CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216403Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Application Information

Application type	NDA
Application number(s)	216403
Priority or standard	Priority
Submit date(s)	3/17/2022
Received date(s)	3/17/2022
PDUFA goal date	2/17/2023
Division/office	Division of Cardiology and Nephrology (DCN)
Review completion date	2/16/2023
Established/proper name	sparsentan
(Proposed) proprietary name	Filspari
Pharmacologic class	endothelin and angiotensin II receptor antagonist
Other product name(s)	RE-021
Applicant	Travere Therapeutics, Inc.
Dosage form(s)/formulation(s)	tablets
Dosing regimen	Initiate treatment with Filspari at 200 mg once daily. After 14 days, increase to the recommended dose of 400 mg once daily, as tolerated.
Applicant-proposed indication(s)/population(s)	To reduce proteinuria in adults aged 18 years and older for treatment of primary immunoglobulin A nephropathy (IgAN) at risk for disease progression
SNOMED CT code for proposed indication disease term(s)[1]	Primary immunoglobulin A nephropathy (disorder) – SCTID 68779003|
Regulatory action	Accelerated approval
Approved dosage (if applicable)	200 mg and 400 mg tablets
Approved indication(s)/population(s) (if applicable)	Filspari is indicated to reduce proteinuria in adults with primary immunoglobulin A nephropathy (IgAN) at risk of rapid disease progression, generally a urine protein to creatinine ratio (UPCR) ≥1.5 g/g. This indication is approved under accelerated approval based on a reduction of. It has not been established whether Filspari slows kidney function decline in patients with IgAN. Continued approval for this indication may be contingent upon verification and description of clinical benefit in a confirmatory clinical trial.
SNOMED CT code for approved indication disease term(s)[1]	Primary immunoglobulin A nephropathy (disorder)

[1] For internal tracking purposes only.
Abbreviations: PDUFA, Prescription Drug User Fee Act; SNOMED CT, Systematized Nomenclature of Medicine Clinical Terms

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Glossary

ACE	angiotensin-converting enzyme
ACEI	angiotensin-converting enzyme inhibitor
ADME	absorption, distribution, metabolism, and excretion
AE	adverse event
AESI	adverse event of special interest
AKI	acute kidney injury
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ARA	acid reducing agents
ARB	angiotensin receptor blocker
AST	aspartate aminotransferase
AT_1	angiotensin II type 1
AT_1R	angiotensin II type 1 receptor
ATS	Antithymocyte Serum
AUC	area under the concentration-time curve
AUC_{AD}	AUC calculated using average dose
AUC_{MD}	AUC calculated using maximum dose
BCRP	breast cancer resistance protein
BLA	biologics license application
BMI	body mass index
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CKD	chronic kidney disease
CL	clearance
CL/F	apparent clearance
C_{max}	maximum plasma concentration
CrCL	creatine clearance
DBP	diastolic blood pressure
DDI	drug-drug interaction
DHN	Division of Hepatology and Nutrition
DILI	drug-induced liver injury
DMC	data monitoring committee
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
E-R	exposure-response
ERA	endothelin receptor antagonist
ET_A	endothelin type A
ET_AR	endothelin type A receptor
FAS	full analysis set
FDA	Food and Drug Administration
FMQ	FDA Medical Dictionary for Regulatory Activities query
FSGS	focal segmental glomerulosclerosis
GCP	good clinical practice

GFR	glomerular filtration rate
GLP	good laboratory practice
GOF	goodness of fit
HDL	high-density lipoprotein
HLM	human liver microsomes
HR	heart rate
HV	healthy volunteer
IA	interim analysis
IAS	interim analysis set
IC_{50}	half maximal inhibitory concentration
IgAN	immunoglobulin A nephropathy
IND	investigational new drug
IRB/IRC	Institutional Review Board/Independent Ethics Committee
IV	intravenous
KA	absorption rate constant
K_i	inhibition constant
k_{inact}	maximal rate of inactivation
K_I	half of the maximal rate of inactivation
MI	multiple imputation
MRHD	maximum recommended human dose
NDA	new drug application
NOAEL	no observed adverse effect level
NR	normal range
NSAIDs	nonsteroidal anti-inflammatory drugs
PBPK	physiologically based pharmacokinetic
pcVPC	prediction-corrected visual predictive check
PI	Prescribing Information
PK	pharmacokinetic
PMR	postmarketing requirement
PP	per protocol
PT	preferred term
QD	once daily
RD	risk difference
REMS	risk evaluation and mitigation strategy
RI	renal impairment
SAE	serious adverse event
SAP	statistical analysis plan
TA	transaminase
TB	total bilirubin
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
ULN	upper limit of normal
UP/C	urine protein to creatinine ratio
U.S.	United States

I. Executive Summary

1. Summary of Regulatory Action

On March 17, 2022, Travere Therapeutics submitted a new drug application (NDA) for Filspari (sparsentan) to "reduce proteinuria in adults aged 18 years and older for treatment of primary immunoglobulin A nephropathy (IgAN) at risk for disease progression." The Applicant is seeking approval under the provisions of 21 CFR Part 314, Subpart H, utilizing proteinuria as a reasonably likely surrogate endpoint. Filspari is an endothelin and angiotensin II receptor antagonist. Per the Applicant, the endothelin type A receptor (ET_AR) and the angiotensin II type 1 receptor (AT_1R) mediate processes that lead to IgAN, such as hemodynamic actions and mesangial cell proliferation, increased expression, and activity of proinflammatory and profibrotic mediators, podocyte injury, and oxidative stress.

Overview of Disease and Available Therapies

IgAN is a serious kidney disease and an important cause of chronic kidney disease and kidney failure. Although the most common cause of primary glomerular disease worldwide, its prevalence varies by region and ethnicity, with the highest frequency observed in individuals of East Asian ancestry, followed by Caucasians. In the United States, it is estimated to affect approximately 169,000 individuals.

Clinically, IgAN is characterized by hematuria, varying degrees of proteinuria, and, in some patients, with progressive loss of kidney function leading to kidney failure. Treatment strategies include blood pressure control, inhibition of the renin-angiotensin system via maximally tolerated doses of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs), and lifestyle modification including weight reduction, exercise, smoking cessation, and dietary restriction. To date, one pharmacologic treatment, Tarpeyo (budesonide) delayed release capsules, a corticosteroid with systemic adverse effects, has been approved specifically for the treatment of IgAN. This product is approved under the accelerated approval pathway to reduce proteinuria (considered a reasonably likely surrogate for effects on the loss of kidney function) in adults with IgAN at risk of rapid disease progression. Dapagliflozin, a sodium-glucose cotransporter-2 inhibitor, was recently approved to reduce the risk of adverse kidney and cardiac outcomes in patients with chronic kidney disease at risk of progression and may be increasingly used in patients with IgAN moving forward.

Data Supporting Efficacy

The Applicant has submitted the results of an interim analysis of an ongoing, adequate, and well-controlled phase 3 study in adults with biopsy-verified primary IgAN as principal support for effectiveness. The Applicant has also submitted the results of a phase 2 study, DUET, in patients with focal segmental glomerulosclerosis (FSGS), a rare glomerular disease, as confirmatory evidence of effectiveness; however, given the highly persuasive findings for proteinuria, the reasonably likely surrogate endpoint supporting accelerated approval, the review team has concluded that the data from this interim analysis are the scientific equivalent of data from two clinical investigations.

The PROTECT study is a randomized, double-blind, active-controlled, multicenter study in patients with biopsy-proven IgAN, estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2, and total urine protein ≥1.0 g/day on a maximized stable dose of RAS inhibitor treatment. A prespecified interim analysis of this study is being used to support accelerated approval; the same study will be used to verify and describe the clinical benefit.

Following discontinuation of ACE inhibitor and/or ARB therapy, patients were randomized (1:1) to either sparsentan (400 mg once daily following 200 mg once daily for 14 days) or irbesartan (300 mg once daily following 150 mg once daily for 14 days) for 110 weeks. The protocol prespecified an unblinded interim analysis performed 36 weeks after randomization of at least 280 subjects to evaluate the primary efficacy endpoint (i.e., endpoint for accelerated approval) based on change in proteinuria at Week 36. The confirmatory endpoint will assess for an effect on the loss of kidney function (eGFR) over 110 weeks following initiation of randomized therapy and will be evaluated in the full study population.

PROTECT met its primary endpoint of the relative change from baseline in urine protein to creatinine ratio (UP/C) at Week 36. The geometric mean ratio of UP/C at 36 weeks relative to baseline was 35% lower (95% CI: 23% to 45% lower) for the sparsentan arm compared to the irbesartan arm ($p<0.0001$). Efficacy findings were consistent across key subgroups, including key demographic and baseline disease characteristics (e.g., baseline proteinuria).

Safety

FDA's safety evaluation focused on the 404 patients (202 sparsentan, 202 irbesartan) who received at least one dose of study treatment up to the interim data lock date in the PROTECT study. Up to this date, the median duration of exposure to sparsentan was 73 weeks. Data from two ongoing studies in patients with FSGS were also used to assess for a signal for hepatotoxicity.

