CYP1A2 for SGE-03212, indicating potential non-NADPH mediated metabolism of test compound into a more potent inhibitor species.

For CYP2C9, a greater IC_{50} value was observed after pre-incubation for 30 min as compared to zero-min incubation. The reason for this change is unknown.

In sum, SGE-03211 showed time-dependent inhibition for CYP1A2, CYP2B6, and CYP3A4/5. However, it is noteworthy, that even though the time dependent shift was observed, the IC50 values were all 10 µM or much higher.

3. *The total concentrations for metabolites (at highest dose of 90 ug/kg/h) are anticipated as follows:*
   - *M133 = ~210 ng/mL = ~ 630 nM*
   - *M136 = ~ 225 ng/mL = ~ 675 nM*
   - *M137 = ~ 225 ng/mL = ~ 675 nM*

Direct inhibition

According to the draft drug interaction guidance, the predicated ratio of victim drug's AUC in the presence and absence of an inhibitor for basic models of reversible inhibition is calculated as following.

Table 5. Predicted ratio of victim drug's AUC in the presence and absence of an inhibitor

Metabolites	*Basic Models of Reversible Inhibition*						
	CYP isoform	*IC_{50} (μM)*	*K_i (μM)[1]*	*Plasma Protein Binding[2]*	*I_{max} (μM)*	*$I_{max,u}$ (μM)*	*R_1*
SGE-03211 (M133)	*2B6*	*49*	*24.5*	*1%*	*0.63*	*0.063*	*1.003*
	2C8	*17*	*8.5*				*1.007*
	2C9	*40*	*20*				*1.003*
SGE-03212 (M136)	*2B6*	*42*	*21*	*1.4%*	*0.675*	*0.0095*	*1.0005*
	2C8	*12*	*6*				*1.002*
	3A4/5	*13*	*6.5*				*1.002*
		*$I_{max,u}$ = Imax * Plasma protein binding%. $R_1 = 1 + (I_{max,u} / K_i)$* *[1]$K_i$ is calculated assuming competitive mode of inhibition.* *[2]According to the draft drug interaction guidance, considering uncertainties in the protein binding measurements, the unbound fraction in plasma should be set to 1% (fraction unbound in the plasma (fu,p) = 0.01) if experimentally determined to be < 1%.*					

The calculated values turned out to be smaller than the recommended cutoff value of 1.02 for CYP2B6, CYP2C8, CYP2C9 and CYP3A4/5 for SGE-03211 and SGE-03212. Per Guidance recommendation, an in vivo study to evaluate the inhibition potential for CYP-mediated drug interactions is not warranted.

Time-dependent inhibition

Although time-dependent inhibition kinetic constants were observed for CYP1A2, CYP2B6, and CYP3A4/5 for SGE-03211. Considering relatively low clinical exposure of SGE-03211 ($I_{max,u}$ = 0.063 μM) as compared to its IC_{50} values (27 – 49 μM), the likelihood for CYP-mediated drug interaction by SGE-03211 is low. Further in vivo drug interaction studies are not required.

4. *The sponsor has not assessed the UGT inhibition potential for the major circulating metabolites. The in vitro determination of UGT inhibition potential by major circulating metabolites is recommended.*

Study # SSN-411
Title: In Vitro Evaluation of SAGE-547 as an Inducer of Cytochrome P450 Expression in Cultured Human Hepatocytes
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-411\

- **Objective**

To investigate the effects of treating primary cultures of cryopreserved human hepatocytes with SAGE-547 on the expression of CYP1A2. CYP2B6, CYP3A4 and UGT1A9.

- **Methods**

Three cryopreserved preparations of cultured human hepatocytes from three separate livers were treated once daily for three consecutive days with dimethyl sulfoxide (DMSO, 0.1% v/v, vehicle control), acetonitrile (1% v/v, vehicle control), flumazenil (25 µM, negative control), one of six concentrations of SAGE-547 (0.3, 0.5, 3, 5, 30 or 50 µM) or one of three known human CYP inducers, namely, omeprazole (50 µM), phenobarbital (750 µM) and rifampin (20 µM). After treatment, the cells were incubated in situ with the appropriate marker substrates for the analysis of phenacetin O-dealkylation (marker for CYP1A2), bupropion hydroxylation (marker for CYP2B6) and midazolam 1′-hydroxylation (marker for CYP3A4/5) by LC/MS/MS. Following the in situ incubation, the same hepatocytes from the same treatment groups were harvested with
Buffer RLT to isolate RNA, which was analyzed by qRT-PCR to assess the effect of SGE-102 on CYP2B6, CYP2C9, CYP3A4 and UGT1A9 mRNA levels.

- **Analytical Method:** LC-MS/MS
- **Results**

Figure 1. CYP1A2 activity fold increase: The effect of treating cultured human hepatocytes with SAGE-547 (SGE-102) or prototypical inducers on the rate of phenacetin O-dealkylase activity

Activity Fold Increase		HC10-1	HC3-22	HC5-25
Phenacetin *O*-dealkylation (CYP1A2)	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	0.0273	-0.0865	-0.0521
	0.5 µM SGE-102	-0.0998	-0.129	-0.0503
	3 µM SGE-102	-0.0739	-0.135	-0.0115
	5 µM SGE-102	-0.0499	-0.132	-0.0184
	30 µM SGE-102	-0.194	-0.307	-0.0259
	50 µM SGE-102	-0.798	-0.293	0.0909
	25 µM Flumazenil	0.142	-0.0866	0.0338
	50 µM Omeprazole	4.45	9.77	34.5

Fold increase = fold change – 1

CYP1A2 activity: Treatment of hepatocytes with up to 50 µM SAGE-547 had little or no effect (individual range of 0.202- to 1.09-fold) in CYP1A2 activity from all three hepatocyte cultures. However, there were decreases in CYP1A2 activity at concentrations above 0.3 µM SGE-102 in HC3-22 (12.9, 13.5, 13.2, 30.7 and 29.3% for 0.5, 3, 5, 30 or 50 µM, respectively) and at 30 and 50 µM SAGE-547 in HC10-1 (19.4 and 79.8%, respectively).

Figure 2. CYP2B6 activity fold increase: The effect of treating cultured human hepatocytes with SAGE-547 or prototypical inducers on the rate of bupropion hydroxylase activity

Activity Fold Increase		HC10-1	HC3-22	HC5-25
Bupropion hydroxylation (CYP2B6)	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	0.141	-0.122	0.177
	0.5 µM SGE-102	-0.0750	-0.152	0.264
	3 µM SGE-102	0.219	-0.0970	0.436
	5 µM SGE-102	0.166	-0.132	0.381
	30 µM SGE-102	2.18	0.326	1.46
	50 µM SGE-102	-0.000700	0.937	3.21
	25 µM Flumazenil	0.693	0.0639	0.170
	750 µM Phenobarbital	3.48	4.88	6.34

Fold increase = fold change – 1

Figure 3. CYP2B6 mRNA fold increase: The effect of treating cultured human hepatocytes with SAGE-547 on CYP2B6 mRNA levels

mRNA Fold Increase		HC10-1	HC3-22	HC5-25
CYP2B6	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	0.0991	0.156	0.311
	0.5 µM SGE-102	-0.0182	-0.0406	0.239
	3 µM SGE-102	0.186	-0.0850	0.494
	5 µM SGE-102	0.0970	0.0225	0.448
	30 µM SGE-102	0.811	0.265	0.822
	50 µM SGE-102	-0.00999	0.894	1.82
	25 µM Flumazenil	0.433	0.198	0.331
	750 µM Phenobarbital	2.53	5.01	8.71

mRNA Fold increase = fold change – 1

Figure 4. CYP2B6 mRNA percent positive control: The effect of treating cultured human hepatocytes with SGE-102 on cytochrome P450 (CYP mRNA levels)

Percent Positive Control mRNA Fold	CYP2B6			Mean ± Std Dev	n
	HC10-1	HC3-22	HC5-25		
0.1% DMSO	0	0	0	0 ± 0	3
1% Acetonitrile	0	0	0	0 ± 0	3
0.3 µM SGE-102	3.92	3.11	3.57	3.53 ± 0.41	3
0.5 µM SGE-102	-0.721	-0.810	2.75	0.405 ± 2.028	3
3 µM SGE-102	7.35	-1.70	5.67	3.77 ± 4.81	3
5 µM SGE-102	3.84	0.448	5.14	3.14 ± 2.42	3
30 µM SGE-102	32.1	5.29	9.43	15.6 ± 14.4	3
50 µM SGE-102	-0.395	17.8	20.9	12.8 ± 11.5	3
25 µM Flumazenil	17.1	3.95	3.80	8.29 ± 7.65	3
750 µM Phenobarbital	100	100	100	100 ± 0	3

CYP2B6 activity: Treatment of cultured human hepatocytes with SAGE-547 (up to 50 µM) had little to no effect (<2.0-fold change) on CYP2B6 activity in hepatocyte culture HC3-22, but caused increases in CYP2B6 activity of 3.18- and 2.46-fold, in HC10-1 and HC5-25 at 30 µM SGE-102, respectively and 4.21-fold in HC5-25 at 50 µM SAGE-547. There was a concentration-dependent increasing trend in all three cultures; however, a 2.0-fold change was never reached in culture HC3-22.

CYP2B6 mRNA levels: Treatment of hepatocytes with up to 50 µM SAGE-547 had little or no effect (individual range of 0.915- to 2.82-fold) in CYP2B6 mRNA levels with the exception of HC5-25 at 50 µM SGE-102 which reached 2.82-fold at 50 µM.

Figure 5. CYP2C9 mRNA fold increase: The effect of treating cultured human hepatocytes with SAGE-547 on CYP2C9 mRNA levels

mRNA Fold Increase		HC10-1	HC3-22	HC5-25
CYP2C9	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	-0.0139	0.234	0.0362
	0.5 µM SGE-102	0.0229	0.284	0.0275
	3 µM SGE-102	0.0621	0.0864	0.0511
	5 µM SGE-102	0.0109	0.233	-0.0496
	30 µM SGE-102	-0.0131	-0.0230	-0.112
	50 µM SGE-102	-0.214	0.192	-0.0664
	25 µM Flumazenil	0.0350	0.0590	0.0489
	20 µM Rifampin	1.61	0.815	1.34

mRNA fold increase = fold change – 1

CYP2C9 mRNA levels: Treatment of cultured human hepatocytes with up to 50 µM SAGE-547 caused little to no change in CYP2C9 mRNA levels (individual range of 0.786- to 1.28-fold).

Figure 6. CYP3A4/5 activity fold increase: The effect of treating cultured human hepatocytes with SAGE-547 or prototypical inducers on the rate of midazolam 1′-hydroxylase activity

Activity Fold Increase		HC10-1	HC3-22	HC5-25
Midazolam 1′-hydroxylation (CYP3A4/5)	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	0.0789	-0.0489	-0.00518
	0.5 µM SGE-102	-0.0811	-0.0808	0.111
	3 µM SGE-102	-0.0205	-0.130	0.0910
	5 µM SGE-102	-0.135	-0.188	0.0729
	30 µM SGE-102	-0.312	-0.395	0.0839
	50 µM SGE-102	-0.880	-0.460	0.384
	25 µM Flumazenil	0.205	-0.000890	0.0326
	20 µM Rifampin	1.60	3.73	5.97

Fold increase = fold change – 1

Figure 7. CYP3A4 mRNA fold increase: The effect of treating cultured human hepatocytes with SAGE-547 on CYP3A4 mRNA levels.

mRNA Fold Increase		HC10-1	HC3-22	HC5-25
CYP3A4	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	0.0429	0.188	-0.0887
	0.5 µM SGE-102	-0.268	0.206	-0.0401
	3 µM SGE-102	0.00659	0.0320	-0.109
	5 µM SGE-102	-0.212	0.0559	-0.0245
	30 µM SGE-102	0.218	-0.182	0.0119
	50 µM SGE-102	-0.192	0.661	0.559
	25 µM Flumazenil	0.593	0.0530	0.0702
	20 µM Rifampin	6.11	5.03	9.03

mRNA fold increase = fold change – 1

CYP3A4/5 activity: treatment of hepatocytes with up to 50 µM SGE-102 had little or no effect (individual range of 0.120- to 1.38-fold) in CYP3A4/5 activity. However, two cultures, HC10-1 and HC3-22, resulted in concentration-dependent decreases in CYP3A4/5

activity (decreased by 88.0 and 46.0% at 50 µM SAGE-547, respectively, compared to the vehicle control).

CYP3A4/5 mRNA levels: treatment of hepatocytes with up to 50 µM SAGE-547 had little or no effect (individual range of 0.732- to 1.66-fold) in CYP3A4 mRNA levels, however slight decreases in CYP3A4 mRNA levels were observed in all three cultures of hepatocytes (26.8, 21.2 and 19.2% at 0.5, 5 and 50 µM in HC10-1; 18.2% at 30 µM in HC3-22; and 10.9% at 3 µM SGE-102 in HC5-25).

Figure 8. UGT1A9 mRNA fold increase: The effect of treating cultured human hepatocytes with SAGE-547 on UGT1A9 mRNA levels

mRNA Fold Increase		HC10-1	HC3-22	HC5-25
UGT1A9	0.1% DMSO	0	0	0
	1% Acetonitrile	0	0	0
	0.3 µM SGE-102	-0.235	0.0198	-0.0491
	0.5 µM SGE-102	-0.168	0.245	-0.0990
	3 µM SGE-102	-0.183	0.0566	-0.156
	5 µM SGE-102	-0.290	0.0200	-0.0448
	30 µM SGE-102	-0.501	-0.256	-0.199
	50 µM SGE-102	-0.730	-0.125	-0.126
	25 µM Flumazenil	0.125	0.112	-0.0577
	50 µM Omeprazole	-0.650	-0.642	-0.282
	750 µM Phenobarbital	-0.367	-0.456	-0.0733
	20 µM Rifampin	-0.711	-0.577	-0.306

mRNA fold increase = fold change – 1

UGT1A9 mRNA levels: Treatment of cultured human hepatocytes with up to 50 µM SAGE-547 caused decreases in UGT1A9 mRNA levels in all three cultures of hepatocytes (23.5, 16.8, 18.3, 29.0, 50.1 and 73.0% at 0.3, 0.5, 3, 5, 30 and 50 µM SGE-102 in HC10-1, 25.6 and 12.5% at 30 and 50 µM SGE-102 in HC3-22 and 15.6, 19.9 and 12.6% at 3, 30 and 50 µM SAGE-547 in HC5-25). The decreases observed in mRNA fold were concentration dependent in hepatocyte culture HC10-1.

- **Sponsor's conclusions:**

Treatment of cultured human hepatocytes with up to 50 µM (15,925 ng/mL) SAGE-547 caused little or no increase in CYP1A2 or CYP3A4 activities or in CYP3A4 mRNA levels, or in CYP2C9 and UGT1A9 mRNA levels. SAGE-547 yielded equivocal results with CYP2B6, showing a modest concentration-dependent increase (above 2-fold) in activity but only one donor demonstrated a concomitant increase in CYP2B6 mRNA.

- **Reviewer's Comments:**

1. *For CYP2B6, a ≥ 2-fold increase in mRNA and a response ≥ 20% of the response of the positive control was observed at 50 µM in HC5-25 hepatocyte cultures, indicating SAGE-547 is an inducer for CYP2B6. However, the systemic exposure of SAGE-7 ($I_{u,max}$ = 0.038 µM) is more than 100 fold lower than the concentration that shown potent induction (50 µM). Additionally, given that brexanolone is going to be marketed as a one time infusion product, clinical exposure to brexanolone is expected only for a short duration of time (<5 days) which makes the induction of enzymes very unlikely. Thus, the potential for clinical drug interaction mediated by CYP2B6 induction is low, and no*

further in vivo assessment for CYP2B6 induction is required.

2. *Although the specific activity of the induced CYPs determine the relative change in drug metabolism activity at the protein level, in some cases both induction and inhibition may occur, masking the level of induction when catalytic activity only is measured. SAGE-547 is not an inhibitor for CYP3A4/5. However, two metabolites (e.g., SGE-03211 and SGE-03212) were determined to be inhibitors of CYP3A4/5. Thus, it is possible that the concentration dependent decrease in CYP3A4/5 activity was SAGE-547 metabolites at relatively high in vitro exposures. Moreover, the mRNA expression after three-day treatment of SAGE-547 was analyzed for CYP3A4/5. The results show that there was no significant change (individual range of 0.732- to 1.66-fold) in the mRNA expression in three cultures of hepatocytes, indicating no induction potential for CYP3A4/5. In addition, the systemic exposure of SAGE-547 (0.036 μM) was significantly lower than the concentrations that shown potent induction for CYP3A4/5 (> 3 μM). Thus, SAGE-547 is unlikely to induce CYP3A4/5 during clinical use.*

3. *The Positive and negative controls for UGT1A9 did not work. Thus, the results for UGT1A9 is inconclusive.*

Study # SSN-01925
Title: In Vitro Evaluation of SAGE-547 as an Inducer of CYP1A2 in Cultured Human Hepatocytes
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-01925\

- **Objective:**

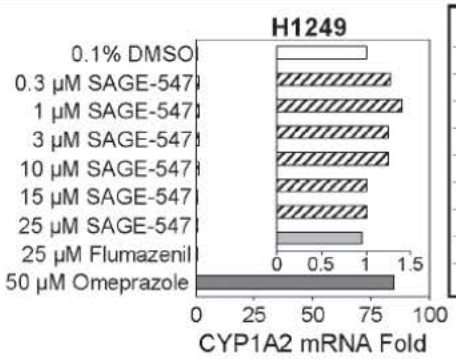
To evaluate the effect of SAGE-547 (0.3 to 25 μM) on the expression of CYP1A2 in three cultures of primary cryopreserved human hepatocytes.