Sparsentan is an antagonist of the ET_AR and the AT_1R and the results of safety analyses were as a whole consistent with risks that might be expected given the known risks of endothelin and angiotensin II receptor antagonists. Common adverse reactions that were reported in PROTECT at a numerically greater incidence in the sparsentan as compared to irbesartan groups included peripheral edema, hypotension, dizziness, hyperkalemia, anemia, acute kidney injury and transaminase elevations (alanine aminotransferase [ALT] or aspartate aminotransferase [AST]). A total of 8% of sparsentan-treated patients discontinued drug due to adverse events; most of the adverse events that led to discontinuation of study drug were consistent with expected risks of sparsentan.

Some endothelin receptor antagonists can cause severe liver injury including liver failure. A case level analysis of potential drug-induced liver injury (DILI) cases in the PROTECT trial and two ongoing studies in patients with FSGS revealed eight cases that were categorized as either probably or possibly related to sparsentan. In general, these cases were characterized by elevations in AST or ALT, a long latency period after initiation of sparsentan, at least a partial resolution after discontinuation of sparsentan, and in most cases, reoccurrence after sparsentan was reinitiated. None of the cases met Hy's Law criteria. Animal reproduction studies also indicate that sparsentan can cause fetal harm when administered to a pregnant patient.

Proteinuria as a Reasonably Likely Surrogate and Verifying the Benefit in the Postmarketing Setting

Kidney failure is associated with significant morbidity and mortality; however, it is also a late outcome of chronic kidney diseases. As such, surrogate endpoints are typically used to assess whether a drug is effective in reducing the risk of progression to kidney failure. In the current case, treatment effects on proteinuria are being used to support accelerated approval, and treatment effects on eGFR will be used to verify the clinical benefit in the postmarketing setting.[1] Data supporting the use of proteinuria as a reasonably likely surrogate endpoint in IgAN include epidemiologic data showing a strong and consistent relationship between the level and duration of proteinuria and loss of kidney function, as well as trial-level analyses of data from randomized controlled trials in IgAN showing an association between treatment effects on percent reduction of proteinuria and treatment effects on a composite of time to doubling of serum creatinine (which reflects a marked loss of kidney function), kidney failure, and death (Thompson et al. 2019).

Given the available data, the Division of Cardiology and Nephrology (the Division) accepts a substantial reduction in proteinuria as a reasonably likely surrogate endpoint in IgA nephropathy and as a basis for accelerated approval. To be granted accelerated approval, the magnitude of the effect on proteinuria in the premarket phase of the study should be sufficient to provide confidence that the postmarketing phase of the study is adequately powered to detect a treatment effect on the endpoint that will be used to verify the benefit in the postmarketing setting. The Division has also held that available eGFR data at the time of submission of an application for accelerated approval should be assessed to provide additional confidence that the postmarketing phase of the study is adequately powered to confirm the clinical benefit.

(b) (4)

PROTECT is also fully enrolled and, as such, is expected to complete in a timely manner following accelerated approval.

Conclusion

The review team believes that the submitted data provide substantial evidence of effectiveness in reducing proteinuria, a reasonably likely surrogate for a treatment's effect on kidney failure in patients with IgAN who are at high risk of disease progression. Although cases of severe DILI with sparsentan have not been observed in clinical trials, the size of the safety database is currently limited, cases of liver enzyme elevation have been observed with sparsentan, and some

[1] Treatment effects on the rate of loss of kidney function (eGFR) that are indicative of a progressive irreversible loss of kidney function can be used as a basis for full approval of drugs intended to treat chronic kidney disease. For example, in 2018, FDA approved a drug to slow kidney function decline in adults at risk of rapidly progressing autosomal dominant polycystic kidney disease based on evidence that the drug slowed the loss of kidney function in earlier and later stages of disease and that the benefit accrued over time (1 to 3 years). Based on such data, FDA concluded that the drug, when used chronically, would have a meaningful impact on the risk of progression to kidney failure (Otsuka 2018).

endothelin receptor antagonists (ERAs) can cause severe liver injury. Because of the uncertainties surrounding this potential risk and the need for monitoring, as well as the risk of birth defects, sparsentan will only be available through a restricted distribution program under a risk evaluation and mitigation strategy (REMS). Other identified risks can be adequately mitigated through labeling.

The Accelerated Approval Program allows for earlier approval of drugs that treat a serious condition and that provide a meaningful therapeutic benefit over existing treatment based on a reasonably like surrogate endpoint. IgAN is a serious disease, and the submitted data indicate the sparsentan provides a meaningful therapeutic benefit over existing treatment.[2] Given the currently available data on the efficacy and safety of the product and the intent of the Accelerated Approval Program, labeling will limit the indicated population to "adults with primary IgAN at risk of rapid disease progression, generally a UP/C ≥1.5 g/g." The available data support the conclusion that this population is at particular risk of rapid disease progression over a relatively short time frame and there is regulatory precedent for using this particular threshold to define a population at risk of rapid disease progression for the purpose of accelerated approval in this therapeutic area.

The Applicant will have a postmarketing requirement to conduct an adequate and well-controlled clinical trial to verify and describe the clinical benefit. This requirement will be addressed by the completion of the PROTECT study. The Applicant will also have a postmarketing requirement to conduct a prospective, single-arm safety study to assess and characterize the risk of drug-induced liver injury. In addition, other postmarketing requirements will be issued to further evaluate for drug-drug interaction liability.

[2] As noted in the Agency's guidance "Expedited Programs for Serious Conditions—Drugs and Biologics", a drug would not be considered available therapy if the drug is granted accelerated approval based on a surrogate endpoint and clinical benefit has not been verified by postapproval studies. As such, at this time, Tarpeyo (budesonide) delayed release capsules would not be considered available therapy for purposes of this approval pathway.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of condition	• Immunoglobulin A nephropathy (IgAN) is a rare, serious kidney disease that is estimated to affect approximately 169,000 individuals in the United States. IgAN can present at any age and has a peak incidence during the second and third decades of life. IgAN occurs with the greatest frequency in East Asians and Caucasians and is relatively rare in individuals of African ancestry. Patients tend to present with proteinuria and hematuria. IgAN is associated with high morbidity and mortality; approximately 50% of patients progress to kidney failure within 30 years of diagnosis. • IgAN is caused by the deposition of immune complexes containing galactose-deficient immunoglobulin A1 (Gd-IgA1) in the kidney, which lead to inflammation of the kidney and eventual loss of kidney function. • IgAN is diagnosed by kidney biopsy; deposits containing IgA can be seen in the kidney mesangium using immunofluorescence.	IgAN is a rare and serious kidney disease that can lead to chronic kidney disease and kidney failure, resulting in the need for long-term dialysis or a kidney transplant to maintain life.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Current treatment options	• Current treatment strategies include blood pressure control, inhibition of the renin-angiotensin system via maximally tolerated doses of angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) and lifestyle modification including weight reduction, exercise, smoking cessation and dietary restriction. In patients who are considered to be at high risk of progression despite maximal supportive care, often defined as patients with persistent proteinuria >1 g/day, glucocorticoids may be used. • The corticosteroid, Tarpeyo (budesonide) delayed release capsules, is approved under FDA's accelerated approval pathway to reduce proteinuria in adults with IgAN at risk of rapid disease progression, generally a UP/C ≥1.5 g/g. • The sodium-glucose cotransporter-2 (SGLT2) inhibitor, dapagliflozin, is approved to reduce the risk of sustained eGFR decline, end-stage kidney disease, cardiovascular death, and hospitalization for heart failure in adults with chronic kidney disease at risk of progression. Dapagliflozin is expected to be more widely used in patients with IgAN moving forward.	To date, no pharmacologic treatment has been approved specifically to slow the loss of kidney function in patients with IgAN. The corticosteroid, Tarpeyo (budesonide) delayed release capsules, is FDA-approved under accelerated approval to reduce proteinuria in adults with IgAN at rapid risk of progression. Tarpeyo is a systemically available corticosteroid and is expected to cause related toxicities, including immunosuppression, elevated blood pressure, peripheral edema, new onset diabetes, and weight gain. As such, there is unmet need for treatments that can slow the loss of kidney function in patients with IgAN who are at high risk for disease progression.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Benefit	• Based on regulatory precedent and currently available data, the Division accepts a substantial reduction in proteinuria as a reasonably likely surrogate endpoint for disease progression (loss of kidney function) in IgAN and as a basis for accelerated approval. • The Applicant has submitted the results of an interim analysis of an ongoing, phase 3 study (PROTECT). PROTECT is a randomized, double-blind, active-controlled, multicenter study in patients with biopsy-proven IgAN, eGFR ≥30 mL/min/1.73 m^2, and total urine protein ≥1.0 g/day on a maximized stable dose of RAS inhibitor treatment. • 281 patients who reached the 36-week treatment period were included in the analysis for accelerated approval. The study met its primary endpoint of the relative change from baseline in UP/C at Week 36. The geometric mean ratio of UP/C at 36 weeks relative to baseline was 35% lower (95% CI: 23% to 45% lower) for the sparsentan arm compared to the irbesartan arm ($p<0.0001$). Efficacy findings were consistent across key subgroups, including key demographic and baseline disease characteristics (e.g., baseline proteinuria).	The submitted data demonstrate that sparsentan reduces proteinuria in patients with IgAN. The results were highly statistically persuasive, and consistent findings across key subgroups, including key demographic and baseline disease characteristics (e.g., baseline proteinuria) strengthen confidence in results. Existing data on the relationship between changes in proteinuria and disease progression suggest that the size of the treatment effect on proteinuria seen in the PROTECT study is reasonably likely to predict clinical benefit (i.e., slow the loss of kidney function and, with chronic use, reduce the risk of kidney failure).

Risk and risk management	• Of the 404 patients who received at least one dose of study drug in the PROTECT study (i.e., safety population), 202 received at least one dose of sparsentan and 202 received at least one dose of irbesartan. Serious adverse events occurred in 14% of patients receiving sparsentan and 13% of patients receiving irbesartan. Approximately 8% of patients in the sparsentan arm and 5% of patients in the irbesartan arm discontinued study drug because of an adverse event. • Risks of sparsentan observed in the PROTECT study were generally consistent with its mechanism of action. Most events were classified as mild or moderate. For the 404 patients in the safety population: – The most frequently reported TEAEs were hypotension (including dizziness and orthostatic hypotension) (24% sparsentan versus 10% irbesartan), hyperkalemia (11% sparsentan versus 9% irbesartan), peripheral edema (12% sparsentan versus 6% irbesartan), and acute kidney injury (4% sparsentan versus 1% irbesartan). – The incidence of hemoglobin decrease >2 g/dL compared to baseline and hemoglobin below the lower limit of normal at any time was higher for the sparsentan arm (11%) compared to the irbesartan arm (5%). This decrease is thought to be in part due to hemodilution. – A greater incidence of SAEs was reported for sparsentan versus irbesartan for hypotension (1.5% sparsentan versus 0.5% irbesartan), acute kidney injury (2% sparsentan versus 1% irbesartan), and anemia (0.5% sparsentan versus 0% irbesartan). • ERAs have caused elevations of aminotransferases, hepatotoxicity, and liver failure. FDA-approved ERAs include bosentan, macitentan, and ambrisentan, which are all indicated for the treatment of pulmonary arterial hypertension. In the PROTECT study, there was a signal for drug-induced liver injury with sparsentan compared to irbesartan. Approximately 6% of patients in the sparsentan	The incidence of hypotension, hyperkalemia, peripheral edema, acute kidney injury, and decrease in hemoglobin were all higher in the sparsentan group compared to the irbesartan group. These are likely drug-related as sparsentan is an antagonist of the endothelin type A receptor (ETAR) and the angiotensin II type 1 receptor (AT1R) and these are expected adverse reactions with drugs that target these receptors. Most of the adverse events that led to discontinuation of study drug were consistent with expected risks of sparsentan. ERAs have caused elevations of aminotransferases, hepatotoxicity, and liver failure. In the PROTECT study, there was a signal for drug-induced liver injury with sparsentan compared to irbesartan. The characterization of the risk of hepatoxicity with sparsentan is limited due to the small existing safety database. The Applicant will have a postmarketing requirement to conduct a prospective, single-arm safety study of patients exposed to sparsentan with 2 years of follow-up to assess and characterize the risk of drug-induced liver injury (DILI). Sparsentan will only be available through a restricted distribution program under a REMS due to the risk of hepatotoxicity and embryo-fetal toxicity. The goals of the REMS are to ensure that patients who can become pregnant are not pregnant before initiating sparsentan, minimize exposure in patients who may become pregnant while taking sparsentan, and to monitor for elevations in liver enzymes in patients exposed to sparsentan. As part of the REMS, patients who can become pregnant will be counseled about the risk of embryo-fetal toxicity and will undergo monthly pregnancy testing. All patients on sparsentan will be counseled on the risk of hepatotoxicity and undergo monthly liver monitoring for the first year of treatment and then every 3 months during treatment as required under the REMS. This required frequency of monitoring is sufficient as it exceeds the required liver testing frequency in the clinical trials (in which no serious liver outcomes occurred), at least in the first year, and matches the monitoring thereafter (i.e., every three months).

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	arm and 4% of patients in the irbesartan arm had a hepatic-related TEAE. • Transaminases were monitored every three months in the PROTECT study. Sparsentan caused at least a 3-fold ULN elevation of AST or ALT in up to 2.5% of patients in the sparsentan arm, including cases with positive rechallenge. The characterization of the risk of hepatoxicity with sparsentan is limited due to the small existing safety database. • Safety analyses for the subgroup of patients who completed at least 36 weeks of treatment by the data cut-off date (148 sparsentan, 147 irbesartan) in the PROTECT study were consistent with the safety analyses of the 404 patients who received at least one dose of study drug (i.e., safety population). • Based on data from animal reproduction studies, sparsentan can cause fetal harm when administered to a pregnant woman.	Labeling should include a boxed warning for hepatoxicity and embryo-fetal toxicity. Labeling will warn about the need for contraception and monthly pregnancy tests, as well the need for monthly liver monitoring for the first year of treatment and then every 3 months during treatment. Given the safety findings in the development program and larger experience with the pharmacologic class, labeling should include Warnings and Precautions for hepatotoxicity, embryo-fetal toxicity, hypotension, acute kidney injury, hyperkalemia, and fluid retention.

Abbreviations: ALT, alanine transferase; AST, aspartate transaminase; eGFR, estimated glomerular filtration rate; ERA, endothelin receptor antagonist; ESKD, end stage kidney disease; FSGS, focal segmental glomerulosclerosis; RAS, renin-angiotensin system; REMS, risk evaluation and mitigation strategy; SAE, serious adverse event; TEAE, treatment-emergent adverse event; ULN, upper limit of normal; UP/C, urine protein to creatinine ratio

2.2. Conclusions Regarding Benefit-Risk

IgAN is a rare and serious kidney disease that can lead to chronic kidney disease and kidney failure, resulting in the need for long-term dialysis or a kidney transplant to maintain life. Therapeutic options for the treatment of IgAN are limited and as such there is unmet medical need.

Sparsentan is expected to confer clinical benefit to patients by slowing chronic kidney disease (CKD) progression in patients with IgAN, and effectiveness will be verified in the postmarket setting. Although the magnitude of the reduction in CKD progression (i.e., extent of clinical benefit) will not be described until the confirmatory study is completed postmarketing, the existing data on the relationship between changes in proteinuria and disease progression suggest that the size of the treatment effect on proteinuria seen in the PROTECT study will likely result in a clinically meaningful benefit.

The major safety issues of hepatotoxicity and embryo-fetal toxicity necessitate a REMS for sparsentan to ensure its benefits outweigh its risks. Because sparsentan is an endothelin receptor antagonist, there is a concern for potential drug-induced liver injury. Although cases of severe drug induced liver injury with sparsentan have not been observed in clinical trials to date, the size of the available safety database is limited, and further safety data collection (single arm observational study) will be required in the postmarketing setting to better assess and characterize the risk. At this time, because of the uncertainties surrounding this potential DILI risk and because of the risk of embryo-fetal toxicity, sparsentan will only be available through a restricted distribution program under the REMS.

Other identified and potential risks can be adequately mitigated through labeling.

To further optimize benefit-risk considerations, particularly in light of the fact that the clinical benefit has not yet been verified, the drug will be approved for IgAN patients at relatively higher risk of rapid disease progression, generally a UP/C $\geq$1.5 g/g.

On balance, given the measures that will be put in place, sparsentan's benefits outweigh its risks in patients with IgAN at risk of rapid disease progression.

II. Interdisciplinary Assessment

3. Introduction

Sparsentan is an endothelin (endothelin type A receptor [ET_AR]) and angiotensin II receptor (AT_1R) antagonist. Endothelin-1 and angiotensin II are thought to contribute to the pathogenesis of immunoglobulin A nephropathy (IgAN) via the ET_AR and AT_1R pathway, respectively. By antagonizing both of these receptors, sparsentan reduces proteinuria.

On March 17, 2022, the Applicant submitted an NDA for sparsentan for the "treatment of immunoglobulin A nephropathy (IgAN) in adults aged 18 years and older." The Applicant is seeking approval under the provisions of 21 CFR Part 314, Subpart H, utilizing proteinuria as a reasonably likely surrogate endpoint. The Applicant has an ongoing study, PROTECT, that is intended to confirm the clinical benefit in the postmarketing setting.

Disease Background

IgAN is a rare and serious disease that is estimated to affect approximately 169,000 individuals in the United States (2:1 male-to-female predominance). Although patients with IgAN can present at any age, the peak incidence appears to be during the second and third decades of life. IgAN occurs with the greatest frequency in East Asians and Caucasians and is relatively rare in individuals of African ancestry. IgAN is associated with high morbidity and mortality; approximately 50% of patients progress to end-stage kidney disease within 30 years of diagnosis (Moriyama et al. 2014). IgAN is diagnosed by kidney biopsy; deposits containing IgA can be seen in the kidney mesangium using immunofluorescence.