- **Methods:**

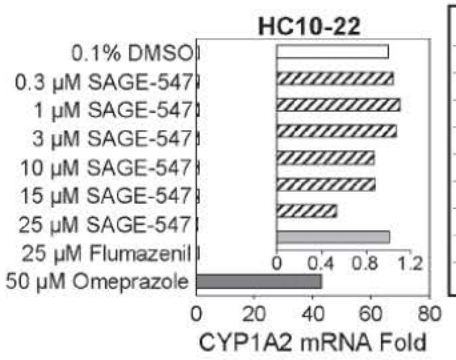
This study was designed to allow any inductive effects of SAGE-547 to be classified relative to a clinically relevant CYP1A2 inducer, namely omeprazole (an AhR activator) (Parkinson et al. 2013). To this end, three preparations of cultured human hepatocytes from three separate livers were treated once daily for three consecutive days with DMSO (0.1% v/v, vehicle control), flumazenil (25 μM, negative control), one of six concentrations of SAGE-547 (0.3, 1, 3, 10, 15 or 25 μM) or a known human CYP1A2 inducer, namely, omeprazole (50 μM).
After treatment, the cells were harvested with Buffer RLT to isolate RNA, which was analyzed by qRT-PCR to assess the effect of SAGE-547 on mRNA levels.

- **Analytical Method:** LC-MS/MS
- **Results:**

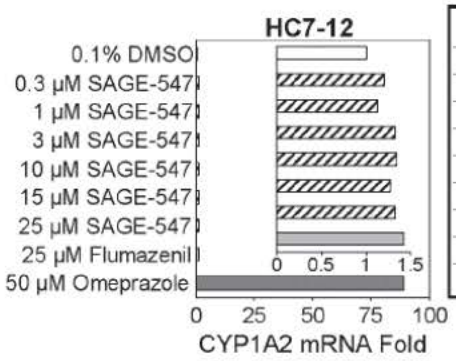
Figure 1. CYP1A2 mRNA fold change: The effect of treating cultured human hepatocytes with SAGE-547 on CYP1A2 mRNA levels



mRNA Fold Change	n
1.00	4
1.27	4
1.40	4
1.25	4
1.25	4
1.01	4
1.00	4
0.962	4
84.9	4



mRNA Fold Change	n
1.00	4
1.05	4
1.11	4
1.07	4
0.867	4
0.881	4
0.531	4
1.01	4
42.9	3



mRNA Fold Change	n
1.00	3
1.21	4
1.13	4
1.33	4
1.35	4
1.27	4
1.33	4
1.42	4
88.8	4

- **Sponsor's conclusions:**

Treatment of cultured human hepatocytes with up to 25 µM SAGE-547 had little or no effect (i.e., < 2-fold change) on CYP1A2 mRNA levels.

- **Reviewer's Comments:**

The sponsor's conclusions seem reasonable.

Study # SSN-01157
Title: (SAGE-547) SGE-00102-03-A: Assessment as an Inhibitor of P-gp, BCRP and MRP-2 in Human Caco-2 Cells
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-01157\

- **Objective:**

To assess whether SAGE-547 (SGE-00102-03-A) is an inhibitor of BCRP, P-gp or MRP-2 using Caco-2 cells.

- **Methods:**

Caco-2 cells were co-dosed with either 10 µM rosuvastatin (BCRP substrate, reported K_m = 10 µM), or 10 µM talinolol (P-pg and MRP-2 substrate, reported K_m = 100 µM) alone or with SAGE-547 at concentrations of 0.25, 2.5, 7.5, 15 and 25 µM.

- **Analytical Method:**
- **Results:**

Table 1 Summary of Caco-2 Permeability Results for Assessment of Inhibition of BCRP Transporter

Compound ID	Assay Conc. (µM)	Mean P_{app}, A-B (10^{-6} cm/s)	Mean P_{app}, B-A (10^{-6} cm/s)	Mean (B-A/A-B) Efflux Ratio	Mean % Recovery	A-B Permeability Ranking
Rosuvastatin	10	0.107	9.04	84.7	87.6	Lower
Rosuvastatin_Gefitinib (10 µM)	10	0.750	3.33	4.44	90.8	Lower
Rosuvastatin_Ko134 (10 µM)	10	0.858	6.55	7.63	92.2	Lower
Rosuvastatin_SAGE-547 (0.25 µM)	10	0.389	8.96	23.0	89.9	Lower
Rosuvastatin_SAGE-547 (2.5 µM)	10	0.450	7.99	17.7	91.6	Lower
Rosuvastatin_SAGE-547 (7.5 µM)	10	0.458	8.52	18.6	91.8	Lower
Rosuvastatin_SAGE-547 (15 µM)	10	0.464	8.65	18.6	94.1	Lower
Rosuvastatin_SAGE-547 (25 µM)	10	0.490	9.05	18.5	91.3	Lower
Controls:						
Ranitidine	10	0.320	0.908	2.84	90.9	Lower
Warfarin	10	37.6	26.0	0.692	96.3	Higher

Notes: Gefitinib is a non-specific inhibitor
Ko134 is a specific inhibitor

Permeability Ranking: lower is < $1x10^{-6}$ cm/s; higher is > $1x10^{-6}$ cm/s.
An efflux ratio >2 indicates potential for the compound to be a substrate for P-gp or other active transporter.

Table 2 Summary of Caco-2 Permeability Results for Assessment of Inhibition of P-gp and MRP-2 Transporters

Compound ID	Assay Conc. (µM)	Mean P_{app}, A-B (10^{-6} cm/s)	Mean P_{app}, B-A (10^{-6} cm/s)	Mean (B-A/A-B) Efflux Ratio	Mean % Recovery	A-B Permeability Ranking
Talinolol	10	0.514	6.67	13.0	93.5	Lower
Talinolol_Verapamil (25 µM)	10	1.66	2.40	1.44	88.5	Higher
Talinolol_Haloperidol (100 µM)	10	0.868	5.31	6.12	92.3	Lower
Talinolol_SAGE-547 (0.25 µM)	10	0.575	6.72	11.7	93.2	Lower
Talinolol_SAGE-547 (2.5 µM)	10	0.447	5.06	11.3	92.8	Lower
Talinolol_SAGE-547 (7.5 µM)	10	0.457	5.38	11.8	90.1	Lower
Talinolol_SAGE-547 (15 µM)	10	0.565	5.29	9.37	90.1	Lower
Talinolol_SAGE-547 (25 µM)	10	0.713	4.97	6.98	87.1	Lower
Controls:						
Ranitidine	10	0.320	0.908	2.84	90.9	Lower
Warfarin	10	37.6	26.0	0.692	96.3	Higher

Notes: Verapamil is a non-specific P-gp inhibitor
Haloperidol is a specific P-gp inhibitor

Permeability Ranking: lower is < $1x10^{-6}$ cm/s; higher is > $1x10^{-6}$ cm/s.
An efflux ratio >2 indicates potential for the compound to be a substrate for P-gp or other active transporter.

- **Sponsor's conclusions:**

SAGE-547 does not appear to be an inhibitor of BCRP, P-gp or MRP-2. This is evidenced by its inability to block the efflux of the BCRP substrate (rosuvastatin) and the P-gp/MRP-2 substrate (talinolol) in a dose-dependent manner. The addition of SAGE-547 over a

concentration range of 0.25 to 25 µM did not significantly change the permeability or efflux of the substrates. The binning of rosuvastatin and talinolol remained the same with or without SAGE-547 at any of the concentrations tested.

- **Reviewer's Comments:**

The study done to determine the BCRP inhibition potential of SAGE-547 clearly demonstrates that the efflux ratio for the probe substrate was decreased significantly. However, it is possible that this change in the efflux ratio is due to an inaccurate measurement of the absolute value of A to B permeability for rosuvastatin, as an abnormally high efflux ratio of 84.7 for the substrate without any concomitant medications. In addition, there is no dose-response for SAGE-547 between 0.25 to 25 µM for the permeability or efflux ratio of the substrate. Therefore, we agree with the sponsor's conclusion that SAGE-547 is a non-inhibitor of Pg-p, BCRP and MRP2.

Study # SSN-01311
Title: In vitro Interaction Studies of SAGE-547 with human BSEP, MRP3 and MRP4 Efflux (ABC) Transporters, and with human MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-01311\

- **Objective:**

The purpose of this study was to provide data on the interaction of SAGE-547 with the human ABC (efflux) transporters: BCRP, BSEP, MDR1, MRP3 and MRP4; and the human SLC (uptake) transporters: MATE1, MATE2-K, OATP1B1, OATP1B3, OAT1, OAT3, OCT1 and OCT2.

- **Methods:**

Kinetic solubility assessment of SAGE-547 in assay buffers

The aqueous solubility of SAGE-547 was determined by optical microscopy evaluation (20 × magnification) for all buffers, and in addition by spectrophotometric measurements for uptake and VT buffers.

Non-specific binding assays

The non-specific binding of SAGE-547 (at 0.2 and 2 µM) was determined by incubating SAGE-547 in tissue culture plates in the absence of cells or membranes. Samples in triplicates were taken after the appropriate incubation time and the amount of SAGE-547 in the wells was determined using liquid scintillation counting.

Transporter assays

1. *Vesicular transport inhibition and substrate assays*

Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing human ABC transporters BSEP, MRP3 and MRP4. SAGE-547 was incubated with membrane vesicle preparations and the probe substrate. Incubations were carried out in the presence of 4 mM ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. After specific incubation time, reactions were quenched by adding ice-cold washing buffer

and immediate filtration. The filters were washed, dried and the amount of substrate inside the filtered vesicles was determined by liquid scintillation counting.

Transporter	Applying protocol	Protein content/well (µg)	Incubation time (min)	Probe substrate	Reference inhibitor
human BSEP	VT-HTS-BSEP-Hi5-TC*	50	5	TC (2 µM)	Cyclosporin A (20 µM)
human MRP3	VT-HTS-MRP3-HEK293-E217βG	50	10	$E_2$17βG (10 µM)	Sulfasalazine (1000 µM)
human MRP4	VT-HTS-MRP4-HEK-DHEAS	50	4	DHEAS (0.5 µM)	MK571 (150 µM)

* In the BSEP vesicular transport substrate assay betagal-Hi5 vesicles were used as negative control

2. Experimental method for vesicular transport substrate assay

The accumulation of SAGE-547 into membrane vesicles was determined using inside-out membrane vesicles prepared from cells overexpressing the human BSEP transporter as well as from control cells. Two incubation time points (2 and 20 min) and two concentrations (0.1 and 1.0 µM) of SAGE-547 were tested in the presence of ATP or AMP, to determine whether SAGE-547 is actively transported into the vesicles. Reactions were quenched by the addition of ice-cold washing buffer and immediate filtration. The amount of accumulated SAGE-547 retained inside the vesicles was determined by liquid scintillation counting.

3. Experimental method for uptake transporter inhibition experiments

Uptake experiments were performed using CHO, MDCKII, or HEK293 cells stably expressing OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, MATE1 and MATE2-K. After specific incubation time with containing the probe substrate and SAGE-547 (0.003, 0.008, 0.025, 0.074, 0.222, 0.667 and 2.00 µM), reference inhibitor or solvent (for controls), cells were washed with ice cold HK buffer and lysed. Radiolabelled probe substrate transport was determined by measuring an aliquot from each well for liquid scintillation counting.

4. Experimental method for uptake transporter substrate experiments

The uptake of SAGE-547 by OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, MATE1 and MATE2-K were determined using cells overexpressing the respective uptake transporter and control cells, at two incubation time points (2 and 20 min) and at two concentrations (0.1 and 1.0 µM) of SAGE-547 to determine whether or not SAGE-547 was actively taken up into the cells. The amount of SAGE-547 in the cell lysate was determined by liquid scintillation counting.

5. Experimental method for bidirectional permeability (substrate assessment)

Bidirectional transport of SAGE-547 was determined through parental MDCKII, MDCKII-BCRP and MDCKII-MDR1 cell monolayers. Cells were preincubated in assay buffer for 10 minutes to allow cells adjusting to the medium. Assay buffer containing SAGE-547 at two concentrations (0.16 and 1.6 µM) was then added to the appropriate apical (400 µL) or basolateral chamber (800 µL). The prazosin (BCRP) or digoxin (MDR1) efflux ratio was determined as a positive control for BCRP or MDR1 function, respectively.

- **Analytical Method:** liquid scintillation counting

- **Results:**

Kinetic solubility assessment

SAGE-547 was soluble up to 2 μM in assay buffers for vesicular and uptake transport assays, yet precipitated at higher concentrations.

Vesicular transport inhibition assays

SAGE-547 did not influence the BSEP, MRP3 or MRP4-mediated probe substrate accumulation up to the highest concentration of 2 μM.

Vesicular transport substrate assays

The ATP-dependent accumulation of SAGE-547 in BSEP expressing and control vesicles was similar in the presence of ATP and AMP (ATP-dependent fold accumulation values were < 2), indicating no active accumulation of SAGE-547.

Uptake transporter inhibition assays

SAGE-547 inhibited the OCT1- and OCT2-mediated metformin accumulation at the applied concentrations in a dose-dependent manner with a maximum inhibition of 80 % for OCT1 and 45% for OCT2. The calculated IC_{50} value for OCT1 was 0.41 μM. As the inhibition did not reach 50%, no IC_{50} was calculated for OCT2.

SAGE-547 did not influence the transporter-mediated probe substrate accumulation at the applied concentrations in the other uptake transporter inhibition assays (MATE1, MATE2-K, OAT1, OAT3, OATP1B1 and OATP1B3).

Uptake transporter substrate assays

Accumulation of SAGE-547 was similar in the OATP1B1, OATP1B3 and OCT1-expressing and the control cells for all transporters tested (transporter specific fold accumulation values were < 2), indicating no active accumulation of SAGE-547.

Monolayer substrate assays

Permeability of SAGE-547 measured in the MDCKII (parental), MDCKII-BCRP and MDCKII-MDR1 monolayer was similar in both apical to basolateral (A-B) and basolateral to apical (B-A) direction, indicating SAGE-547 is unlikely to be a substrate for either BCRP or MDR1.

Table 1. Summary of the obtained results

	Inhibition results		Substrate results	
Transporter and assay type	Maximum inhibition (% of control)	IC_{50} (µM)	Maximum efflux ratio or fold accumulation	Substrate
BCRP ML	NT	NT	~1 ER	Unlikely
MDR1 ML	NT	NT	~1 ER	Unlikely
BSEP VT	NIO	NIO	< 2 fold	Unlikely
MRP3 VT	NIO	NA	NT	NT
MRP4 VT	NIO	NA	NT	NT
MATE1 UPT	NIO	NA	NT	NT
MATE2-K UPT	NIO	NA	NT	NT
OAT1 UPT	NIO	NA	NT	NT
OAT3 UPT	NIO	NA	NT	NT
OATP1B1 UPT	NIO	NA	< 2 fold	Unlikely
OATP1B3 UPT	NIO	NA	< 2 fold	Unlikely
OCT1 UPT	80	0.41	< 2 fold	Unlikely
OCT2 UPT	45	NA	NT	NT

ML: Monolayer assay, NIO: no interaction observed, defined as inhibition being <20%, NA: not applicable, NT: not tested, UPT: uptake assay VT: vesicular transport assay

- **Sponsor's conclusions:**

1. SAGE-547 did not inhibit human efflux transporters BSEP, MRP3 and MRP4 up to the highest soluble concentration of 2 µM.
2. SAGE-547 was not detected as and is therefore unlikely a substrate of human efflux transporters BCRP, BSEP and MDR1.
3. SAGE-547 did not significantly inhibit (no or <20% inhibition) human uptake transporters MATE1, MATE2-K, OAT1, OAT3, OATP1B1 and OATP1B3, but inhibited human uptake transporters OCT1 and OCT2 in a dose dependent manner (OCT1: IC_{50} = 0.41 µM, OCT2: maximum relative inhibition = 45%).
4. SAGE-547 was not detected as and is therefore unlikely a substrate of human uptake transporters OATP1B1, OATP1B3 and OCT1.