The most common presentation of IgAN is gross hematuria (40% to 50% of cases), often accompanied by an upper respiratory infection. Approximately 30% to 40% of patients present with microscopic hematuria and subnephrotic proteinuria; less than 10% of patients present with either nephrotic syndrome or an acute, rapidly progressive glomerulonephritis.

There is an unmet need for approved therapies for IgAN. Initial treatment for IgAN includes optimized supportive care, which includes dietary sodium restriction, smoking cessation, weight control, control of blood pressure, and interventions to address cardiovascular risk. Angiotensin-converting enzyme inhibitors (ACEIs) or angiotensin receptor blockers (ARBs) are recommended in all patients with IgAN and proteinuria >0.5 g/day for controlling blood pressure, reducing proteinuria, and slowing the progression of renal disease. Patients who have persistent proteinuria of >1 g/day despite maximal supportive care are generally considered to be at "high risk" of progression (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work 2021). The current Kidney Disease Improving Global Outcomes Clinical Practice Guideline for the Management of Glomerular Diseases (Kidney Disease: Improving Global Outcomes Glomerular Diseases Work 2021) suggests that patients with IgAN who remain at high risk of progressive chronic kidney disease (CKD) despite maximal supportive care be considered for a 6-month course of systemic corticosteroid therapy.

Budesonide, a corticosteroid, is the first and currently only approved therapy for IgAN and was granted accelerated approval in 2021 "to reduce proteinuria in adults with primary

immunoglobulin A nephropathy (IgAN) at risk of rapid disease progression, generally a urine protein to creatinine ratio (UP/C) ≥1.5 g/g." The confirmatory study to verify and describe the clinical benefit is on-going. The sodium-glucose cotransporter-2 inhibitor, dapagliflozin, was FDA-approved in 2021 for reducing the risk of kidney disease progression in patients with CKD at risk for progression. By reducing sodium reabsorption, dapagliflozin is thought to decrease intraglomerular pressure, and therefore, is thought to target a common pathway for CKD. The study to support approval of dapagliflozin included patients with IgAN and the findings in this subgroup were consistent with the findings in the larger study population. Moving forward, dapagliflozin is expected to be more widely used in patients with IgAN.

Regulatory History

There were numerous interactions with the Applicant over the course of the development program; see Section 12 for further discussion of the regulatory history. Several interactions addressed the magnitude of the effect on UP/C that would need to be shown in the premarketing study to provide confidence that the postmarketing study is adequately powered to detect the anticipated clinical benefit.

3.1. Review Issue List

The review team identified two key review issues that had a significant impact on the overall determination of approvability, one related to the ability of the second phase of the trial to resolve the residual uncertainty about clinical benefit and one related to the risk of hepatotoxicity.

3.1.1. Key Efficacy Review Issues

(b) (4)

3.1.2. Key Safety Review Issues

3.1.2.1. Hepatoxicity

3.2. Approach to the Clinical Review

Table 3 provides an overview of the clinical studies submitted in support for efficacy and safety. The PROTECT study provides principal support for safety and effectiveness in the proposed population. The DUET study, conducted in patients with focal segmental glomerulosclerosis (FSGS), provides data to support the dosing regimen that was used in the PROTECT study.

Table 3. Clinical Studies/Trials Submitted in Support of Efficacy and/or Safety Determinations[1] for Sparsentan

Study/Trial Identifier (NCT#)	Study/Trial Population	Study/Trial Design	Regimen (Number Treated), Duration	Primary and Key Secondary Endpoints	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
PROTECT (021IGAN17001) (on-going)	Adults with biopsy-verified primary IgAN, on a stable dose of maximally tolerated ACEI or ARB therapy, eGFR ≥30 mL/min/1.73 m^2, proteinuria ≥1 g/day	Control type: active Randomization: randomized Blinding: double-blind	Drug and Dosage: sparsentan 400 mg or irbesartan 300 mg (active control) daily Number treated (interim analysis): 141 sparsentan, 140 irbesartan Duration (quantity and units): at least 36 wk	Primary (accelerated approval): change from baseline (Day 1) in the UPCR based on a 24-hour urine sample at Week 36 Confirmatory: rate of change in eGFR over a 110-week period following the initiation of randomized therapy	Planned:380 (total) Actual: 406 (total)	156 sites, 18 countries
DUET (RET-D-001)	Patients aged 8 to 75 years with biopsy-verified primary FSGS, eGFR ≥30 mL/min/1.73 m^2, UPCR ≥1.0 g/g	Control type: active Randomization: randomized Blinding: double-blind	Drug and dosage: sparsentan 200, 400, or 800 mg or irbesartan 300 mg (active control) daily Number treated: 73 sparsentan, 36 irbesartan Duration (quantity and units): 8 wk	Primary: change from baseline to Week 8 visit of the natural log of the UPCR	Planned: 100 Actual: 109	45 sites, 3 countries

Source: Reviewer.
[1] Includes all submitted clinical studies, even if not reviewed in-depth, except for phase 1 and pharmacokinetic studies.
[2] If no randomization, then replace with "Actual Enrolled."
Abbreviations: ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BID, twice daily; d, day; DB, double-blind; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; h, hour; IgAN, Immunoglobulin A nephropathy; LTE, long-term extension; MC, multicenter; mo, month(s); N, number of subjects; NCT, national clinical study; OL, open-label; PC, placebo-controlled; PG, parallel group; R, randomized; UPCR, urine protein to creatinine ratio; wk, week(s); y, year(s)

4. Patient Experience Data

The review team considered the experience and perspectives shared by patients and caregivers during an Externally-led Patient Focused Drug Development meeting hosted by the National Kidney Foundation and IgA Nephropathy Foundation on August 19, 2019, in its benefit-risk assessment. The Applicant included a summary of the report with the NDA submission.

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical Outcome Assessment Data Submitted in the Application		
☐	Patient-reported outcome	
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other Patient Experience Data Submitted in the Application		
☒	Patient-focused drug development meeting summary	
☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	If no patient experience data were submitted by Applicant, indicate here.	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☒	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

5.1. Nonclinical Assessment of Potential Effectiveness

5.1.1. Primary Pharmacology

Sparsentan is an endothelin receptor and angiotensin II receptor antagonist that has a high binding affinity to ET_AR (inhibition constant [K_i]=12.8nM) and AT_1R (K_i =0.36nM), with a >500-fold selectivity over endothelin type B and AT_2 subtype receptors. Sparsentan has approximately 36-fold higher affinity for AT_1R than ET_AR.

The inhibitory effect of sparsentan on receptor function was assessed using cells that express human ET_AR or AT_1R (PathHunter). In these in vitro studies, endothelin-1-stimulated (5.6nM) and angiotensin II-stimulated (1.0nM) calcium mobilization was inhibited with half maximal inhibitory concentration (IC_{50}) values of 46.55nM and 13.2nM, respectively, and endothelin-1-stimulated (5nM) and angiotensin II-stimulated (3.9nM) β-arrestin translocation was inhibited with IC_{50} values of 521nM and 7.86nM, respectively. In an in vivo functional study conducted in healthy male rats, sparsentan inhibited endothelin-1- and angiotensin II-mediated pressor responses with an effective dose 50 (ED_{50}) of 7.6 mg/kg for ET_AR and 0.9 mg/kg for AT_1R.

5.1.2. Animal Model Data Showing Proof of Concept for Efficacy

Sparsentan showed proof of concept for efficacy in the following animal models relevant to human IgAN. The endpoints measured in these animal models assessed different aspects underlying the pathophysiology of glomerular diseases like IgAN, which include proteinuria, glomerular sclerosis, mesangial cell activation and proliferation, and tissue inflammation. In these models, sparsentan attenuated glomerular injury which was reflected by improved renal function or retained structural features.

Animal models relevant to IgAN include the following:

- Grouped ddY (gddY) mouse model: This model exhibits early onset of an IgAN-like disease state marked by albuminuria due to renal damage resulting from glomerular deposition of IgA, immunoglobulin G (IgG), and complement 3, leading to a progressive mesangioproliferative glomerulonephritis. Sparsentan (180 mg/kg or 360 mg/kg) administered in chow, daily for 12 days, starting at 4 weeks of age, reduced the elevated urine albumin levels and glomerulosclerosis.
- Passive mouse IgAN model: This model is developed by injecting EICs (human polymeric galactose-deficient (Gd) IgA1 protein and a recombinant human IgG autoantibody specific for Gd-IgA1) to the athymic (nude) mice every other day for 12 days (total six intravenous (IV) injections). This model demonstrates microscopic features of IgAN-related renal pathology and incites a mesangioproliferative injury. Sparsentan treatment (60 or

120 mg/kg/day, PO, daily for 12 days beginning on the day of EIC administration) attenuated mesangial cellularity and glomeruli proliferation, and it ameliorated increased plasma creatinine levels.