- **Reviewer's Comments:**

1. *According to the FDA draft guidance for drug interaction, since SAGE-547 is minimally excreted in the urine, in vitro studies to evaluate whether the drug is a substrate for renal transporters of OAT1/3, OCT2, MATE1 and MATE2-K are not required.*
2. *OCT1: $I_{max,u}/IC_{50}$ = 0.0053 /0.41 = 0.01, which is smaller than the cut-off value of 0.1. Thus, the in vivo DDI study is not required.*

Study: # SSN-02081
Title: In vitro Interaction Studies of SGE-03211 (M133), SGE-03212 (M136), and SGE-03227 (M137) with human BCRP, BSEP, MDR1, MRP3 and MRP4 Efflux (ABC) Transporters, and with human MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2 Uptake Transporters
EDR link: \\cdsesub1\evsprod\NDA211371\0001\m4\42-stud-rep\422-pk\4224-metab\ssn-

02081\

- **Objective:**

The purpose of this study was to provide data on the interaction of SGE-03211 (M133), SGE-03212 (M136), and SGE-03227 (M137) with the human ABC (efflux) transporters: BCRP, BSEP, MDR1, MRP3 and MRP4; and the human SLC (uptake) transporters: MATE1, MATE2-K, OAT1, OAT3, OATP1B1, OATP1B3, OCT1 and OCT2.

- **Methods:**

1. Vesicular transport inhibition and substrate assays

Vesicular transport assays were performed with inside-out membrane vesicles prepared from cells overexpressing BCRP, BSEP, MDR1, MRP3, MRP4. M133, M136 and M137 (2.5 and 25 µM) were incubated with membrane vesicle preparations and the probe substrate. Incubations were carried out in the presence of 4 mM ATP or AMP to distinguish between transporter-mediated uptake and passive diffusion into the vesicles. After specific incubation time, reactions were quenched by adding ice-cold washing buffer and immediate filtration. The filters were washed, dried and the amount of substrate inside the filtered vesicles was determined by liquid scintillation counting.

2. Experimental method for uptake transporter inhibition experiments

Uptake experiments were performed using MDCKII, or HEK293 cells stably expressing OAT1, OAT3, OATP1B1, OATP1B3, OCT1, OCT2, MATE1 and MATE2-K. After specific incubation time with containing the probe substrate and M133, M136 and M137 (2.5 and 25 µM), reference inhibitor or solvent (for controls), cells were washed with ice cold HK buffer and lysed. Radiolabelled probe substrate transport was determined by measuring an aliquot from each well for liquid scintillation counting.

Table 1. Test articles and transporter assays requested by Sage Therapeutics

Test article	Transporter	Assay	Applied concentration range
SGE-03211, SGE-03212 and SGE-03227	BCRP BSEP MDR1 MRP3 MRP4	Vesicular transport assay	2.5 and 25 µM
	MATE1 MATE2-K OAT1 OAT3 OATP1B1 OATP1B3 OCT1 OCT2	Uptake transporter assay	2.5 and 25 µM

- **Analytical Method:**
- **Results:**

Table 1. Summary of the obtained results

	maximum inhibition (% of control)		
Transporter and assay type	**SGE-03211**	**SGE-03212**	**SGE-03227**
BCRP VT	24*	NIO*	NIO*
BSEP VT	88	89	89
MDR1 VT	46	42	NIO
MRP3 VT	NIO	78	99
MRP4 VT	71	81	32
MATE1 UPT	NIO	NIO	NIO
MATE2-K UPT	NIO	NIO	NIO
OAT1 UPT	NIO	NIO	27
OAT3 UPT	44	61	NIO
OATP1B1 UPT	90	90	53
OATP1B3 UPT	98	95	54
OCT1 UPT	35	NIO	23
OCT2 UPT	NIO	NIO	NIO

VT: vesicular transport assay, UPT: uptake assay

* - all three compounds stimulated BCRP-mediated E3S transport

- **Sponsor's conclusions:**

Vesicular transport inhibition assays

1. SGE-03211 inhibited the following efflux transporters: BCRP, BSEP, MDR1 and MRP4, with a maximum of 24%, 88%, 46% and 71%, respectively. SGE-03211 showed no interaction with the human MRP3.
2. SGE-03212 inhibited the following efflux transporters: BSEP, MDR1, MRP3 and MRP4, with a maximum of 89%, 42%, 78% and 81%, respectively. SGE-03212 showed no interaction with the human BCRP.
3. SGE-03227 inhibited the following efflux transporters: BSEP, MRP3 and MRP4, with a maximum of 89%, 99% and 32%, respectively. SGE-03227 showed no interaction with the human BCRP and MDR1.

Uptake transporter inhibition assays

4. SGE-03211 inhibited the following uptake transporters: OAT3, OATP1B1, OATP1B3 and OCT1, with a maximum of 44%, 90%, 98% and 35%, respectively. SGE-03211 showed no interaction with the human MATE1, MATE2-K, OAT1 and OCT2 transporters.
5. SGE-03212 inhibited the following uptake transporters: OAT3, OATP1B1 and OATP1B3, with a maximum of 61%, 90% and 95%, respectively. SGE-03212 showed no interaction with the human MATE1, MATE2-K, OAT1, OCT1 and OCT2 transporters.
6. SGE-03227 inhibited the following uptake transporters: OAT1, OATP1B1, OATP1B3 and OCT1, with a maximum of 27%, 53%, 54% and 23%, respectively. SGE-03227 showed no interaction with the human MATE1, MATE2-K, OAT3 and OCT2 transporters.

- **Reviewer's Comments:**

All three metabolites are BSEP inhibitors (~ 50% inhibition at 2.5 µM). BSEP is expressed on the canalicular membrane of hepatocyte, where it functions in the secretion of bile salts from the liver into the bile canaliculi. Although the intracellular concentrations of metabolites are not known, considering the systemic exposure (~ 0.65 µM), these metabolites could have the hypothetical potential to cause BSEP inhibition in vivo. It has been reported that BSEP inhibition is associated with cholestatic liver injury. Thus, the clinical safety information for potential liver toxicity was further reviewed, and there no sign for bile flow reduction or liver damage during the treatment of SAGE-547. Thus, an additional clinical study to assess the impact of BSEP inhibition by SAGE- 547 is not required.

22.5. **Additional Clinical Outcome Assessment Analyses**

[Add your Text and Figures/Tables Here]

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Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
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STATISTICAL REVIEW AND EVALUATION

CLINICAL STUDIES

NDA/BLA #:	NDA 211371 (reference IND 122279)
Drug Name:	Zulresso (brexanolone 5mg/mL intravenous injection)
Indication(s):	Treatment of postpartum depression
Applicant:	Sage Therapeutics
Date(s):	Received 4/19/2018; PDUFA due date 9/19/2018
Review Priority:	Priority review
Biometrics Division:	Division of Biometrics VI/Office of Biostatistics (DBVI)
Statistical Reviewer:	Feng Zhou, M.S., DBVI/OB/OTS
Secondary Reviewer:	Ling Chen, Ph.D., DBVI/OB/OTS
Concurring Reviewer:	Qianyu Dang, Ph.D., Team Leader, DBVI/OB/OTS
Biometrics Division Director:	Yi Tsong, Ph.D. DBVI/OB/OTS
CSS Reviewer:	Shalini Bansil, M.D., Ph.D. OD/CSS
Project Manager:	Sandra Saltz, OD/CSS
Medical Division:	Controlled Substance Staff

Keywords: Crossover design; Drug abuse potential study; Self-reported endpoint; Multiple endpoints

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EXECUTIVE SUMMARY

Study 547-CLP-102 was a randomized, double-blind, active- and placebo-controlled, double-dummy, 6-way crossover study to determine the abuse potential of single doses (90 µg/kg IV) intravenously administered SAGE-547 compared to placebo and 2 doses (1.5 mg and 3 mg) of alprazolam in healthy, nondependent, recreational central nervous system (CNS) depressant users.

Of the 40 subjects who were randomized to the Treatment Phase, 36 subjects (80%) were included in the PK Population and 25 subjects (62.5%) completed the study. The reviewer's analysis was based on the completers. This study was designed before the FDA 2017 guidance was published. Thus, the test value (or margin) for each test was zero. The primary endpoint was Emax of Drug Liking VAS. Per CSS request, this reviewer also studied five secondary abuse potential measures: High VAS, Good Effects, Bad Effects, Overall Drug Liking VAS, and Take Drug Again VAS.

The statistical analysis results show that:

- The mean of Emax for Drug Liking was significantly higher for alprazolam 1.5 mg and 3.0 mg compared to placebo, thereby establishing the study validity.

- On the average, the responses to SAGE-547 90 µg/kg were significantly lower than those to both doses of alprazolam for the primary and five selected secondary endpoints. There was no significant mean (or median) difference between SAGE-547 90 µg/kg and placebo.

- On the average, the responses to SAGE-547 180 µg/kg were significantly lower than those to both doses of alprazolam for the primary and five selected secondary endpoints except the primary endpoint for the comparison of SAGE-547 180 µg/kg versus 1.5 mg alprazolam (p=0.029). However, the responses to SAGE-547 180 µg/kg were significantly larger than those to placebo.

- On the average, the responses to SAGE-547 270 µg/kg were not significantly different from those to both doses of alprazolam for the primary endpoint and selected secondary endpoints except Bad Effects Emax. SAGE-547 270 µg/kg had significantly lower mean of Bad Effects Emax compared to both doses of alprazolam.

In summary, the therapeutic dose of SAGE-547 (90 µg/kg) is not euphoric, and has less liking, and high as well as take drug again compared to alprazolam. However, the mid dose of SAGE-547 showed significantly higher mean response to placebo, and the high dose of SAGE-547 did not show significantly lower mean response to alprazolam 3.0 mg for the primary endpoint and the selected secondary endpoints. The rapidly raising mean response of SAGE-547 at hour 0.5 and less sedative effects raise more concern of the abuse potential of SAGE-546. Based on both the primary and secondary analyses, this reviewer concludes that the abuse potential of SAGE-547 may not be lower than alprazolam.

1 INTRODUCTION

1.1 Overview

1.1.1 Background Information

Sage Therapeutics has submitted NDA 211371 for brexanolone (5mg/mL) intravenous injection for the treatment of postpartum depression (PPD). The compound was investigated under IND 122279 and received breakthrough therapy on 8/23/16. The Sponsor's application included a drug abuse potential assessment (Study 547-CLP-102), which was part of a Phase 1 program in support of the development of brexanolone. This consult review responded to a request by the Controlled Substance Staff (CSS) to review this study.

SAGE-547 injection (hereafter referred to as "SAGE-547") is a proprietary formulation of allopregnanolone, also referred to as brexanolone, an endogenous neuroactive steroid and major metabolite of progesterone, which is unscheduled. Allopregnanolone has been available as an unscheduled substance for several decades. Based on the available evidence, there were no signals of abuse, misuse, diversion, dependence or withdrawal with allopregnanolone using a variety of public post-marketing data sources. The Sponsor stated that, "the abuse potential and physical dependence potential of brexanolone is low, which supports the proposed recommendation that brexanolone should not be scheduled as a controlled substance". The Joint Meeting of the Psychopharmacologic Drugs Advisory Committee and the Drug Safety and Risk Management Advisory Committee was scheduled on 11/2/2018.

1.1.2 Specific Studies Reviewed

The applicant, Sage, submitted one list of preclinical and clinical study reports related to abuse potential assessment that were conducted and cited in the NDA submission. This review only focuses on the human abuse potential study 547-CLP-102 (Table 1).

Table 1: List of Studies Included in this Review

Study ID (Duration)	Location	Design	Primary Endpoints	Treatments	Number of Subjects
547-CLP-102 12/21/2015 – 8/8/2016	1 site in CANADA	R, DB, AC, PC, MD, 6-arms crossover to evaluate the abuse potential of intravenously administered drug	Emax for Drug Liking	A: placebo [PBO] B: alprazolam 1.5 mg PO [ALZ1.5] C: alprazolam 3.0 mg PO [ALZ3.0] D: SAGE-547 90 µg/kg IV [SAGE90] E: SAGE-547 180 µg/kg IV [SAGE180] F: SAGE-547 270 µg/kg IV [SAGE270]	40 randomized and 25 subjects completed all treatment periods

Abbreviations: DB = double blind; PC = placebo-controlled; AC = active-controlled; R = randomized; MD=multi-dose

1.2 Data Sources

All data was supplied by the applicant to the CDER electronic data room in SAS transport format. The data and final study report for the electronic submission were archived under the network path location \\...\211371.enx. The information needed for this review was contained in submission modules 5.3.4 modules and datasets.

2 STATISTICAL EVALUATION

2.1 Data and Analysis Quality

Data for study 547-CLP-102 was submitted on 4/19/2018 (NDA 211371/S0001). In general, the data and analysis quality are acceptable.

2.2 Human Abuse Potential Stud 547-CLP-102

2.2.1 Overview

547-CLP-102 was a randomized, double-blind, active- and placebo-controlled, double-dummy, 6-way crossover study to determine the abuse potential of intravenously administered SAGE-547 in healthy, nondependent, recreational central nervous system (CNS) depressant users.

Objectives of the Study
The primary objective of the study was to assess the abuse potential of intravenously infused SAGE-547 relative to placebo and orally administered alprazolam (schedule C-IV) in nondependent, recreational central nervous system (CNS) depressant users.

Secondary Objectives were mainly to assess the pharmacokinetics (PK) of SAGE-547 in plasma when administered by intravenous (IV) infusion in nondependent, recreational CNS depressant users and to assess the safety of intravenously infused SAGE-547 relative to placebo and orally administered alprazolam in nondependent, recreational CNS depressant users.

Reviewer's comment: This review report wasonly for the primary objective of the study

2.2.1.1 Study Design

In order to determine the appropriate maximum dose of SAGE-547 to be used in the main study, this study was conducted in 2 parts: Part A (Dose Selection Phase) and Part B (Treatment Phase). The purpose of Part A was conducted an exploratory Dose Selection Phase to identify the appropriate doses of SAGE-547 to be used in the Treatment Phase (Part B). The study objectives were addressed in Part B.

Dose Selection Phase (Part A): consisted of a Screening Visit, Dose Selection Visit, and Follow-Up Visit. The Dose Selection Phase employed an exploratory single-dose, randomized, double-blind, placebo-controlled study to evaluate the safety and tolerability of escalating dose of SAGE-547 given as an IV infusion over 1 hour. Each SAGE-547 dose level was tested in cohorts of eight new subjects. With subjects in each cohort randomized to receive a dose of either SAGE-547 (n=6) or placebo (n=2).

Within 30 days of screening, eligible subjects were admitted to the research clinic on Day -1 for their Dose Selection Visit and dosed on Day 1 with blinded drug, either SAGE-547 or matching placebo, infused over 1 hour. Subjects were discharged on Day 2, approximately 24 hours after dosing, at the discretion of the investigator or designee. Subjects returned for a safety Follow-Up Visit 5 to 10 days after dosing or at the time of early withdrawal from the study.

Dose selection began with a SAGE-547 dose of 60 µg/kg administered via a 1-hour IN infusion, a dose approximating those that have been previously shown to be well tolerated in past studies in conscious subjects. After the completion of each cohort, available safety data were un-blinded and reviewed by the Investigator, Sage, and designees. Following the review, dose escalation occurred if a higher dose could have been safely administered and if a maximum dose had not been identified. Subsequent cohorts were dosed with the next higher dose in 30 µg/kg increments, with a maximum of eight cohorts (60, 90, 120, 150, 180, 210, 240, and 270 µg/kg). The maximum dose (270 µg/kg) represented a three-fold increase in the maximum therapeutic dose planned for the postpartum depression clinical development program (90 µg/kg).

Treatment Phase (Part B): was a single-dose, randomized, double-blind, and placebo- and active-controlled cross-over study with 7 treatment visits per subject.

The abuse potential of 3 doses of SAGE-547 (90 µg/kg IV, 180 µg/kg IV, or 270 µg/kg IV) was compared to that of placebo or 1.5 mg and 3.0 mg alprazolam (active control) in healthy, male and female, nondependent, recreational CNS depressant users. Subjects participated in a Screening Visit, one 5-day Qualification Phase, a Treatment Phase consisting of six 3-day in-clinic Treatment Visits (each separated by a minimum 7-day washout period), and a safety Follow-up visit.

Within 30 days of a standard medical screening, subjects attended a randomized, double-blind Qualification Phase in which they received either 2.0 mg alprazolam (treatment Y) or matching placebo (treatment X) in a cross-over manner, each separated by approximately 48 hours, to ensure that subjects could discriminate and show positive effect of the positive control effects of alprazolam.