- Antithymocyte Serum (ATS)-induced glomerular injury model: In this model, administration of ATS (0.6 mL/100 g, IV) induces injury to the mesangial cell membranes which results in features of IgAN, including mesangiolysis mediated via complement activation, inflammation, interstitial expansion of profibrotic mediators, matrix accumulation, and proteinuria. Sparsentan treatment (20, 60, or 180 mg/kg/day, PO, daily for 7 days beginning 24 hr after the ATS injection), dose-dependently attenuated the elevated proteinuria, glomerular injury, and inflammatory response induced by ATS.

Sparsentan exhibits inhibitory activity against the endothelin type A (ET_A) and angiotensin II type 1 (AT_1) receptors, which have been implicated in the pathophysiology underlying renal disease (Dhaun et al. 2011). To assess the contribution of both these activities, the effect of sparsentan was compared to the effect of individual ET_AR or AT_1R antagonists in several animal models of FSGS. Despite the differences between the initial stimuli that trigger glomerular injury in IgAN and FSGS, these models do exhibit multiple overlapping structural and functional attributes of renal injury. The information from these animal models may also support an efficacious effect of ET_A and AT_1 receptor antagonism. In TRPC6 transgenic mice (FSGS-Tg model), sparsentan showed efficacy while losartan alone (AT_1R antagonist) was ineffective or had weaker effects on several glomerular hemodynamic endpoints and on the frequency of p57-positive podocytes. In the adriamycin-induced nephropathy model, losartan and atrasentan administered alone was less effective than sparsentan on improving several structural endpoints including glomerular sclerosis, glycocalyx staining, and podocyte number. These results support the view that inhibition of both the ET_A and AT_1 receptors may confer greater benefit in certain aspects of renal diseases relative to antagonism at a single receptor. However, this comparative data in the animal models should be interpreted with caution as there was no clear justification for the losartan and atrasentan dose selection with respect to their receptor binding affinities (K_i values).

As an additional analysis, the Applicant developed a pharmacokinetic (PK)/pharmacodynamic model to evaluate the relationship between estimated ET_A and AT_1 receptor occupancy and the reduction in proteinuria observed in the ATS-induced glomerular injury model, an animal model relevant to IgAN. Receptor occupancy was estimated in part using plasma exposure levels of sparsentan derived from separate studies. The analysis identified an attenuation in proteinuria at the 50% receptor occupancy of AT_1 receptors, followed by a further attenuation in proteinuria when the receptor occupancy of ET_A receptors was approaching 50%. This analysis lends further support that a greater attenuation in proteinuria may be achieved with antagonism of both ET_A and AT_1 receptors than antagonism at a single receptor in this animal model.

5.2. Clinical Pharmacology / Pharmacokinetics

Table 5. Summary of Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established pharmacologic class (EPC)	Sparsentan is an endothelin and angiotensin II receptor antagonist.
Mechanism of action	Sparsentan is a single molecule that functions as a high-affinity antagonist of both the ET_AR (K_i =12.8nM) and AT_1R (K_i =0.36nM), with greater than 500-fold selectivity over endothelin type B receptor and angiotensin II subtype 2 receptor.
Active moieties	Sparsentan
QT prolongation	A thorough QT (TQT) study demonstrated that sparsentan was not associated with any potential to cause QTc interval prolongation (i.e., >10 msec) after single doses of 800 mg and 1600 mg. A single-dose of 1600 mg adequately covers the highest potential clinical exposures.
	General Information
Bioanalysis	Plasma sparsentan concentrations were measured using a validated turbo ion spray liquid chromatography-mass spectrometry (LC-MS/MS) method.
Healthy subjects versus patients	There is no clinically relevant difference in the pharmacokinetics of sparsentan between patients with IgAN, FSGS and healthy subjects, as evaluated by population pharmacokinetic (PopPK) analysis.
Drug exposure at steady state following the therapeutic dosing regimen (or single dose, if more relevant for the drug)	**Parameter** / **Mean (CV%) (400 mg)** AUC_{ss} (µg·h/mL) / 114 (23.6%) C_{minss} (µg/mL) / 1502 (69.4%) (Simulated exposure for patients with IgAN using PopPK model)
Range of effective dose(s) or exposure	Starting dose of 200 mg once daily (QD) titrated to the target dose of 400 mg based on tolerability.
Maximally tolerated dose or exposure	A maximum tolerated dose was not identified for sparsentan. A maximum single dose of 1600 mg was studied in healthy subjects in Study RTRX-RE021-103 and multiple doses of 1600 mg daily for 14 days were studied in healthy subjects in Study RTRX-RE021-103.
Dose proportionality	Sparsentan steady-state exposure (C_{max} and AUC) increased in a dose proportional manner over the dose range of 50 to 200 mg and in a less than dose-proportional manner over the dose range of 200 to 1600 mg.
Accumulation	No significant accumulation is observed at steady state with once daily dosing.
Time to achieve steady-state	Because there is no significant accumulation upon repeat once-daily dosing, the steady state exposures of sparsentan can be expected to be achieved by Day 2 or Day 3.
Bridge between to-be-marketed and clinical trial/study formulations	N/A. To-be-marketed tablets were used in the pivotal phase 3 study, PROTECT.
	Absorption

Characteristic	Drug Information
Bioavailability	The absolute bioavailability of sparsentan was not determined.
T_{max}	The median time to peak plasma concentrations of sparsentan is approximately 3 hours (ranging from 2 to 5 hours).
Food effect (fasted/fed) Geometric least square mean and 90% CI	**Dosage** | **AUC_{0-72}, GMR (90% CIs)** | **C_{max}, GMR (90% CIs)** | **T_{max}, median** 200 mg | 86% (90% CI: 73.8%, 100.5%) | 122% (90% CI: 107.0%, 138.5%) | 3.9 h 2× 400 mg | 122% (90% CI: 104.7%, 142.5%) | 208% (90% CI: 183.0%, 236.6%) | 4.5 h (Effect of Food on the Pharmacokinetic Parameters of Sparsentan) The effect of food on exposure of a single 400 mg tablet has not been tested, however, the expected effect is bracketed by the results observed with 200 mg and 800 mg. The pivotal clinical study, PROTECT, was conducted by administering the drug prior to the first meal of the day, without postdose food restriction. The review team recommends sparsentan be taken prior to the morning or evening meal, without postdose food restriction, allowing flexibility while still maintaining similar instructions to those implemented in the clinical study.
	Distribution
Volume of distribution	The mean volume of distribution is 61.4 L.
Plasma protein binding	Sparsentan is >99% bound to human plasma proteins with >90% binding to albumin (concentration independent). Binding to α1-acid glycoprotein is concentration dependent, with approximately 50% at 10 and 40µM, and approximately 80% at 1µM with a larger coefficient of variation.
Drug as substrate of transporters	Sparsentan is a substrate of P-gp and BCRP.
	Elimination
Mass balance results	Following administration of 400 mg [^{14}C]-sparsentan, 2.2% of the dose is recovered in urine and 80.2% is recovered in feces. Unchanged sparsentan represented about 9% of the administered dose in feces and <1% in urine (Study 021HVOL16005).
Clearance	The clearance of sparsentan is time-dependent. Following a single dose of 400 mg sparsentan, the geometric mean of apparent clearance (CL/F) was 4.82 L/h, and after a single dose of 800 mg sparsentan, mean CL/F was 4.97 L/h. Following multiple doses of 400 mg QD sparsentan for 14 days, mean CL/F was 6.29 L/h, and after multiple doses of 800 mg QD sparsentan for 14 days, mean CL/F was 8.25 L/h. Based on a PopPK analysis in patients with FSGS, after an oral dose of 400 mg and 800 mg sparsentan, the mean CL/F was 3.88 L/h and 5.47 L/h, respectively, increasing to 5.11 L/h and 7.21 L/h, respectively, at steady state.
Half-life	Mean $t_{1/2}$ of sparsentan ranged from 10.2 to 12.2 hours after single dose administration of 400 or 800 mg sparsentan, and 10.8 and 13.9 hours after multiple doses of 400 or 800 mg sparsentan. The steady state $t_{1/2}$ of sparsentan was estimated to be 9.6 hours.
Metabolic pathway(s)	Sparsentan is primarily metabolized by CYP3A4, with minor contribution from CYP2C8 and CYP2C9.
	Intrinsic Factors and Specific Populations
Body weight	Based on PopPK analyses, body weight is not a statistically significant covariate on sparsentan exposure.
Age	Based on PopPK analyses, age is not a statistically significant covariate on sparsentan exposure.
Renal impairment	A dedicated renal impairment study was not conducted. Renal elimination is not expected to be the major elimination pathway of sparsentan. Only 2.2% was recovered in the urine in the mass balance study with unchanged sparsentan only in trace amounts. PopPK analysis showed no significant difference in systemic exposure of sparsentan between patients with mild or