Following Qualification, it was planned that approximately 45 healthy female and male subjects aged 18 to 55 years (inclusive), who had used CNS depressants for recreational, nontherapeutic reasons at least five times in the past year and at least once in the 8 weeks prior to screening and were non-substance or alcohol dependent within the past 2 years and who had passed the pharmacologic qualification, were randomized in the Treatment Phase. The treatments in the Treatment Phase were:

- Treatment A (placebo [PBO])
- Treatment B (alprazolam 1.5 mg PO [ALZ1.5])
- Treatment C (alprazolam 3.0 mg PO [ALZ3.0])
- Treatment D (SAGE-547 90 µg/kg IV [SAGE90])
- Treatment E (SAGE-547 180 µg/kg IV [SAGE180])
- Treatment F (SAGE-547 270 µg/kg IV [SAGE270])

Subjects were to receive all six treatments in the order specified by the treatment sequence according to a 6x6 Williams square design below:

	Treatment by Period					
Treatment Sequence	**1**	**2**	**3**	**4**	**5**	**6**
1	A	B	F	C	E	D
2	B	C	A	D	F	E
3	C	D	B	E	A	F
4	D	E	C	F	B	A
5	E	F	D	A	C	B
6	F	A	E	B	D	C

However, there was an error in the specification of the actual randomization schedule, rather than using the 6x6 Williams square design; subjects received all sex treatments in the order specified below. This issue was discussed in section 2.2.2.2 in this review.

Treatment Sequence	Treatments by Period					
	1	2	3	4	5	6
1	A	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	A	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E

Treatment visits were separated by a washout interval of at least 7 days. Subjects returned for the safety follow-up visit approximately 5 to 10 days following the last study drug administration.

2.2.1.2 Abuse Potential Measures

The following pharmacodynamics assessments were administered to evaluate the subjective and objective effects of SAGE-547.

Primary endpoint: was the maximum (peak) effect (Emax) for Drug Liking ("at this moment"), assessed as a bipolar (0 to 100 point) visual analog scale (VAS): Emax of Drug Liking VAS

Secondary endpoints: were included for the following measures:

- Balance of effects:
  - Drug Liking VAS (Emin, and TA_AUE)
  - **Overall Drug Liking VAS (Emax/Emin, end-of-day and next day scores)**
  - **Take Drug Again VAS (Emax, end-of-day and next day scores)**
- Positive effects:
  - **High VAS (Emax** and TA_AUE**)**
  - **Good Effects VAS (Emax** and TA_AUE**)**
- Negative effects:
  - **Bad Effects VAS (Emax** and TA_AUE**)**
- Other drug effects:
  - Alertness/Drowsiness (Emax, Emin, TEmax, Temin, and TA_AUE)
  - Agitation/Relaxation (Emax, Emin, TEmax, Temin, and TA_AUE)
  - Any Effects VAS (Emax, TEmax, and TA_AUE)
- Objective assessment of drug effects:
  - Choice Reaction Time (CRT)
    - Total Reaction Time (TRT) (CFBmax and TA_AUE)
    - Recognition Reaction Time (RRT) (CFBmax and TA_AUE)
    - Motor Reaction Time (MRT) (CFBmax and TA_AUE)
    - % Correct (CFBmin and TA_AUE)
- Percentage correct responses (Emin and TA_AUE)
  - Divided Attention Test (DAT)
    - Mean of 3 Flights RMS (Emax and CFBmax)
    - Mean of 3 Flights % Over Road (Emin and CFBmin)
    - Mean of 3 Flights Furthest Diagonal Distance (Emax and CFBmax)
    - Mean of 3 Flights Mean Hit Latency (Emax and CFBmanx)
    - Mean of 3 Flights False Alarms (Emin and CFBmin)
    - Target Hits (%) (Emin and CFBmin)
    - Stanford Sleepiness Scale (Score) (Emax and TEmax)
    - Sternberg Short Term Memory (SSTM) Task (Dprime, Pooled (Emin and CFBmin)
    - Mean Hit Reaction Time (Emax and CFBmax)

Reviewer's Comments: There were too many abuse potential measures in this study. After discussed with the CSS reviewer, this reviewer only focused on the secondary endpoints above in bold.

2.2.1.3 Analysis Population and Sample Size

For subjects randomized in the treatment phase, most subjects were male (76%), White (76%), and not Hispanic or Latino (92%). The mean (SD) age was 39.0 (7.9) years and ranged from 28 to 53 years (See Table 11 in Appendix. for detail). The primary analysis was based on the completer population. No imputation was performed for any missing measurements. A total of 40 subjects were randomized to the treatment phase and 25 subjects completed all 6 treatment periods.

The sponsor claimed that as determined by a paired t-test with a 2-sided significance level of 0.05, a sample size of 24 subjects had at least 90% power to detect a difference of 15 in Drug Liking VAS Emax (bipolar scale) with a standard deviation (SD) of 20. Assuming an approximate 25% dropout rate, 36 subjects (six subjects per sequence) were to be randomized into the treatment phase, with the intention to complete approximately 24 subjects (four subjects per sequence).

2.2.1.4 Statistical Methodologies used in the Sponsor's Analyses

The primary endpoint analyses were based on Drug Liking VAS Emax and the following pairwise treatment comparisons were performed:

- Each dose of alprazolam (1.5 mg, 3.0 mg) compared with placebo
- Each dose of SAGE-547 (low, intermediate, high) compared with each dose of alprazolam (1.5 mg, 3.0 mg)
- Each dose of SAGE-547 (low, intermediate, high) compared with placebo

The treatment comparison analyses were performed using a mixed-effects model for a crossover study. The model included effects for treatment sequence, treatment, and period as fixed effects, baseline (pre-dose) measurement as a covariate, where applicable, and subject nested within treatment sequence as a random effect. Contrasts were used to calculate least square means for each treatment, pairwise differences between treatments, and the corresponding 95% confidence intervals and p-values for each difference between treatments.

The residuals from the mixed-effect model were investigated for normality using the Shapiro-Wilk W-test. Parameters were analyzed as having a normal distribution if the probability value is $\geq$0.05. Parameters that did not meet this criterion were analyzed nonparametrically. Nonparametric tests of overall treatment effect were assessed using Friedman's test (using a Freq procedure); pairwise treatment comparisons were assessed using the sign test on the within-subject differences (using a Univariate procedure).

For study validity purposes, the primary endpoint, Emax for Drug Liking VAS, was compared between the investigational positive control (alprazolam) and placebo. The comparison would assess the null hypothesis that the mean difference in Emax VAS for Drug Liking for alprazolam minus placebo was less than or equal to 0 against the alternative hypothesis that the mean

difference was greater than zero. The hypotheses could be expressed as the following (where μ_A is the mean for alprazolam and μ_P is the mean for placebo):

$$H_0: \mu_A - \mu_P \leq 0 \text{ vs } H_1: \mu_A - \mu_P > 0$$

If statistically significant, it would confirm the sensitivity of the study and allow for the comparison of the other pairwise comparisons including SAGE-547 doses.

The Univariate procedure in SAS software returns a two-sided p-value (noted "P > |M|"). To get a one-sided p-value focusing on upper tail (noted "P > M"), the following transformation had to be made regarding the sign of M (statistic M being the sign test value).

- If M is positive, then P > |M| = (P < - M) + (P > M) leading to P > M = (P > |M|) / 2.
- Else if M is negative then P > |M| = (P < M) + (P > - M) leading to P > M = 1 – ((P > |M|) / 2).

Reviewer's comment: According to FDA Guidance for Industry: Assessment of Abuse Potential of Drugs published in January 2017, hierarchically testing the following three hypotheses for mean differences:

1. H_0: $\mu_c - \mu_p \leq \delta_1$ *versus* H_a: $\mu_c - \mu_p > \delta_1$, *where* $\delta_1 > 0$.
2. H_0: $\mu_c - \mu_T \leq \delta_2$ *versus* H_a: $\mu_c - \mu_T > \delta_2$, *where* $\delta_2 \geq 0$.
3. H_0: $\mu_T - \mu_p \geq \delta_3$ *versus* H_a: $\mu_T - \mu_p < \delta_3$, *where* $\delta_3 > 0$.

The actual values of δ_1, δ_2, and δ_3 should be pre-specified in the protocol according to such factors as subjective measures, drug class, and route of drug administration. All tests are at 0.05-alpha significance level.

The Statistical Analysis Plan (SAP) for this study was finalized on 9/27/2016, before the guidance was published. Therefore, it is acceptable the sponsor's analyses using margin as 0 with a significance level of 0.025 (1-sided) except the comparison between test drug and placebo.

2.2.1.5 Changes in the Conduct of the Study

The Statistical Analysis Plan (SAP) was finalized on 9/27/2016. As the sponsor reported, the following changes were made in the planned statistical analysis.

1. Due to the randomization schedule error, Section 6.10.1 has been updated to remove the inclusion of a first-order carryover effect and skip the first stage of the analysis which was planned to test the carryover effect at the 25% significance level. Instead, it will be assumed that there is no first-order carryover effect. The assumption of no carryover effect is considered acceptable based on the 6-day washout between treatment periods and the estimated half-lives of the study drugs used.
2. The confidence intervals for the mixed effects models were changed from 90% to 95% CIs.
3. The Wilcoxon rank test was changed to the sign test.

2.2.1.6 Sponsor's Summary and Conclusions

Summary

- The Emax for Drug Liking was significantly higher for alprazolam 1.5 mg and 3.0 mg compared to placebo, thereby establishing study validity.

- The Emax for Drug Liking, Good Effects, High Effects, and Any Effects was significantly lower for SAGE-547 90 μg/kg and 180 μg/kg compared to alprazolam 1.5 mg. There was no significant difference between SAGE-547 270 μg/kg and alprazolam 3.0 mg.

- The Emax for Drug Liking, Good Effects, High Effects, and Any Effects was significantly higher for both SAGE-547 180 μg/kg and 270 μg/kg compared to placebo. For SAGE-547 90 μg/kg, the Emax for Good Effects, High Effects, and Any Effects was significantly higher compared to placebo. The difference between SAGE-547 and placebo increased with increasing dose of SAGE-547.

- Drowsiness and sleepiness ratings were significantly lower (ie, less drowsiness/sleepiness) for SAGE-547 90 μg/kg and 180 μg/kg compared to alprazolam. The SAGE-547 270 μg/kg dose produced sedative effects similar to alprazolam.

- Performance on motor, attention, and short-term memory tasks were generally better for all SAGE-547 doses compared to each alprazolam dose.

Conclusion

Alprazolam demonstrated effects consistent with the known profile for abuse potential as a Schedule IV controlled substance.

The therapeutic dose of SAGE-547 (90 μg/kg) demonstrated some potential for abuse, but did not differentiate from placebo on several parameters including Emax and TA_AUE for Drug Liking. The SAGE-547 90 μg/kg dose also had minimal effects on drowsiness, relaxation, and sleepiness and did not show evidence of decreasing short-term memory or attention.

Supratherapeutic doses of SAGE-547 did not exceed the abuse potential or performance effects of alprazolam. On many parameters, the supratherapeutic SAGE-547 dose (180 μg/kg) demonstrated significantly less abuse potential and better performance on motor and attention tasks compared to both alprazolam doses. The supratherapeutic SAGE-547 dose (270 μg/kg) demonstrated abuse potential in the range of therapeutic alprazolam doses, but was associated with less drowsiness/sleepiness and often better performance on attention and short-term memory tasks compared to alprazolam 3.0 mg.

2.2.2 Reviewer's Assessment

This reviewer focused on the primary endpoint Emax of Drug Liking VAS and selected secondary endpoints (High VAS, Good Effects, Bad Effects, Overall Drug Liking VAS, and Take Drug Again VAS). All analyses were based on completer population. In the reviewer's tables and figures PBO, ALZ1.5, ALZ3.0, SAGE90, SAGE180, and SAGE270 denote placebo, alprazolam 1.5 mg, 3 mg, SAGE-547 90 μg/kg IV, 180 μg/kg IV, and 270 μg/kg IV, respectively.

This reviewer followed the sponsor's SAP, all analyses used margin as 0 with a significance level of 0.025 (1-sided) and based on the completer population. The heat maps in this report were using the heat map methods proposed by Chen and Wang (2012).

2.2.2.1 Missing Data Issue

All sponsor's analyses were based on the PD population. According to FDA guidance, all analyses in this review were based on the completer population.

Figure 1 – 3 show the individual time course response profiles for two doses of alprazolam and high dose of SAGE-547 for Drug Liking VAS. The orange line separates the responses by gender. The subjects above the orange line are females, and the subjects below the orange line are males. Colors blue, white, and red denote dislike, neutral and like, respectively. The grey color indicates missing data. From Figure 1, one may see that for ALZ3.0 24% (6/25), 36% (9/25), 24% (6/25) and 16% (4/25) of subjects have missing data at hours 0.66, 1, 1.33, and 1.66, respectively. The missing data situation for ALZ3 is similar to what has been observed in past human drug abuse potential studies which included alprazolam 3 mg as a positive control. Figure 2 showed much less missing data for ALZ1.5 than observed for ALZ3.0, only one subject had missing data at hour 2. Figure 3 shows that only one subject had missing data at hour 1 for SAGE270.

Figure 1: Individual Time Course Response Profiles for Drug Liking VAS (ALZ3)

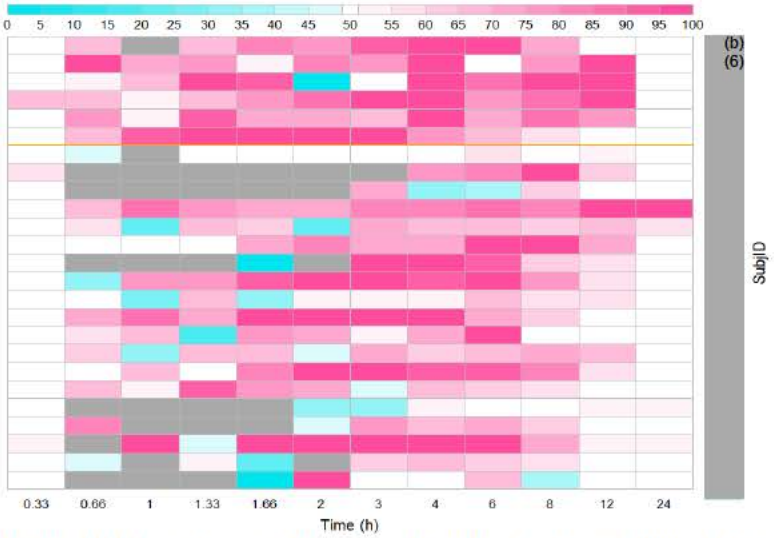


Figure 2: Individual Time Course Response Profiles for Drug Liking VAS (ALZ1.5)

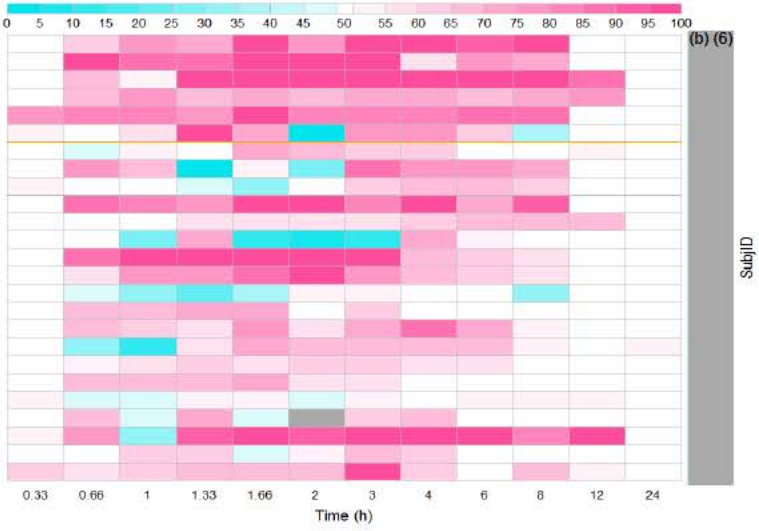
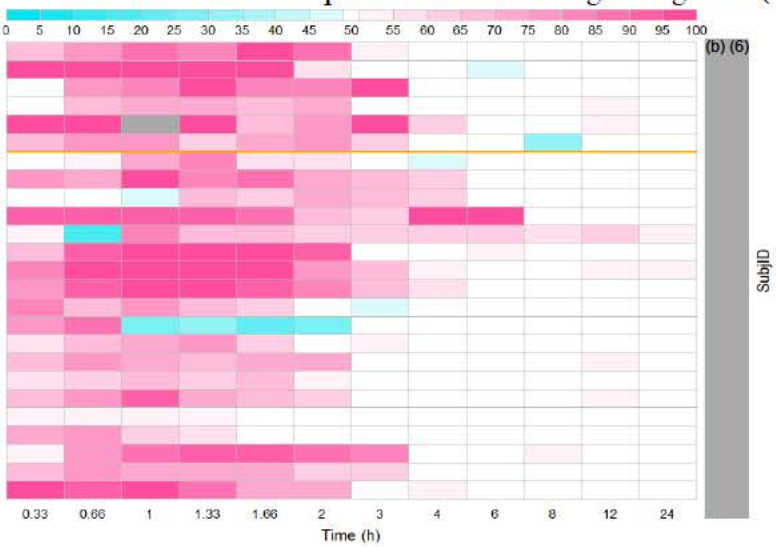


Figure 3: Individual Time Course Response Profiles for Drug Liking VAS (SAGE270)



In past Human Abuse Potential (HAP) studies with alprazolam as a positive control, some sponsor reported that all subjects with missing data points in the alprazolam 3 mg treatment period had somnolence sufficient to prevent them from providing an accurate assessment (i.e. fell asleep) and were not easily awakened. It is standard practice at the site conducting the study to attempt to arouse the subject at least once. If the subject is extremely sedated and is not easily aroused, the investigator must assess the subject in order for the collection of PD data to be skipped. The similar situation may also occur in this study.

Because this was a crossover study and the primary endpoint was Emax, the missing data were not imputed in either the Sponsor's analysis or the reviewer's analysis. Since SAGE270 had the similar missing status as ALZ1.5, therefore, in this reviewer's opinion, ALZ1.5 may be a proper dose of the active control in this study.

2.2.2.2 Study Design Issues

According to the sponsor's report, during the conduct of the study, after the initiation of the Qualification and Treatment Phases, it was discovered that the 6x6 Williams square design was incorrectly implemented in the randomization scheme of the study. The order of treatments within sequence in the final randomization scheme was not the same as defined in the Williams square design table provided in the protocol.

6x6 Williams square design table:

	Treatment by Period					
Treatment Sequence	**1**	**2**	**3**	**4**	**5**	**6**
1	A	B	F	C	E	D
2	B	C	A	D	F	E
3	C	D	B	E	A	F
4	D	E	C	F	B	A
5	E	F	D	A	C	B
6	F	A	E	B	D	C

Implemented design table:

Treatment Sequence	Treatments by Period					
	1	2	3	4	5	6
1	A	B	C	D	E	F
2	B	C	D	E	F	A
3	C	D	E	F	A	B
4	D	E	F	A	B	C
5	E	F	A	B	C	D
6	F	A	B	C	D	E

Note:

- Treatment A (placebo [PBO])
- Treatment B (alprazolam 1.5 mg PO [ALZ1.5])
- Treatment C (alprazolam 3.0 mg PO [ALZ3.0])
- Treatment D (SAGE-547 90 µg/kg IV [SAGE90])
- Treatment E (SAGE-547 180 µg/kg IV [SAGE180])
- Treatment F (SAGE-547 270 µg/kg IV [SAGE270])

The second design table shows placebo after the high dose of SAGE-547 and the low dose of SAGE-547 after the high dose of alprazolam. As shown in Figure 4, the heat map indicates a possibility of carryover effects. For example, subjects (b) (6) took placebo after taking the high dose of test drug, and had scores of Emax of Drug Liking 70 and 74 for placebo, respectively.

Figure 4: Heat Map for Emax of Drug Liking VAS by Sequence and Treatment (N=25)

The statistical model used in the reviewer's analysis was the linear mixed-effects model with period, sequence, treatment, and the first-order carryover effect as fixed effects, subject as a random effect. The first-order carryover effect was significant only for the primary endpoint (p-value=0.1394 < 0.25 alpha level). This reviewer performed the sensitivity analyses for the primary endpoint. The analysis results based on the model including the first-order carryover effect were similar to those excluding the first-order carryover effect (see Table 12 and Table 3 for the details). The analysis results based on the first period data from the PD population (N=33) were similar to those based on the all data from completer population (N=25) (see Table 13 and Table 3 for the details). Therefore, this reviewer concluded that there were some carryover effects due to the design issue. However, the carryover effects did not significantly change the conclusion of the primary analysis. Therefore, the first-order carryover effect was dropped from the model in both reviewer's primary and secondary analyses.

2.2.2.3 Placebo Response

Thirty six percent (9/25) of subjects had placebo responses (Emax > 60) to Drug Liking VAS, as shown in Figure 8. As shown in Figure 5 and Figure 6, the individual time course response profiles of SAGE-547 90 µg/kg and placebo are similar. The mean Emax of Drug Liking of placebo responses is 59.8 with a standard deviation of 13.7 (see Table 2). The large placebo responses may reduce the mean difference between SAGE-547 90 µg/kg and placebo. Hence there may be a serious consequence of concluding no abuse potential of SAGE-547 90 µg/kg, when it may not be true.

Figure 5: Individual Time Course Response Profile for Placebo for Drug Liking VAS (N=25)

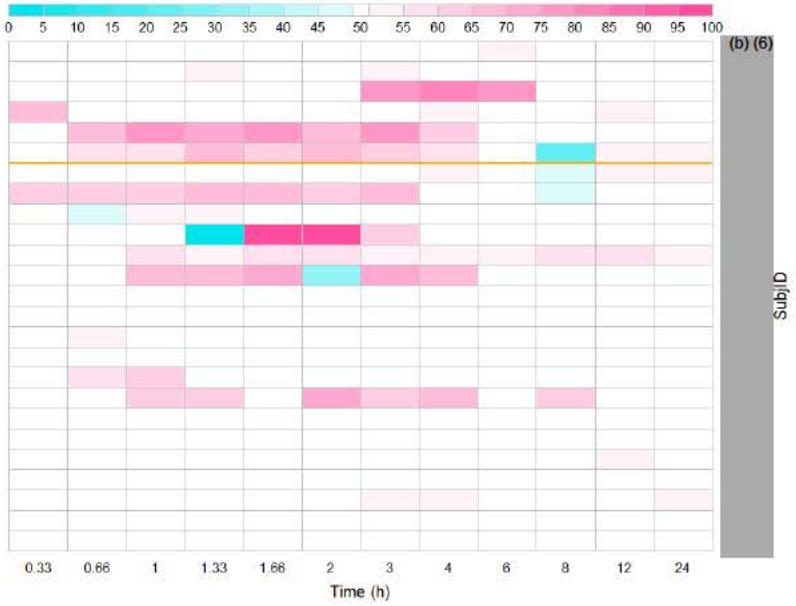
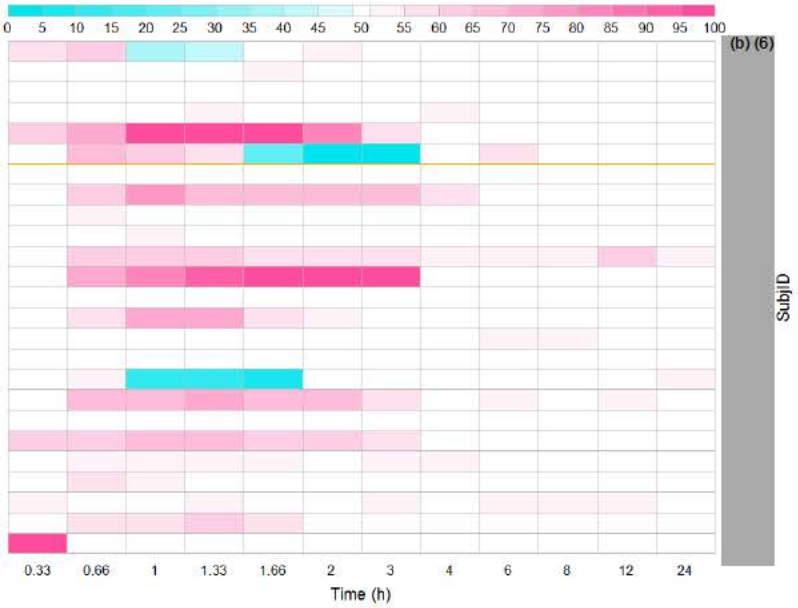


Figure 6: Individual Time Course Response Profile for SAGE-547 90 µg/kg for Drug Liking VAS (N=25)



2.2.2.4 Primary Analysis

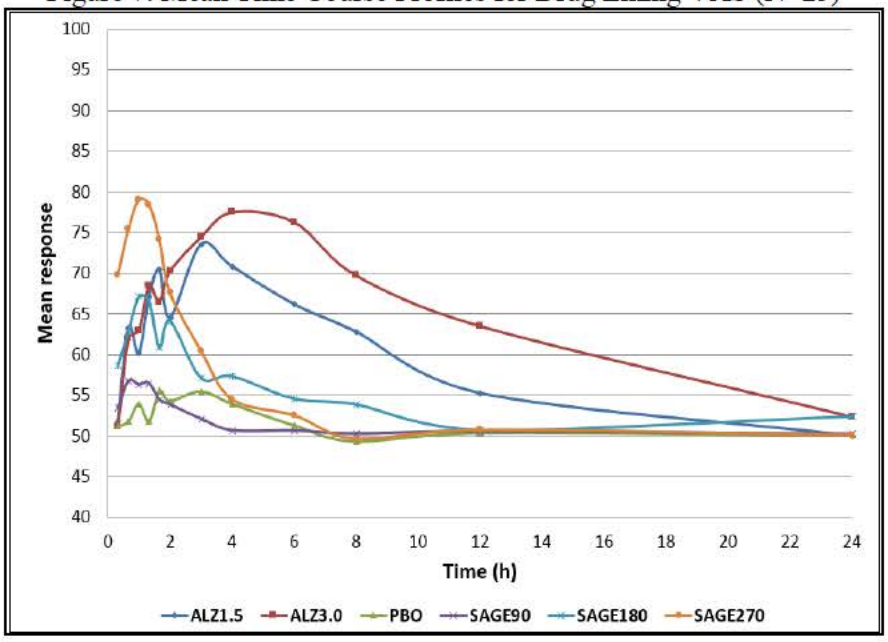
2.2.2.4.1 Descriptive Statistics

The primary PD measure for this study was the 0- to 100-point bipolar VAS score for Drug Liking. A score of 0 indicates strong disliking, a score of 50 indicates neither liking nor disliking, and a score of 100 indicates strong liking. As showed in Table 2, the third quartiles of ALZ1.5 and ALZ3.0 are 100. It means that even for a schedule IV drug, alprazolam, the Emax of Drug Liking VAS is extremely large in approximately 25% of subjects. One may notice that the means and medians of the differences between alprazolam two doses and SAGE-547 low and mid doses are all positive. The means and medians of the differences between SAGE-547 and placebo are all positive. SAGE270 are lower than ALZ3.0 and higher than ALZ1.5 in term of mean of Emax of Drug Liking VAS.

Table 2: Summary Statistics for Emax of Drug Liking VAS (N=25)

TRT or Comparison	Mean	Std	Minimum	Q1	Median	Q3	Maximum
PBO	59.8	13.7	50	50	51	69	100
ALZ1.5	82.1	16.6	51	71	76	100	100
ALZ3.0	89.5	15.6	51	75	100	100	100
SAGE90	62.8	16.4	50	51	55	69	100
SAGE180	75.7	16.5	50	67	72	82	100
SAGE270	86.9	13.3	51	76	90	100	100
ALZ1.5 vs PBO	22.3	17.6	-1	11	20	32	50
ALZ3.0 vs PBO	29.7	17.2	0	17	31	49	50
ALZ3.0 vs SAGE90	26.7	19.7	-1	10	24	49	50
ALZ1.5 vs SAGE90	19.3	20.1	-25	2	19	34	50
SAGE90 vs PBO	3.0	18.8	-45	-1	0	13	50
ALZ3.0 vs SAGE180	13.8	18.2	-15	0	6	25	50
ALZ1.5 vs SAGE180	6.4	15.5	-20	0	2	17	48
SAGE180 vs PBO	15.9	18.0	-24	0	16	22	50
ALZ3.0 vs SAGE270	2.6	12.1	-23	-1	0	4	30
ALZ1.5 vs SAGE270	-4.8	11.1	-33	-10	-3	0	22
SAGE270 vs PBO	27.2	16.4	0	15	26	42	50

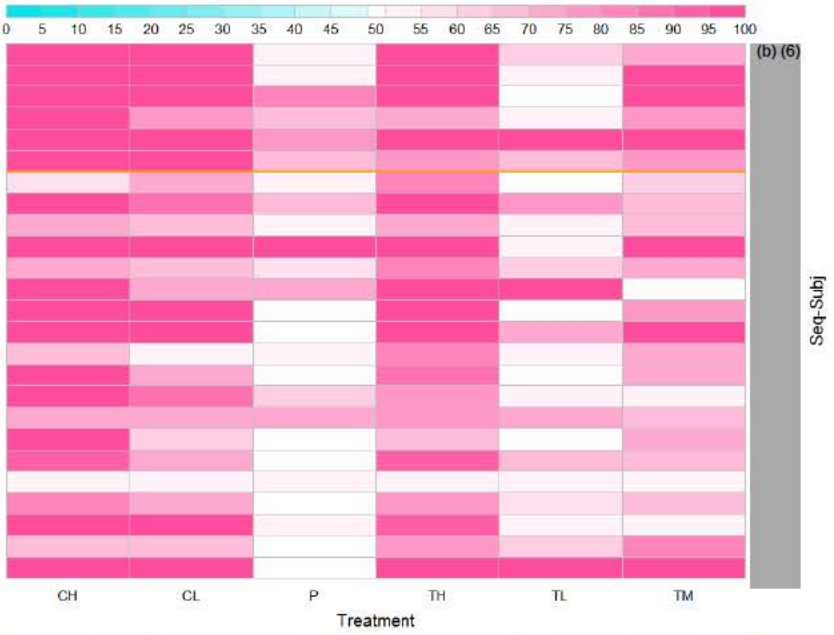
Figure 7: Mean Time Course Profiles for Drug Liking VAS (N=25)



As shown in Figure 7, the mean Drug Liking VAS scores for the subjects took with SAGE-547 increase rapidly, with a steep rate of rise and drop rapidly after 2 hours then gradually approach to baseline value after 4 hours. The onset of effect is at approximately 0.5-hour post-dose for mid and high doses of SAGE-547. On contract, the curves for ALZ3.0 and ALZ1.5 increase gradually and reach the peak around hour 4 and gradually drop to baseline at hour 24. The peak mean response of ALZ3.0 is larger than that of ALZ1.5 and lower than SAGE270. The profile for SAGE90 is very similar to that of placebo.

In the heat map (Figure 8), the intensity of blue indicates the degree of the disliking, the intensity of red indicates the degrees of the liking and white denotes neutral score of 50. From this graph, one may notice that some subjects have high placebo responses. The responses to SAGE90 and PBO are very similar. Overall, more subjects highly like ALZ1.5, ALZ3.0, and SAGE270 compared to SAGE90 and SAGE180. The light pink in the category [51, 60] occurs in many subjects for PBO and SAGE90. One may notice that in Table 2, the medians of Emax of Drug Liking VAS are 55 and 51 for SAGE90 and PBO respectively.

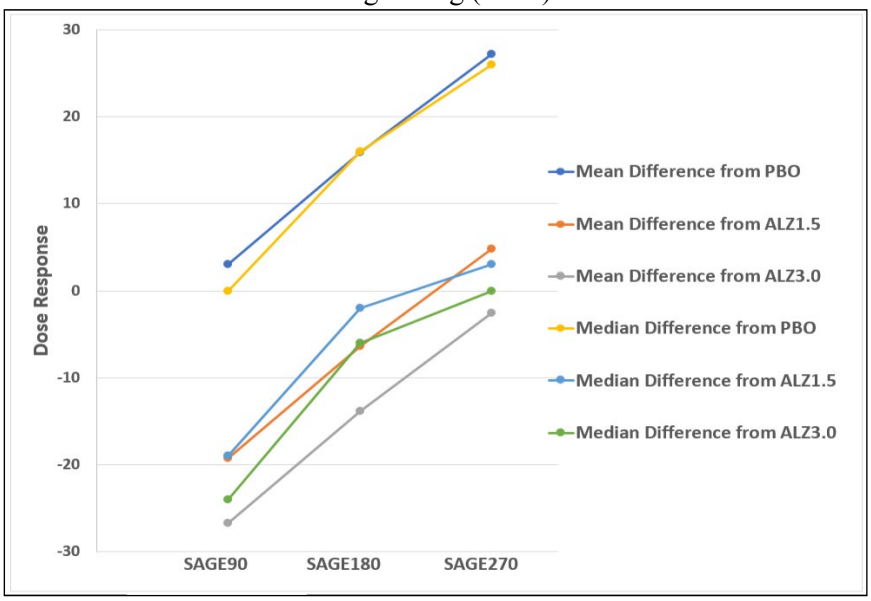
Figure 8: Heat Map for Emax of Drug Liking VAS by Treatment (N=25)



(Note: in the figure, CH=ALZ3.0, CL=ALZ1.5, P=PBO, TH=SAGE270, TL=SAGE90, and TM=SAGE180)

Because this was a crossover study, the dose response curves for the test drug relative to the active control and placebo may be also useful. As show in Figure 9, positive dose responses of SAGE-547 were observed.

Figure 9: Dose Response Curves of SAGE-547 and Alprazolam in difference from Placebo for Emax of Drug Liking (n=25)



2.2.2.4.2 Inferential Statistics

The statistical model used in the reviewer's analysis was the linear mixed-effects model with period, sequence, and treatment as fixed effects, and subject as a random effect. There were no significant period and sequence effects. The reviewer checked assumptions in the model for the equal variances and the normality. The normal assumption was not violated for Drug Liking VAS. However, the assumption of equal variances was not satisfied. The SAS proc mixed procedure can adjust the unequal variances using Tukey-Kramer's method. All tests were at a significance level of 0.025 (1-sided).