Characteristic	Drug Information
	moderate renal impairment and normal renal function. The effect of severe renal impairment and end stage kidney disease including dialysis is unknown.
Hepatic impairment	Mild and moderate hepatic impairment did not appear to significantly affect the total systemic exposure of sparsentan. The mean C_{max} unbound and AUC_{last} unbound values of the moderate hepatic impairment group were approximately 2 times those of the normal group; however, because of the large variability in measurement of unbound concentrations, robust conclusions cannot be made using unbound concentration data. The effect of severe hepatic impairment (Child-Pugh Class C) on the PK of sparsentan is unknown.
	Drug Interaction Liability (Drug as Perpetrator)
Inhibition/induction of metabolism	In vitro studies showed that sparsentan was a potential inhibitor of CYP2C8 and CYP3A4, and a potential inducer of CYP2B6, 2C9, 2C19, and 3A4. **In vivo evaluation:** Co-administration of sparsentan with midazolam, a CYP3A4 substrate, did not significantly change the PK of midazolam upon administration with sparsentan for 7 days. Exposure to bupropion, a CYP2B6 substrate, decreased by 30% upon co-administration with sparsentan for 9 days. **PBPK simulation:** Modeling of co-administration of sparsentan with midazolam, a CYP3A4 substrate, did not suggest a significant change in the PK of midazolam upon administration with sparsentan for 14 days. Modeling of co-administration of sparsentan with tolbutamide (CYP2C9 substrate) or omeprazole (CYP2C19 substrate) suggested a decrease in the exposure of tolbutamide and omeprazole by 42% and 18%, respectively. However, it is unclear whether induction parameters generated from in vitro hepatocyte induction studies reliably predict drug effects on CYP2C induction. As such, the PBPK simulations can only serve as a risk assessment. Please see Section 14.714.7 for details.
Inhibition/induction of transporter systems	In vitro studies showed that sparsentan was a potential inhibitor of P-gp, BCRP and OATP1B3. **In vivo evaluation:** Exposure to pitavastatin (a UGT1A3, UGT2B7, CYP2C9, OATP, P-gp and BCRP substrate) decreased by 30% upon co-administration with sparsentan. No clinical DDI studies were conducted to evaluate the inhibitory effect of sparsentan specifically towards P-gp and BCRP.

Abbreviations: AT_1R, angiotensin II type 1 receptor; AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; CV%, coefficient of variation; CYP, cytochrome P450 isoenzyme; DDI, drug-drug interactions; ET_AR, endothelin type A receptor; FSGS, focal segmental glomerulosclerosis; GMR, geometric mean ratio; IgAN, Immunoglobulin A nephropathy; T_{max}, time to maximum concentration;

6. Efficacy (Evaluation of Benefit)

6.1. Assessment of Dose and Potential Effectiveness

6.1.1. Applicant's Proposed Dosing Regimen

Proposed labeling indicates that renin-angiotensin-aldosterone system inhibitors, endothelin receptor antagonists (ERAs), and aliskiren should be discontinued prior to initiating treatment with sparsentan. The proposed starting dose for sparsentan is 200 mg taken orally once daily (QD). After 14 days, the dose should be increased to the recommended dose of 400 mg QD, as tolerated.

The proposed dosing regimen mirrors the dosing regimen used in the pivotal study.

6.1.2. Selection of Dosing Regimen for Phase 3 Trials

The dose of sparsentan that was selected for the phase 3 study was based on the results of a dose-ranging study in patients with FSGS. A separate dose-ranging study was not conducted in patients with IgAN.

The DUET study (RET-D-001) evaluated doses of 200, 400, and 800 mg QD in patients with FSGS. Analyses of the data from this study demonstrated a statistically significant greater reduction in UP/C among pooled (all doses combined) sparsentan-treated subjects compared with irbesartan-treated subjects at Week 8, with the reduction in UP/C appearing to reach a plateau above 400 mg (Table 6). Following oral administration of multiple doses of sparsentan to patients with FSGS, steady state systemic exposure (area under the concentration-time curve $[AUC]_{0-24}$ and maximum plasma concentration $[C_{max}]$) increased in a less than dose-proportional manner over the 200 to 800 mg doses, with the exposure to sparsentan largely overlapping between 400 mg QD and 800 mg QD (Table 7). A dose of 400 mg QD dose was selected for the phase 3 study because the reduction in UP/C appeared to plateau above a dose of 400 mg and because exposures were similar in the 400 mg QD and 800 mg QD dosing arms in the DUET study.

For further discussion of the efficacy findings in this trial, see Section 16.3.

Table 6. Change From Baseline in Urine Protein to Creatinine Ratio at Week 8

	Treatment Group Comparisons									
	Sparsentan, Pooled (200 mg, 400 mg, and 800 mg) to Irbesartan 300 mg		Sparsentan, Pooled (400 mg and 800 mg) to Irbesartan 300 mg		Sparsentan 400 mg to Irbesartan 300 mg		Sparsentan 800 mg to Irbesartan 300 mg		Sparsentan 200 mg to Irbesartan 300 mg	
Parameter Statistic	Irbesartan (n=32)	Sparsentan (n=64)	Irbesartan (n=25)	Sparsentan (n=51)	Irbesartan (n=17)	Sparsentan (n=21)	Irbesartan (n=8)	Sparsentan (n=30)	Irbesartan (n=7)	Sparsentan (n=13)
Baseline UP/C (g/g)										
n	32	64	25	51	17	21	8	30	7	13
Mean	4.017	4.707	3.816	4.824	3.482	4.749	4.526	4.876	4.734	4.248
(SD)	(2.6717)	(3.7810)	(2.7160)	(4.0506)	(2.6074)	(4.1696)	(2.9842)	(4.0362)	(2.5701)	(2.5304)
Median	3.265	3.620	2.970	3.530	2.850	3.710	3.295	3.225	4.860	3.710
Min, Max	0.88, 10.73	0.43, 18.66	0.88, 10.73	0.43, 18.66	1.10, 10.73	1.25, 18.66	0.88, 9.76	0.43, 14.68	0.98, 8.03	1.50, 10.15
Week 8 UP/C (g/g)										
n	32	64	25	51	17	21	8	30	7	13
Mean	3.164	3.300	2.990	3.208	2.795	2.452	3.405	3.737	3.786	3.663
(SD)	(2.2713)	(3.5719)	(2.3598)	(3.4738)	(2.4581)	(2.6254)	(2.2343)	(3.9177)	(1.9501)	(4.0650)
Median	2.405	1.980	2.390	1.900	2.380	1.730	3.020	2.085	3.640	2.060
Min, Max	0.43, 10.19	0.12, 14.47	0.43, 10.19	0.12, 14.47	0.43, 10.19	0.28, 12.34	1.11, 8.37	0.12, 14.47	1.45, 6.69	0.59, 14.38
Percentage Change from Baseline to Week 8 (95% CI)[a]	-18.5 (-34.6, 1.7)	-44.8 (-52.7, -35.7)	-19.0 (-38.0, 5.9)	-47.4 (-56.3, -36.9)	-28.1 (-47.5, -1.6)	-52.7 (-64.3, -37.2)	-9.3 (-45.3, 50.3)	-41.3 (-54.4, -24.4)	-15.0 (-41.8, 24.2)	-33.1 (-49.3, -11.6)
Ratio (sparsentan/ irbesartan)[b] (95% CI)		0.7 (0.5, 0.9)		0.6 (0.5, 0.9)		0.7 (0.4, 1.0)		0.6 (0.4, 1.1)		0.8 0.5, 1.3
p-value[c]	---	0.006	---	0.011	---	0.056[d]	---	0.127[d]	---	0.298[d]

Abbreviations: ANCOVA = analysis of covariance; CI = confidence interval; LS = least squares; Max = maximum; Min = minimum; SD = standard deviation; UP/C = urine protein to creatinine ratio ; UPE = urinary protein excretion

a Geometric least squares mean, calculated as [exp (LS mean change from baseline in natural log(UP/C)) – 1] x 100. The same transformation was applied to obtain the corresponding 95% CI.

b Ratio (sparsentan/irbesartan) = exp[LS mean change from baseline in natural log(UPE) for sparsentan – LS mean change from baseline in natural log(UPE) for irbesartan]. The same transformation is applied to obtain corresponding 95% CI for the ratio (sparsentan/irbesartan).

c p-value is the treatment effect p-value from the ANCOVA model. An ANCOVA model was fitted with the change in natural log(UP/C) from baseline as the dependent variable, treatment and cohort as the main effects, and baseline natural log(UP/C) as a covariate.

d Nominal p-value.

Source: Applicant's DUET report. Table 14 on page 43.

Table 7. Summary Pharmacokinetic Parameter Data for Sparsentan Following Oral Administration

Dose	AUC_{0-24} (CV%) (ng·h/mL)	C_{max} (CV%) (ng/mL)
200 mg, QD (n=15)	54400 (43%)	4300 (52.9%)
400 mg, QD (n=25)	81400 (69.0%)	7070 (54.6%)
800 mg, QD (n=20)	95500 (65.8%)	7620 (51.7%)

Source: Applicant's DUET PK report. Table 82 on page 128, table 83 on page 129, table 84 on page 130.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CV%, coefficient of variation; QD, once daily.