As show on Table 3, the mean of Emax for Drug Liking is significantly higher for alprazolam 1.5 mg and 3.0 mg compared to placebo, thereby establishing study validity. The mean response to SAGE-547 90 µg/kg is significantly smaller than that to each of alprazolam. There is no significant mean difference between SAGE-547 90 µg/kg and placebo. On the average, the responses to SAGE-547 180 µg/kg are significantly lower than those to 3.0 mg dose of alprazolam only. LSmean of Emax of Drug Liking produced by SAGE-547 180 µg/kg (75.8) was not statistically significantly smaller than those produced by alprazolam 1.5 mg (81.9). However, the responses to SAGE-547 180 µg/kg are significantly larger than those to placebo. LSmean of Emax of Drug Liking produced by SAGE-547 270 µg/kg (86.9) was not statistically significantly smaller than those produced by alprazolam 1.5 mg (81.9) and by alprazolam 3.0 mg (89.7).

Figure 10 graphically displays the treatment comparison of Emax of Drug Liking VAS. The markers represent the mean difference between the treatments and bar represent the 95% confidence interval of the mean difference. The markers fall in the negative area of Emax indicates that the SAGE-547 doses are numerically or significantly lower than comparator in term of Emax. The bar touched the 0 line indicates that the treatment comparison is not statistically significant.

Table 3: Statistical Analysis Results for Emax of Drug Liking (Completer Population)

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (STD)	62.8 (16.4)	75.7 (16.5)	86.9 (13.3)	82.1 (16.6)	89.5 (15.6)	59.8 (13.7)
Median	55	72	90	76	100	51
Q1, Q3	51, 69	67, 82	76, 100	71, 100	75, 100	50, 69
Min, Max	50, 100	50, 100	51, 100	51, 100	51, 100	50, 100
LS mean (SE)	62.3 (3.8)	75.8 (3.3)	86.9 (2.8)	81.9 (3.1)	89.7 (3.2)	59.80 (3.5)
Treatment Comparisons						
Comparison	**LS Mean Difference (SE)**		**95% Confidence Interval**		**p-value**	
ALZ1.5 vs PBO	22.1 (3.3)		(15.3, 28.8)		<.0001	
ALZ3.0 vs PBO	29.9 (3.4)		(23.0, 36.9)		<.0001	
ALZ3.0 vs SAGE90	27.4 (3.7)		(19.9, 34.9)		<.0001	
ALZ1.5 vs SAGE90	19.6 (3.6)		(12.3, 26.9)		<.0001	
SAGE90 vs PBO	2.5 (4.0)		(-5.6, 10.6)		0.2677	
ALZ3.0 vs SAGE180	13.9 (3.2)		(7.4, 20.4)		<.0001	
ALZ1.5 vs SAGE180	6.1 (3.1)		(-0.29, 12.4)		0.0304	
SAGE180 vs PBO	16.0 (3.5)		(8.8, 23.2)		<.0001	
ALZ3.0 vs SAGE270	2.8 (2.7)		(-2.8, 8.3)		0.1572	
ALZ1.5 vs SAGE270	-5.1 (2.5)		(-10.4, 0.2)		0.9709	
SAGE270 vs PBO	27.1 (3.1)		(20.9, 33.4)		<.0001	

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances

Figure 10: Treatment Comparisons for Emax of Drug Liking VAS

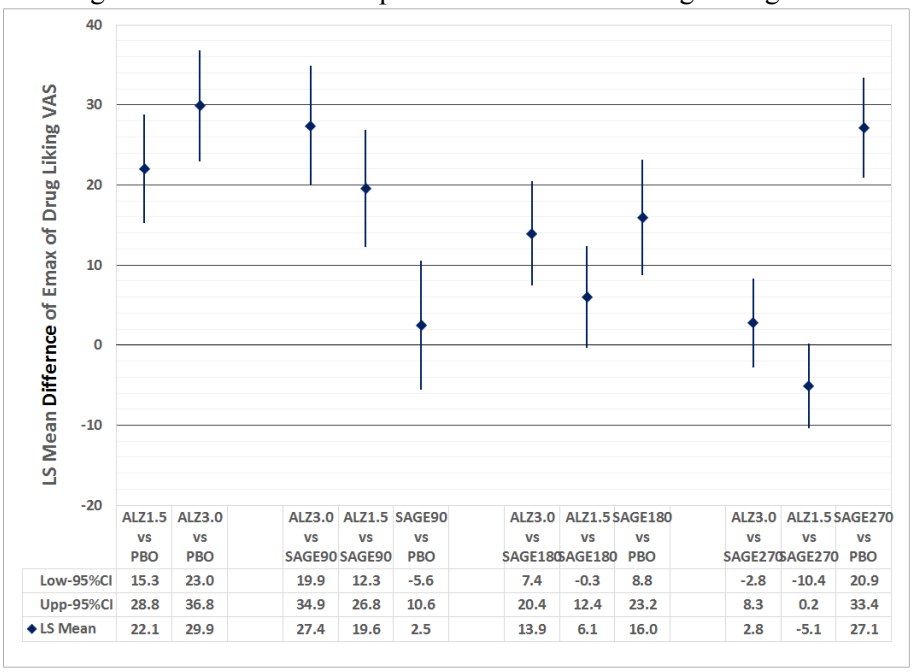


Table 4 displays the primary analysis results based on the PD population. Compare with Table 3, the significant differences are a). The mean produced by 180 µg/kg of SAGE-547 were significantly lower than those produced by 1.5 mg of alprazolam for Emax of Drug Liking; b). The mean produced by 270 µg/kg of SAGE-547 was significantly larger than 1.5 mg of alprazolam. According to FDA guidance, this review's analyses results were based on the completer population.

Table 4: Statistical analysis results for Drug Liking VAS (PD population)

Statistic	SAGE90 N=32	SAGE180 N=32	SAGE270 N=32	ALZ1.5 N=33	ALZ3.0 N=31	PBO N=30
Emax						
Mean (STD)	65.1 (17.3)	75.2 (16.0)	87.3 (12.7)	82.4 (16.0)	88.4 (16.5)	58.7 (13.0)
Median	60	73	88	78	100	51
Q1, Q3	51, 75	66.5, 81	77, 100	71, 100	73, 100	50, 100
Min, Max	50, 100	50, 100	51, 100	51, 100	50, 100	50, 100
LS mean (SE)	64.5 (3.3)	75.5 (2.9)	87.5 (2.2)	82.8 (2.5)	87.8 (3.0)	58.7 (3.1)
Treatment Comparisons						
Comparison	**LS Mean Difference (SE)**		**95% Confidence Interval**		**p-value**	
ALZ1.5 vs PBO	24.1 (3.1)		(17.9, 30.3)		<.0001	
ALZ3.0 vs PBO	29.1 (3.5)		(22.1, 36.1)		<.0001	
ALZ3.0 vs SAGE90	23.4 (3.8)		(31.0, 15.8)		<.0001	
ALZ1.5 vs SAGE90	18.3 (3.4)		(11.5, 25.2)		<.0001	
SAGE90 vs PBO	5.7 (3.8)		(-2, 13.4)		0.0717	
ALZ3.0 vs SAGE180	12.3 (3.3)		(5.7, 19.0)		0.0003	
ALZ1.5 vs SAGE180	7.3 (2.9)		(1.4, 13.1)		0.0080	
SAGE180 vs PBO	16.8 (3.4)		(10.0, 23.6)		<.0001	
ALZ3.0 vs SAGE270	0.3 (2.8)		(-5.4, 6.0)		0.4564	
ALZ1.5 vs SAGE270	-4.8 (2.2)		(-9.4, -0.1)		0.9778	
SAGE270 vs PBO	28.8 (2.9)		(23.0, 34.6)		<.0001	

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances

2.2.2.5 Secondary Analyses

Per the CSS reviewer Dr. Shalini Bansil's requests, the reviewer's secondary analyses included abuse potential measures: High VAS, Good Effects, Bad Effects, Overall Drug Liking VAS, and Take Drug Again VAS. All analyses were based on the completer population.

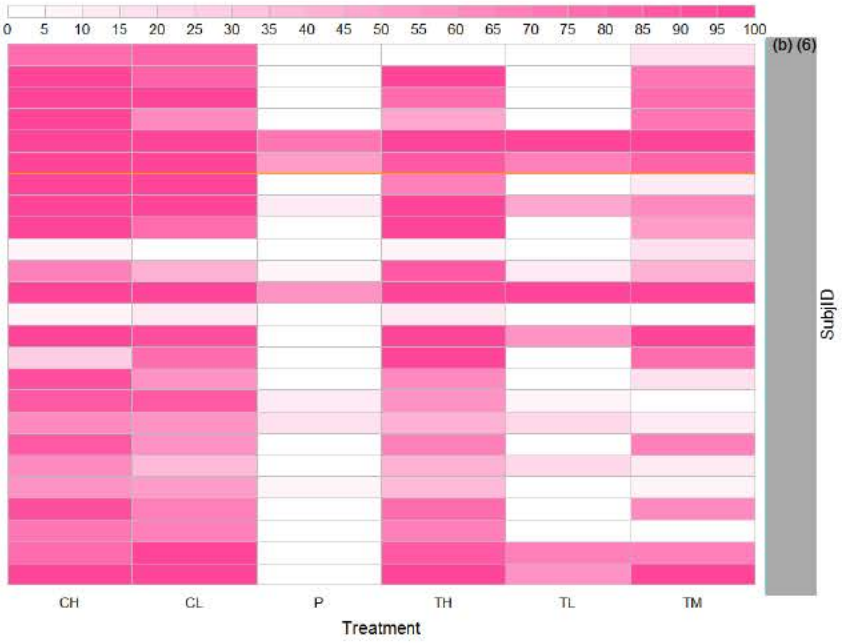
The same methodologies as the primary analysis were used in the secondary analyses. Among five secondary measures, the normal assumption of the model is satisfied for Good Effects VAS, and High VAS. The pairwise comparisons were assessed using paired T-test or Sign-test for those secondary endpoints which the normal assumption of the model is no satisfied. The 95% confidence intervals were the distribution free 95% confidence limits.

2.2.2.5.1 Descriptive Statistics

High VAS

High Effects VAS in on a unipolar scale; a score of 0 indicates feeling not at all high and a score of 100 indicates feeling extremely high. As shown in the heat map (Figure 11), the Emax of high VAS had the similar pattern as that for the Emax of Drug Liking VAS (Figure 8).

Figure 11: Treatment Comparison for High



Good Effect and Bad Effect VAS

Good Effects VAS is on a unipolar scale; a score of 0 indicates feeling good effects not at all and a score of 100 indicates feeling good effects extremely. Bad Effects VAS is on a unipolar scale; a score of 0 indicates feeling bad effects not at all and a score of 100 indicates feeling bad effects extremely.

Before making conclusion, the reviewer examined data from SAGE-547 270 µg/kg as well as alprazolam 3 mg for Good Effect VAS and Bad Effect VAS using bar plots since the high dose of the test drug is the big concern based on the primary analysis.

The bar plot compares the Emax of Good Effects VAS and Emax of Bad Effects VAS for individual subjects on the same plot (Figure 12 and Figure 13). The light blue indicates Emax of Good Effects VAS, and the darker color indicates Emax of Bad Effects VAS. Each subject has two bars standing one in front of the other on the graph. If one bar is higher than the other, this bar is put behind the other bar. For example, Subject #20 had 100 and 46 for Emax of Good Effects VAS and Emax of Bad Effects VAS, respectively. The graph shows the bar for Good Effects VAS behind that for Bad Effects VAS. If only one color shows on the bar, it means that either the other Emax is zero or the values of two Emaxs are the same. For identifying the latter case, a star is marked on the bar.

From Figure 12 for alprazolam 3.0 mg, one may see that approximately 72% (18/25) subjects had score 80 or above, and 60% (15/25) had at least 90 for Emax of Good Effects VAS. As shown in Figure 13, approximately 56% (14/25) subjects did not experience any bad effects from SAGE-547 270 µg/kg. For those experienced bad effects, only 2 of them had larger bad effects than good effects. Ten out of 25 subjects had Emax of Good Effects of 100. Therefore, more subjects showed large good effects and small bad effects for SAGE-547 270 µg/kg dose compared to their responses to alprazolam 3 mg.

Figure 12: Good Effects Emax versus Bad Effects Emax for Alprazolam 3 mg

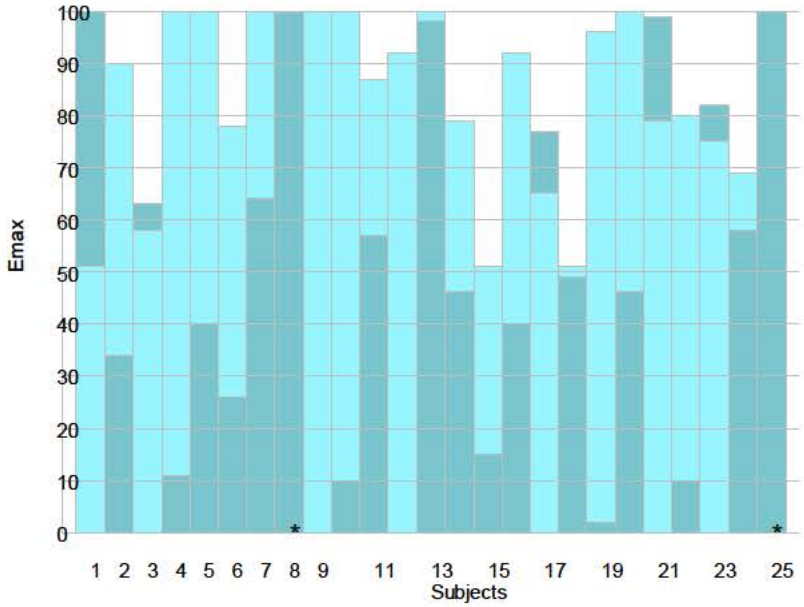
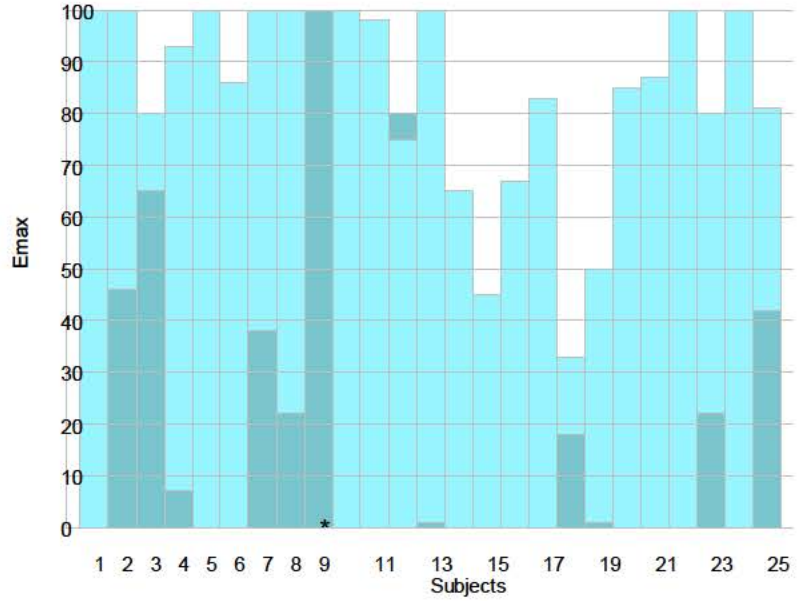


Figure 13: Good Effects Emax versus Bad Effects Emax for 270 µg/kg of SAGE-547



As shown in Figure 14, mean Good Effects VAS scores for the placebo condition were near 0 at all time points (range from 0 to 12.7). Mean Good Effects scores following administration of alprazolam (1.5 mg and 3.0 mg doses) increased rapidly, peaked around 2 to 4 hours and remained above 50 for 3 to 6 hours after dosing. For the 90 µg/kg dose of SAGE-547, mean scores peaked at 1 hour and were less than 30 at all evaluations. For the 180 and 270 µg/kg doses of SAGE-547, mean scores increased rapidly, were above 50 for 1 to 2 hours, and returned rapidly toward placebo levels.

As shown in Figure 15, mean of Bad Effects VAS scores were low following administration of all treatments. The highest mean of Bad Effects VAS score of 22.2 was observed for alprazolam 3.0 mg at 80 minutes after dosing. Note that the mean responses in mean time course profiles at early hours may not be based on 25 observations during to missing data occurred at early time points, especially, for alprazolam 3.0 mg.

Figure 14: Mean time course profiles for Good Effects VAS

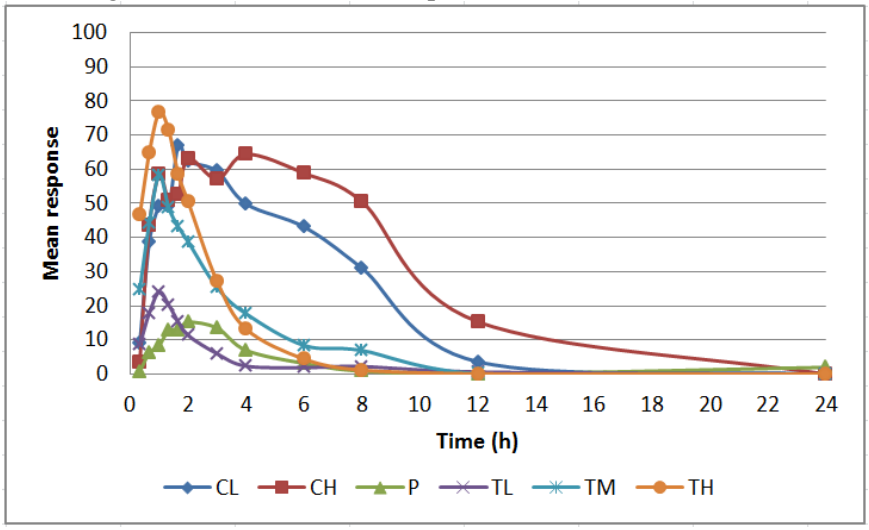
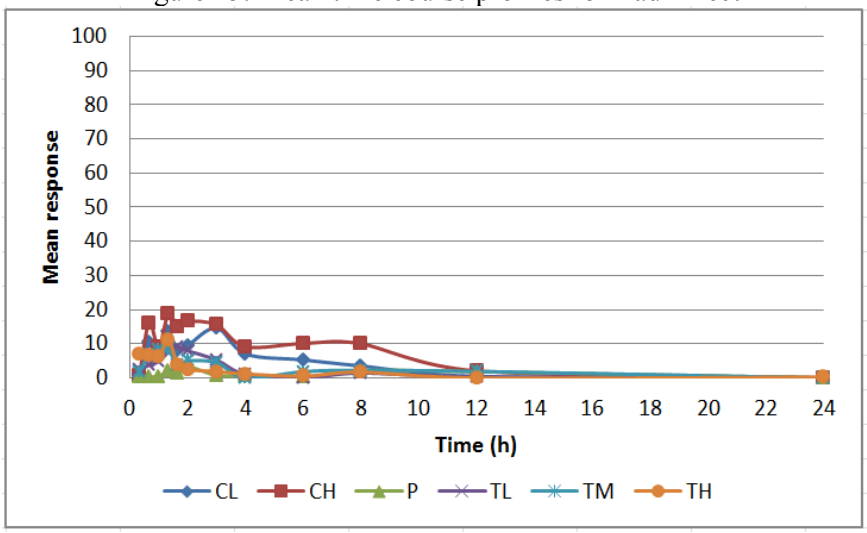


Figure 15: Mean time course profiles for Bad Effect



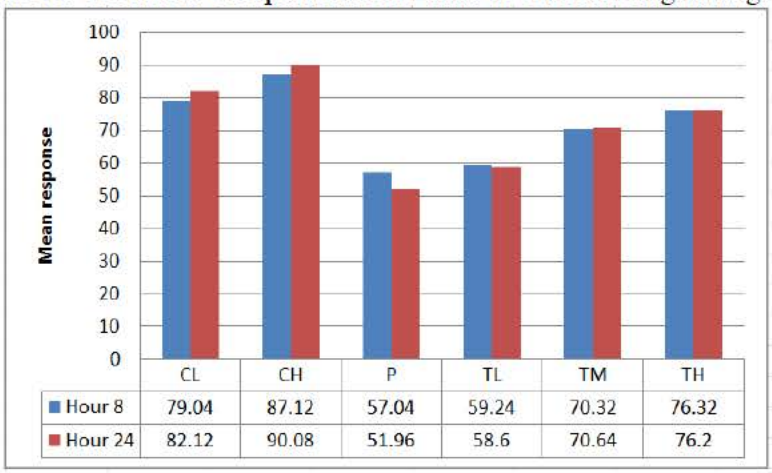
Overall Drug Liking VAS

Overall Drug Liking VAS is on a bipolar scale. The scores 0, 50 and 100 denote strong disliking, neither liking nor disliking, and strong liking, respectively.

The means for Overall Drug Liking VAS scores at hours 8 and 24 are presented in Figure 16. Mean of Overall Drug Liking scores following administration of alprazolam were greater than placebo at 8 and 24

hours. Mean of Overall Drug Liking scores for the SAGE-547 90 µg/kg and 180 µg/kg doses were less than those of both alprazolam doses at both time points assessed. Mean of Overall Drug Liking scores for the highest SAGE-547 dose (270 µg/kg) were like the lower alprazolam dose (1.5 mg).

Figure 16: Treatment Comparisons for Emax of Overall Drug Liking VAS

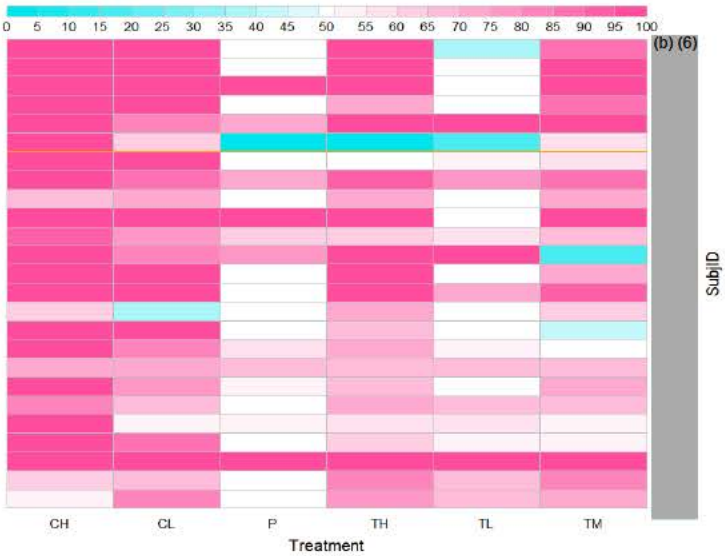


	CL	CH	P	TL	TM	TH
Hour 8	79.04	87.12	57.04	59.24	70.32	76.32
Hour 24	82.12	90.08	51.96	58.6	70.64	76.2

(Note: in the figure, CH=ALZ3.0, CL=ALZ1.5, P=PBO, TH=SAGE270, TL=SAGE90, and TM=SAGE180)

As showed in Figure 17, the Emax of Overall Drug Liking VAS had the similar pattern as that for the Emax of Drug Liking VAS (Figure 8).

Figure 17: Treatment Comparisons for Emax of Overall Drug Liking VAS



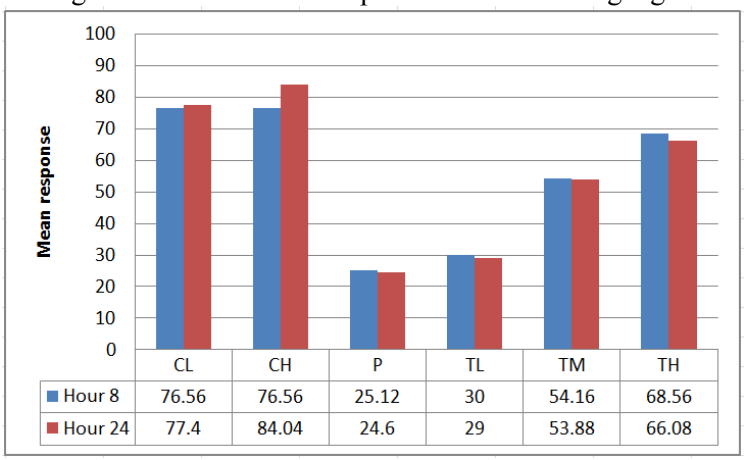
(Note: in the figure, CH=ALZ3.0, CL=ALZ1.5, P=PBO, TH=SAGE270, TL=SAGE90, and TM=SAGE180)

Take Drug Again VAS

Take Drug Again VAS is on a unipolar scale. The scores 0, 50, and 100 denote definitely not take drug again, not sure take drug again, and definitely take drug again, respectively.

Mean of Take Drug Again scores following administration of alprazolam were larger than for placebo at hours 8 and 24. The mean of Take Drug Again VAS scores for all doses of SAGE-547 were less than both doses of alprazolam, but greater than placebo. The score for the highest SAGE-547 dose (270 µg/kg) approached the score for the lowest alprazolam dose (1.5 mg) (Figure 18).

Figure 18: Treatment Comparison for Take Drug Again



(Note: in the figure, CH=ALZ3.0, CL=ALZ1.5, P=PBO, TH=SAGE270, TL=SAGE90, and TM=SAGE180)

2.2.2.5.2 Inferential Statistics

Tables 5 – 9 are summary statistics for 6 treatments in the study and for the treatment differences between SAGE-547 and alprazolam (or placebo) for Emaxs of six selected secondary endpoints (High VAS, Good Effects, Bad Effects, Overall Drug Liking VAS, and Take Drug Again VAS). Some descriptive figures are presented in Appendix.

Table 5: Summary Statistics and Testing Results for Emax of High VAS

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (STD)	23.2 (33.0)	49.4 (35.6)	69.5 (31.1)	73.3 (28.0)	79.0 (27.9)	9.8 (20.1)
Median	2	64	78	80	91	0
Q1, Q3	0, 50	14, 76	50, 100	58, 100	70, 100	0, 7
Min, Max	0, 100	0, 100	1, 100	0, 100	8, 100	0, 73
LS mean (SE)	20.0 (6.8)	47.1 (6.5)	67.2 (6.2)	70.7 (5.3)	76.4 (5.7)	8.0 (6.4)
Treatment Comparisons						
Comparison	**LS Mean Difference (SE)**		**95% Confidence Interval**		**p-value**	
ALZ1.5 vs PBO	62.7 (5.2)		(52.2, 73.2)		<.0001	
ALZ3.0 vs PBO	68.4 (5.6)		(57.1, 79.7)		<.0001	
ALZ3.0 vs SAGE90	56.4 (6.0)		(43.9, 68.8)		<.0001	
ALZ1.5 vs SAGE90	50.7 (5.6)		(39.1, 62.3)		<.0001	
SAGE90 vs PBO	12.1 (6.6)		(-1.7, 25.8)		0.0418	
ALZ3.0 vs SAGE180	29.3 (5.7)		(17.7, 40.8)		<.0001	
ALZ1.5 vs SAGE180	23.6 (5.3)		(12.7, 34.5)		<.0001	
SAGE180 vs PBO	39.1 (6.3)		(26.3, 52.0)		<.0001	
ALZ3.0 vs SAGE270	9.2 (5.3)		(-1.7, 20.0)		0.0480	
ALZ1.5 vs SAGE270	3.5 (4.8)		(-6.5, 13.5)		0.2397	
SAGE270 vs PBO	59.2 (6.0)		(47.0, 71.5)		<.0001	

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances

Table 6: Summary Statistics and Testing Results for Emax of Good Effects VAS

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (STD)	29.5 (34.0)	68.2 (30.9)	84.3 (19.3)	80.7 (23.5)	83.7 (17.5)	21.4 (30.4)
Median	17	74	87	93	90	4
Q1, Q3	0,60	56, 100	80, 100	68, 100	75, 100	0, 100
Min, Max	0, 100	0, 100	33, 100	25, 100	51, 100	0, 100
LS mean (SE)	29.1 (6.5)	68.9 (5.4)	85.0 (4.3)	80.9 (4.8)	84.7 (4.0)	21.5 (6.0)
Treatment Comparisons						
Comparison	**LS Mean Difference (SE)**		**95% Confidence Interval**		**p-value**	
ALZ1.5 vs PBO	59.4 (6.3)		(46.5, 72.3)		<.0001	
ALZ3.0 vs PBO	63.2 (5.8)		(51.3, 75.0)		<.0001	
ALZ3.0 vs SAGE90	55.5 (6.3)		(42.7, 68.4)		<.0001	
ALZ1.5 vs SAGE90	51.8 (6.7)		(38.0, 65.5)		<.0001	
SAGE90 vs PBO	7.6 (7.7)		(-7.9, 23.2)		0.1635	
ALZ3.0 vs SAGE180	15.78 (5.2)		(5.2, -26.3)		0.0023	
ALZ1.5 vs SAGE180	12.0 (5.8)		(0.3, 23.8)		0.0224	
SAGE180 vs PBO	47.4 (6.8)		(33.6, 61.2)		<.0001	
ALZ3.0 vs SAGE270	-0.4 (4.0)		(-8.7, 7.9)		0.5353	
ALZ1.5 vs SAGE270	-4.1 (4.7)		(-13.8, 5.6)		0.8034	
SAGE270 vs PBO	63.5 (6.0)		(51.3, 75.7)		<.0001	

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances

Table 7: Summary Statistics and Testing Results for Emax of Bad Effects VAS

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (STD)	12.6 (29.7)	17.4 (28.3)	17.7 (28.5)	36.7 (34.3)	49.1 (34.6)	6.4 (16.1)
Median	0	1	0	28	46	0
Q1, Q3	0, 5	0, 25	0, 22	7, 54	15, 77	0, 0
Min, Max	0, 100	0, 100	0, 100	0, 100	0, 100	0, 50
Treatment Comparisons [a]						
Comparison	**Mean (STD)/ Median (Q1, Q3)**			**95% Confidence Interval**		**p-value**
ALZ1.5 vs PBO	30.3 (39.5) [T]			(14.0, 46.6)		0.0004
ALZ3.0 vs PBO	42.7 (33.7) [T]			(28.8, 56.6)		<.0001
ALZ3.0 vs SAGE90	35 (10, 58) [S]			(11, 47)		<.0001
ALZ1.5 vs SAGE90	24.1 (43.5) [T]			(6.2, 42.1)		0.0053
SAGE90 vs PBO	0 (0, 0) [S]			(0, 0)		0.5078
ALZ3.0 vs SAGE180	31.7 (43.8) [T]			(13.6, 49.8)		0.0007
ALZ1.5 vs SAGE180	19.3 (41.0) [T]			(2.4, 36.2)		0.0135
SAGE180 vs PBO	0, (0, 7) [S]			(0, 2)		0.0225
ALZ3.0 vs SAGE270	40 (10, 58) [S]			(10, 58)		0.0009
ALZ1.5 vs SAGE270	19.0 (37.2) [T]			(3.6, 34.4)		0.0087
SAGE270 vs PBO	0 (0, 15) [S]			(0, 7)		0.1796

Note: a: T indicated the pairwise comparisons were assessed using paired T-Test and S indicated the pairwise comparisons were assessed using Sign-test P-value was 1-sided p-value

Table 8: Summary Statistics and Testing Results for Emax of Overall Drug Liking VAS

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (SD)	59.6 (19.6)	73.0 (21.0)	78.4 (22.9)	84.0 (16.9)	91.6 (15.4)	58.7 (20.9)
Median	51	72	75	84	100	50
Q1, Q3	50, 68	60, 87	69, 100	74, 100	92, 100	50, 66
Min, Max	16, 100	19, 100	0, 100	39, 100	51, 100	0, 100
Treatment Comparisons [a]						
Comparison	**Mean (STD)/ Median (Q1, Q3)**			**95% Confidence Interval**		**p-value**
ALZ1.5 vs PBO	24 (6, 50) [S]			(11, 50)		<.0001

ALZ3.0 vs PBO	32 (13, 50) [S]	(18, 50)	<.0001
ALZ3.0 vs SAGE90	32.0 (25.4) [T]	(21.5, 42.5)	<.0001
ALZ1.5 vs SAGE90	24.4 (24.7) [T]	(14.12, 34.6)	<.0001
SAGE90 vs PBO	0 (0, 16) [S]	(0, 4)	0.2379
ALZ3.0 vs SAGE180	18.6 (25.4) [T]	(8.1, 29.1)	0.0006
ALZ1.5 vs SAGE180	5 (0, 13) [S]	(1, 13)	0.0015
SAGE180 vs PBO	14.2 (23.9) [T]	(4.4, 24.1)	0.0033
ALZ3.0 vs SAGE270	0 (0, 3) [S]	(0, 25)	0.0768
ALZ1.5 vs SAGE270	0 (-4, 14) [S]	(0, 7)	0.4807
SAGE270 vs PBO	20 (3, 26) [S]	(7, 25)	<.0001

Note: a: T indicated the pairwise comparisons were assessed using paired T-Test and S indicated the pairwise comparisons were assessed using Sign-test P-value was 1-sided p-value

Table 9: Summary Statistics and Testing Results for Emax of Take Drug Again VAS

Statistic	SAGE90 N=25	SAGE180 N=25	SAGE270 N=25	ALZ1.5 N=25	ALZ3.0 N=25	PBO N=25
Emax						
Mean (SE)	34.6 (7.734)	57.08 (7.75)	69.32 (6.74)	81.6 (5.43)	85.88 (4.99)	25.52 (7.46)
Median	25	66	73	100	100	0
Q1, Q3	0, 55	17, 100	50, 100	71, 100	70, 100	0, 52
Min, Max	0, 100	0, 100	0, 100	0, 100	0, 100	0, 100

Treatment Comparisons [a]			
Comparison	**Mean (STD)/ Median (Q1, Q3)**	**95% Confidence Interval**	**p-value**
ALZ1.5 vs PBO	50 (17, 100) [S]	(34, 100)	<.0001
ALZ3.0 vs PBO	54 (45, 100) [S]	(45, 100)	<.0001
ALZ3.0 vs SAGE90	49 (10, 100) [S]	(11, 100)	<.0001
ALZ1.5 vs SAGE90	50 (0, 100) [S]	(1, 100)	0.0106
SAGE90 vs PBO	8 (0, 36) [S]	(0, 35)	0.0127
ALZ3.0 vs SAGE180	14 (0, 83) [S]	(0, 33)	0.0007
ALZ1.5 vs SAGE180	14 (0, 36) [S]	(0, 29)	0.0192
SAGE180 vs PBO	31.6 (43.0) [T]	(13.8, 49.3)	0.0006
ALZ3.0 vs SAGE270	16.6 (39.2) [T]	(0.4, 32.7)	0.0226
ALZ1.5 vs SAGE270	12.3 (36.7) [T]	(-2.9, 27.4)	0.0536
SAGE270 vs PBO	38 (16, 73) [S]	(16, 72)	<.0001

Note: a: T indicated the pairwise comparisons were assessed using paired T-Test and S indicated the pairwise comparisons were assessed using Sign-test P-value was 1-sided p-value

Table 10 summaries the treatment comparison results for the abuse potential measures reviewed. The sign (>) shows that on the average, A was greater than B. The (<) sign denotes that on the average, A was smaller than B. S and NS note significant difference and nonsignificant difference, respectively.