6.1.3. Dose Response

6.1.3.1. Exposure-Response for Safety and Efficacy

The Applicant conducted an exposure-response (E-R) analysis using data from the phase 3 study (PROTECT). A trend between a percentage reduction from baseline of UP/C at Week 36 and increasing sparsentan exposure was observed. However, it should be noted the presented E-R relationship may be confounded by titration and is largely based on a single dose level.

No clear E-R relationship was observed for hypotension of any grade or peripheral edema of worst grade. A statistically significant relationship was observed between exposure and the incidence of hyperkalemia of any grade, with increasing sparsentan exposures associated with a greater risk of hyperkalemia. See Section 14.5.2 for further information.

6.2. Clinical Studies/Trials Intended to Demonstrate Efficacy

6.2.1. Study PROTECT

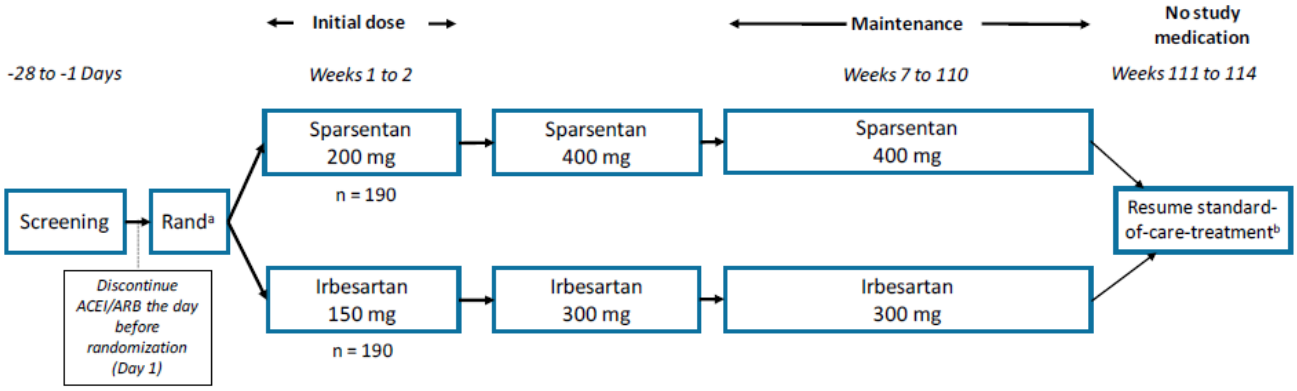
6.2.1.1. Design, Study PROTECT

The PROTECT study is an ongoing, randomized, double-blind, active-controlled, multicenter study comparing sparsentan 400 mg daily to irbesartan 300 mg daily in subjects with IgAN, proteinuria ≥1 g/day, estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2, who are on a stable dose of maximally tolerated angiotensin-converting enzyme (ACE) inhibitor and/or ARB. Subjects were randomized 1:1 to either sparsentan or irbesartan, with randomization stratified by baseline eGFR (30 to <60 mL/min/1.73 m^2 and ≥60 mL/min/1.73 m^2) and baseline urine protein excretion (≤1.75 g/day and >1.75 g/day). ACE inhibitor and/or ARB therapy was discontinued before randomization. Subjects initially received one-half of the dose of study drug (i.e., sparsentan 200 mg or irbesartan 150 mg daily) for 2 weeks and if tolerated[3] based on investigator judgement, they were titrated to the full dose (i.e., target dose) at the Week 2 visit (see Appendix for details on dosing). Patients will be treated with double-blind study drug for 110 weeks. Following the 110-week blinded treatment period, treatment with study medication will be discontinued for 4 weeks and patients will be placed on the same ACE inhibitor and/or ARB treatment regimen the patient was on at study entry. An overview of the study design for the double-blind period is shown in the figure below.

By May 26, 2021, the study was fully enrolled with 406 subjects. The protocol prespecified an unblinded interim analysis performed 36 weeks after randomization of at least 280 subjects to evaluate the primary efficacy endpoint (i.e., endpoint for accelerated approval) based on change in proteinuria at Week 36. The same study will be used to verify and describe the clinical benefit. The confirmatory endpoint will evaluate the rate of change of eGFR over 110 weeks following initiation of randomized therapy and will be evaluated in the full study population.

[3] The investigator evaluated dose tolerance in a blinded manner. Evaluation of dose tolerance was based on blood pressure, “lack of adverse events” at the Week 2 visit, and Week 2 visit labs.

Figure 1. Design, Study PROTECT



Abbreviations: ACEI = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker; eGFR = estimated glomerular filtration rate; RAAS = renin-angiotensin-aldosterone system; Rand = randomization

[a] On Day 1, patients will be randomized 1:1 to sparsentan or irbesartan, stratified by eGFR value (30 mL/min/1.73 m^2 to <60 mL/min/1.73 m^2 and ≥60 mL/min/1.73 m^2) and urine protein excretion (≤1.75 g/day and >1.75 g/day).

[b] Resume standard-of-care treatment, including RAAS inhibitor treatment. Where possible, the same treatment regimen the patient was on at study entry (ie, the same ACEI and/or ARB at the same dose[s]) should be used unless, in the Investigator's opinion, an alternative treatment approach is warranted.

Source: PROTECT Clinical Study Protocol, Amendment 5

6.2.1.2. Objective, Study PROTECT

The objective of the PROTECT study is to determine the effect of sparsentan on proteinuria and preservation of renal function, as compared to an ARB, in patients with IgAN.

6.2.1.3. Eligibility Criteria, Study PROTECT

Key inclusion criteria are as follows:

- Male or female, aged ≥18 years
- Biopsy-proven IgAN
- Urine protein excretion value ≥1.0 g/day at screening (based on a 24-hour urine sample)
- eGFR value of ≥30 mL/min/1.73 m^2 at screening
- On a stable dose of ACEI and/or ARB therapy for at least 12 weeks prior to screening that is the patient's maximum tolerated dose and is at least one-half of the maximum labeled dose
- Blood pressure ≤150/100 mmHg at screening
- Willing to undergo a change in ACEI and/or ARB and antihypertensive medications
- Women of childbearing potential, beginning at menarche, must agree to the use of one highly reliable (i.e., can achieve a failure rate of <1% per year) method of contraception from 7 days prior to the first dose of study medication until 90 days after the last dose of study medication.[4] One additional barrier method must also be used during sexual activity, such as

[4] Highly reliable contraception methods include stable oral, implanted, transdermal, or injected contraceptive hormones associated with inhibition of ovulation, or an intrauterine device in place for at least 3 months.

a diaphragm or diaphragm with spermicide (preferred) or male partner's use of male condom or male condom with spermicide (preferred), from Day 1/Randomization until 90 days after the last dose of study medication

Key exclusion criteria are as follows:

- IgAN secondary to another condition or Henoch-Schoenlein purpura
- Presence of cellular glomerular crescents in >25% of glomeruli on renal biopsy within 6 months of screening
- CKD due to another condition in addition to IgAN
- Undergone any organ transplantation, with the exception of corneal transplants
- Requires any of the prohibited concomitant medications (see Appendix for details)
- Taking any systemic immunosuppressive medications (including corticosteroids) for >2 weeks within 3 months prior to screening
- Documented history of heart failure (NYHA Class II-IV) and/or previous hospitalization for heart failure or unexplained dyspnea, orthopnea, paroxysmal nocturnal dyspnea, ascites, and/or peripheral edema
- Clinically significant cerebrovascular disease and/or coronary artery disease within 6 months prior to screening
- Jaundice, hepatitis, or known hepatobiliary disease (excluding asymptomatic cholelithiasis), or alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) >2 times the upper limit of the normal range at screening
- Screening hematocrit value <27% or hemoglobin value <9 g/dL
- Screening potassium value >5.5 mEq/L

6.2.1.4. Endpoints, Study PROTECT

Endpoints for the Interim Analysis

The primary efficacy endpoint for the interim analysis was the change from baseline (Day 1) in the UP/C based on a 24-hour urine sample at Week 36 (i.e., endpoint for accelerated approval).

Secondary endpoints at the time of the Interim Analysis were supportive (i.e., not included within the testing strategy) and included the following:

- The rate of change in eGFR over a 52-week (approximately 1 year) period following the initial acute effect of randomized therapy (the initial acute effect of randomized therapy is defined as the first 6 weeks of randomized treatment with study medication; thus, the analysis is from 6 weeks postrandomization to 58 weeks postrandomization (i.e., eGFR chronic slope at 1 year)
- The rate of change in eGFR over a 58-week period following the initiation of randomized therapy (i.e., eGFR total slope)

Endpoints for the Confirmatory Trial

The confirmatory endpoint to verify the clinical benefit is the rate of change in eGFR over a 110-week (approximately 2 years) period following the initiation of randomized therapy (thus, the analysis is from Day 1 to 110 weeks postrandomization (i.e., eGFR total slope at 2 years).