Table 10: Summary of the Results from Significance Tests for the Abuse Potential Measures Reviewed

Treatment Comparison	Drug Liking [a]	Overall Drug Liking [b]	High [a]	Good Effects [a]	Bad Effects [b]	Take Drug Again [b]
ALZ1.5 vs PBO	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
ALZ3.0 vs PBO	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
ALZ3.0 vs SAGE90	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
ALZ1.5 vs SAGE90	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
SAGE90 vs PBO	NS (>)	NS (>)	NS (>)	NS (>)	NS (>)	S (>)
ALZ3.0 vs SAGE180	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
ALZ1.5 vs SAGE180	NS (>)	S (>)	S (>)	S (>)	S (>)	S (>)
SAGE180 vs PBO	S (>)	S (>)	S (>)	S (>)	S (>)	S (>)
ALZ3.0 vs SAGE270	NS (>)	NS (>)	NS (>)	NS (<)	S (>)	S (>)
ALZ1.5 vs SAGE270	NS (<)	NS (>)	NS (>)	NS (<)	S (>)	NS (>)
SAGE270 vs PBO	S (>)	S (>)	S (>)	S (>)	NS (>)	S (>)

Note: a: based on mixed model; b: based on sign-test or paired t-test.

2.2.3 Conclusion

As showed in Table 10, this study demonstrated that:

- The mean of Emax for Drug Liking was significantly higher for alprazolam 1.5 mg and 3.0 mg compared to placebo, thereby establishing the study validity.

- On the average, the responses to SAGE-547 90 μg/kg were significantly lower than those to both doses of alprazolam for the primary and five selected secondary endpoints. There was no significant mean (or median) difference between SAGE-547 90 μg/kg and placebo.

- On the average, the responses to SAGE-547 180 μg/kg were significantly lower than those to both doses of alprazolam for the primary and five selected secondary endpoints except the primary endpoint for the comparison of SAGE-547 180 μg/kg versus 1.5 mg alprazolam (p=0.029). However, the responses to SAGE-547 180 μg/kg were significantly larger than those to placebo.

- On the average, the responses to SAGE-547 270 μg/kg were not significantly different from those to both doses of alprazolam for the primary endpoint and selected secondary endpoints except Bad Effects Emax. SAGE-547 270 μg/kg had significantly lower mean of Bad Effects Emax compared to both doses of alprazolam.

In summary, the therapeutic dose of SAGE-547 (90 μg/kg) is not euphoric, and has less liking, and high as well as take drug again compared to alprazolam. However, the mid dose of SAGE-547 showed significantly higher mean response to placebo, and the high dose of SAGE-547 did not show significantly lower mean response to alprazolam 3.0 mg for the primary endpoint and the selected secondary endpoints. The rapidly raising mean response of SAGE-547 at hour 0.5 and less sedative effects raise more concern of the abuse potential of SAGE-547. Based on both the primary and secondary analyses, this reviewer concludes that the abuse potential of SAGE-547 may not be lower than alprazolam.

3 REFERENCE

Chen, L, Wang, Y. Heat Map Displays for Data from Human Drug Abuse Potential Crossover Studies. *Drug Information Journal.* (2012) 46:6, 701-707.

4 APPENDIX

Table 11: Demographics and Baseline Characteristics (Completer Population, Treatment Phase)

	Treatment Sequence						
Characteristics	**ABCDEF N=6**	**BCDEFA N=3**	**CDEFAB N=5**	**DEFABC N=3**	**EFABCD N=4**	**FABCDE N=4**	**OVERALL N=25**
Gender, N (%)							
Male	5 (83.3)	2 (66.7)	4 (80.0)	2 (66.7)	3 (75.0)	3 (75.0)	19 (76.0)
Female	1 (16.67)	1 (33.3)	1 (20.0)	1 (33.3)	1 (25.0)	1 (25.0)	6 (24.0)
Age (years)							
Mean (SD)	37.0 (8.0)	43.0 (12.2)	36.2 (4.0)	38.7 (6.5)	40.8 (6.0)	41.0 (12.8)	39.0 (7.9)
Min, Max	28, 50	29.0, 51.0	30.0, 40.0	32.0, 45.0	33.0, 46.0	29.0, 53.0	28.0, 53.0
Median	36.5	49.0	37.0	39.0	42.0	41.0	39.0
Race, N (%)							
White	4 (66.7)	3 (100)	4 (80.0)	1 (33.3)	4 (100)	3 (75.0)	19 (76.0)
Black/African American	2 (33.3)	0	1 (20.0)	1 (33.3)	0	0	2 (8.0)
Other	0	0	0	1 (33.3)	0	1 (25.0)	4 (16.0)
Ethnicity, N (%)							
Hispanic/Latino	1 (16.7)	0	1 (20.0)	0	0	0	2 (8.0)
Not Hispanic/Latino	5 (83.3)	3 (100)	4 (80.0)	3 (100)	4 (100)	4 (100)	23 (92.0)
Height (cm)							
Mean (SD)	172.2 (10.2)	168.5 (9.2)	178.4 (10.4)	172.1 (11.1)	173.0 (6.0)	172.0 (6.4)	173.1 (8.7)
Min, Max	157.5, 185.6	163.0, 179.1	162.5, 187.4	160.8, 182.9	166.4, 178.6	165.0, 179.1	157.5, 187.4
Median	173.2	163.4	183.8	172.7	173.5	172.0	173.5
Body Weight (kg)							
Mean (SD)	77.1 (11.3)	71.4 (15.1)	88.5 (22.2)	81.8 (11.8)	77.5 (7.6)	77.2 (9.2)	79.3 (13.6)
Min, Max	62.7, 92.1	56.2, 86.3	57.2, 107.9	74.5, 95.4	70.8, 85.9	66.9, 88.1	56.2, 107.9
Median	74.8	71.8	96.4	75.6	76.7	76.8	75.6
Body Mass Index (kg/m2)							
Mean (SD)	26.2 (4.3)	25.0 (3.4)	27.3 (4.1)	27.6 (2.3)	26.0 (2.6)	26.1 (2.3)	26.4 (3.2)
Min, Max	19.9, 31.2	21.0, 27.0	21.7, 31.2	25.0, 29.2	22.4, 28.5	23.7, 28.4	19.9, 31.2
Median	27.4	26.9	28.5	28.5	26.5	25.1	27.0

Table 12: Statistical Analysis Results for Emax of Drug Liking (Completer Population)

Statistic	**SAGE-547 90 µg/kg/IV N=25**	**SAGE-547 180 µg/kg/IV N=25**	**SAGE-547 270 µg/kg/IV N=25**	**Alprazolam 1.5 mg N=25**	**Alprazolam 3.0 mg N=25**	**Placebo N=25**
Emax						
Mean (STD)	62.8 (16.4)	75.7 (16.5)	86.92 (13.3)	82.1 (16.6)	89.5 (15.6)	59.8 (13.7)
Median	55	72	90	76	100	51
Q1, Q3	51, 69	67, 82	76, 100	71, 100	75, 100	50, 69
Min, Max	50, 100	50, 100	51, 100	51, 100	51, 100	50, 100
LS mean (SE)	53.3 (8.2)	68.5 (6.2)	89.4 (5.3)	87.4 (6.4)	100.8 (5.5)	57.3 (6.0)

Treatment Comparisons			
Comparison	**LS Mean Difference (SE)**	**95% Confidence Interval**	**p-value**
ALZ1.5 vs PBO	30.1 (8.7)	(12.5, 47.7)	0.0006
ALZ3.0 vs PBO	43.5 (7.8)	(27.9, 59.1)	<.0001
ALZ3.0 vs SAGE90	47.5 (10.3)	(26.5, 68.5)	<.0001
ALZ1.5 vs SAGE90	34.1 (11.1)	(11.8, 56.5)	0.0018
SAGE90 vs PBO	-4.0 (10.7)	(-25.7, 17.8)	0.6436
ALZ3.0 vs SAGE180	32.3 (8.0)	(16.1, 48.4)	0.0001
ALZ1.5 vs SAGE180	18.9 (8.9)	(0.9, 36.9)	0.0202
SAGE180 vs PBO	11.2 (8.5)	(-5.9, 28.4)	0.0967
ALZ3.0 vs SAGE270	11.4 (7.0)	(-2.9, 25.6)	0.0570
ALZ1.5 vs SAGE270	-2.0 (8.1)	(-18.6, 14.6)	0.5945
SAGE270 vs PBO	32.1 (7.6)	(16.8, 47.5)	<.0001

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances
Note: The model included the first order carryover effect

Table 13: Statistical Analysis Results for Emax of Drug Liking
(PD Population in the First Period Only)

Statistic	SAGE-547 90 µg/kg/IV N=7	SAGE-547 180 µg/kg/IV N=7	SAGE-547 270 µg/kg/IV N=6	Alprazolam 1.5 mg N=7	Alprazolam 3.0 mg N=6	Placebo N=7
Emax						
Mean (STD)	62.9 (16.5)	69.0 (9.1)	86.5 (20.3)	80.6 (14.7)	93.5 (12.1)	57.4 (12.5)
Median	55	70	98	76	100	50
Q1, Q3	51, 75	65, 75	73, 100	69, 100	91, 100	50, 72
Min, Max	50, 94	51, 79	51, 100	62, 100	70, 100	50, 79
LS mean (SE)	62.9 (6.2)	69.0 (3.4)	86.5 (8.3)	80.6 (5.6)	93.5 (4.9)	57.4 (4.7)
Treatment Comparisons						
Comparison	**LS Mean Difference (SE)**		**95% Confidence Interval**		**p-value**	
ALZ1.5 vs PBO	23.1 (7.3)		(7.2, 39.1)		0.0042	
ALZ3.0 vs PBO	36.1 (6.8)		(21.0, 51.1)		0.0001	
ALZ3.0 vs SAGE90	30.6 (7.9)		(13.1, 48.2)		0.0014	
ALZ1.5 vs SAGE90	17.7 (8.4)		(-0.5, 35.9)		0.0279	
SAGE90 vs PBO	5.4 (7.8)		(-11.7, 22.6)		0.2508	
ALZ3.0 vs SAGE180	24.5 (6.0)		(11.0, 38.0)		0.0013	
ALZ1.5 vs SAGE180	11.6 (6.5)		(-3.0, 26.2)		0.0538	
SAGE180 vs PBO	11.6 (5.9)		(-1.3, 24.4)		0.0367	
ALZ3.0 vs SAGE270	7.0 (9.6)		(-15.2, 29.2)		0.2441	
ALZ1.5 vs SAGE270	-5.9 (10.0)		(-28.5, 16.7)		0.7163	
SAGE270 vs PBO	29.1 (9.5)		(7.1, 51.0)		0.0079	

Note: All p-values were from the one-sided t test with 0 025 alpha level, and adjusted by Tukey -Kramer's method for unequal variances

Figure 19: Mean time course profiles for High VAS

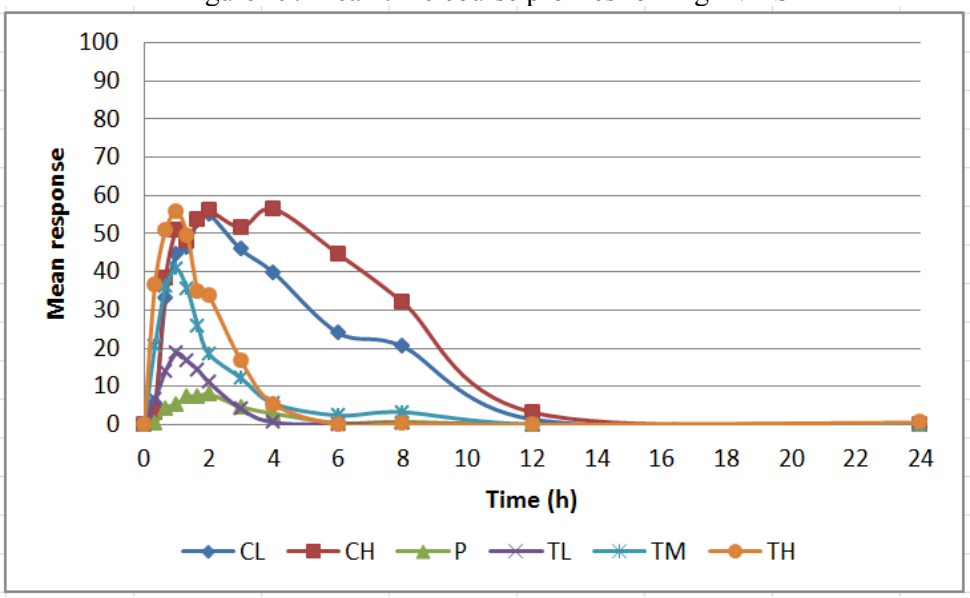


Figure 20: Treatment Comparison for Good Effects Emax

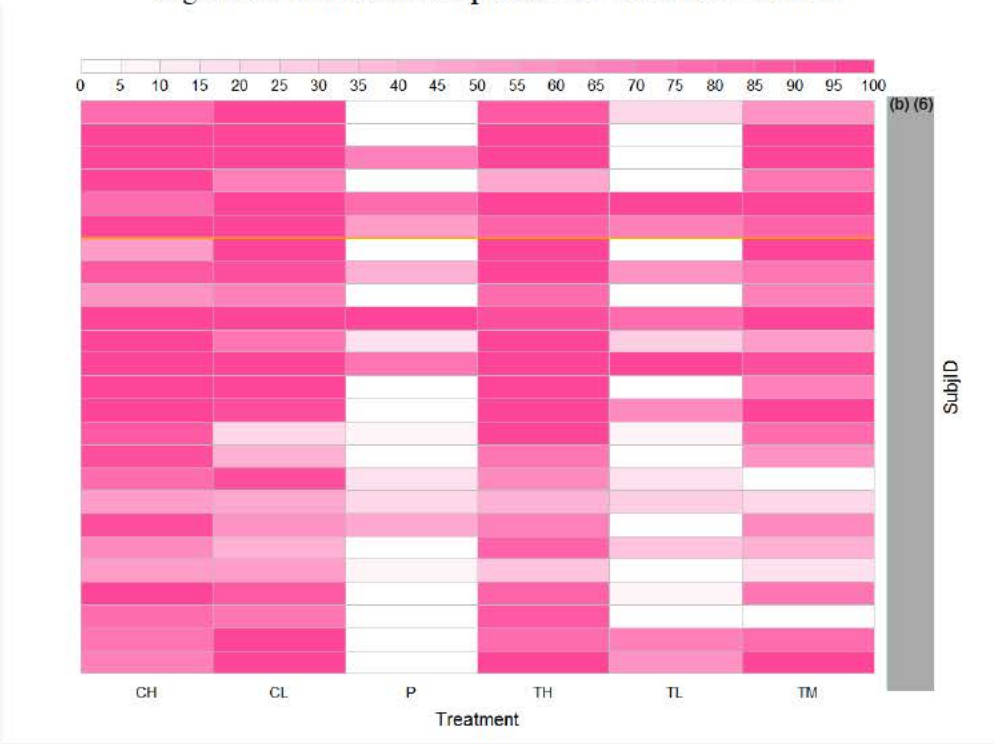


Figure 21: Treatment Comparison for Bad Effects Emax

Figure 22: Treatment Comparison for Take Drug Again

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/s/

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