The confirmatory analysis will include the following key secondary endpoint that will be included within the testing strategy: the rate of change in eGFR over a 104-week (approximately 2 years) period following the initial acute effect of randomized therapy; thus, the analysis is from 6 weeks postrandomization to 110 weeks postrandomization (i.e., eGFR chronic slope at 2 years).

6.2.1.5. Statistical Analysis Plan, Study PROTECT

Version 1 of the Applicant's statistical analysis plan for the interim analysis (IA) (data cutoff date August 1, 2021) was finalized on April 11, 2019, when 28 subjects had been enrolled in the study. The statistical analysis plan (SAP) for the IA was amended twice. The changes that were made to the SAP via these amendments (see Appendix 16.1 for further details) do not raise concerns about the interpretability of the study results.

Efficacy Analysis Sets

The SAP specified three analysis sets:

- The full analysis set (FAS) was to include all patients who were randomized and had taken at least one dose of randomized therapy.
- The primary analysis set (PAS) was to include the subset of patients in the FAS at the time of the data extraction for the primary analysis.
- The Per-Protocol Analysis Set was to include the subset of patients in the FAS who met study eligibility requirements and did not have any protocol deviations that might impact the assessment of efficacy measurements.

The SAP also stated that the PAS would be the same as the FAS if the study was fully enrolled at the time of the interim analysis.

Endpoints

Primary Endpoint at IA

Change from baseline in proteinuria (UP/C).

Proteinuria (UP/C) will be determined based on a 24-hour urine sample. As UP/C is a highly right-skewed variable, analyses will be performed on log-transformed data.

The primary analysis of proteinuria will be conducted on the primary analysis set (PAS) after 36 weeks following randomization of approximately 280 patients to determine whether the primary efficacy endpoint, the change from baseline in UP/C, is statistically significant.

Secondary Endpoints at IA

- Chronic slope: rate of change in eGFR following acute effect of randomized therapy over 52 weeks (Week 6 to Week 58).
- Total slope: rate of change in eGFR over 58 weeks following initiation of randomized therapy.

The eGFR for each baseline and postbaseline visit will be determined using the Chronic Kidney Disease Epidemiology Collaboration, (Levey et al. 2009) formula for adults, based on serum creatinine values from the visit.

Analysis Visit Window

The analysis windows associated with primary and key secondary endpoints at the interim analysis are shown in Table 8.

Table 8. Visit Windows (Study Days)

		Analysis Visit Window (Study Days)	
Analysis Visit	**Relative Target Day**	**eGFR and Safety Labs**	**Quantitative Urinalysis**
Week 6	43	37 – 64	37 – 64
Week 36	253	212 – 295	212 – 295
Week 58	407	373 – 449	373 – 449

Source: Applicant's SAP Table 1
Abbreviations: eGFR, estimated glomerular filtration

Multiplicity Adjustment

Different testing procedures were specified for U.S. and non-U.S. regulatory bodies. For the FDA analysis, the overall family-wise Type I error rate was to be controlled by a combination of a gatekeeping and fixed sequence procedure.

At the interim analysis, only the primary endpoint of UP/C at Week 36 was to be tested at a full alpha of 0.05, and no formal testing was to be conducted on the eGFR rate of change over 6 to 58 weeks. The full testing sequence is as shown below. Only the first endpoint was to be tested at the interim analysis; the other endpoints were to be tested at the final analysis:

- UP/C at 36 weeks
- eGFR rate of change at 110 weeks (2-year total slope)
- eGFR rate of change over 6 to 110 weeks (2-year chronic slope)
- Other secondary endpoints at final analysis

If at any step, the statistical comparison was not statistically significant at the specified significance level of 0.05, then the remaining comparisons would be considered descriptive and exploratory.

Sample Size

For the primary endpoint UP/C at the interim analysis, a total of 280 randomized subjects will provide at least 90% power assuming the following:

- True relative treatment effect on UP/C, sparsentan versus irbesartan, is at least 30%.

- Standard deviation of the log change from baseline in proteinuria at Week 36 is 0.92.
- Two-sided alpha level is 0.05.

The SAP also assumes, based on analyses of an IgAN patient registry database, that a 30% treatment effect on UP/C predicts a difference in eGFR total slope at 104 weeks of 6.4 mL/min/1.73 m^2, with a 95% CI of (0.83, 12.44).

(b) (4)

Primary Analysis

Analysis Method

The change from baseline in proteinuria (UP/C) will be analyzed in the PAS and will be analyzed using an MMRM analysis. The analysis will be performed on log transformed data since UP/C is a highly right -skewed variable. Fixed effects include:

- Treatment group (two levels: sparsentan, irbesartan)
- Baseline UP/C in log scale
- Time (i.e., analysis visit in weeks)
- Treatment group by time interaction
- Randomization stratification variable (4 levels based on Screening eGFR (30 to <60 mL/min/1.73 m^2 and ≥60 mL/min/1.73 m^2) and total urine protein excretion (≤1.75 g/day and >1.75 g/day))

Subjects will be included as a random effect.

An unstructured covariance matrix will be used. If it fails to converge, other prespecified structures will be used. Estimates and CIs will be converted to percentages via the following transformations:

[exp(LS mean change from baseline in natural log(UP/C)) – 1] × 100

Missing Data and Intercurrent Event Handling

Missing data for the primary endpoint were to be imputed using a multiple imputation (MI) procedure under the missing at random assumption. A Bayesian multivariate normal mode for the data was to be fitted using a MCMC approach. The MCMC approach allows either monotone or nonmonotone pattern missing observations to be imputed. Baseline and postbaseline scheduled visits were to be used in the regression option to impute the missing values. Specifically:

- Intermittent missing values before a discontinuation event (i.e., discontinuation of randomized therapy or early permanent dropout): Multiple imputation will be based on MCMC option in SAS PROC MI by treatment group under the missing at random assumption.

- Missing data following a discontinuation event (i.e., discontinuation of randomized therapy or early permanent dropout): Multiple imputation for the post discontinuation missing data by treatment group under the missing at random assumption using the regression option from the monotone statement of SAS PROC MI.

Of note, during the IND phase, the Agency expressed interest in a treatment policy strategy to handle intercurrent events and indicated that if the Sponsor used a different estimand, then the Sponsor should also include supplementary analyses based on all observed data (i.e., a treatment policy approach).

Sensitivity Analyses

To explore the robustness of the primary analysis due to missing data and premature treatment discontinuations, the following sensitivity analyses were prespecified:

- Tipping point approach of the MI analysis
- MMRM using observed data
- MMRM using completers only
- MMRM using observed data including after premature treatment discontinuation (treatment policy estimand)

To assess the impact of changes in systemic immunosuppressive medications:

- Repeat primary analysis by excluding assessments after initiation of renal indication of systemic immunosuppressive medication

To assess the impact of protocol deviations:

- Repeat primary analysis on per protocol (PP) analysis set

Subgroup Analyses

Subgroup analyses on primary endpoint will be performed on age, sex, race, randomization strata, region, baseline BMI, baseline eGFR categories, baseline total urine protein, baseline UP/C, baseline use of antihypertensive medications include diuretics, and history of hypertension.

Key Secondary Analyses

Analysis Method

The rate of change in eGFR over Week 6 to Week 58 (1-year chronic slope) and the rate of change in eGFR over 58 weeks following the initiation of randomized therapy (1-year total slope) will be analyzed via a mixed model random coefficients analysis including fixed effects:

- Treatment group (two levels: sparsentan, irbesartan)
- Baseline eGFR
- Time (i.e., analysis visit in weeks)

- Treatment group by time interaction
- Randomization stratification variable (4 levels based on Screening eGFR and urine protein excretion)

The model will also include a random intercept and random slope for each patient.

Of note, during the IND phase, the agency recommended using a two-slope linear spline mixed effect model to estimate chronic slope since the Applicant's approach did not include data collected before Week 6 and therefore may not be statistically valid.

Missing Data and Intercurrent Event Handling

Same as primary analysis.

Sensitivity Analyses

Same as sensitivity analyses for primary analysis, except that the MMRM is substituted by the mixed random coefficient model.

Additional sensitivity analysis: a two-slope model with knot or change point at Week 6 is used to analyze the available 1-year eGFR data.

6.2.1.6. Results of Analyses, Study PROTECT

Although the SAP indicated that the primary analysis would be based on the FAS (defined as all subjects who were randomized and had taken at least one dose of randomized therapy) if the study was fully enrolled at the date of data-cut off, because a large proportion of subjects in the FAS did not have a 9-month UP/C measurement at the time of the interim analysis, FDA's analyses do not use the FAS. Instead, the main analyses results shown in this section are based on the interim analysis set (IAS), defined as the first 281 randomized subjects. This analysis set includes subjects who either completed or were supposed to have completed the Week 36 proteinuria assessment by the interim data cut-off date of August 1, 2021.

Patient Disposition

The disposition for all screened subjects is shown in Table 9. The study was fully enrolled at the date of data cut-off. As shown in the table, 671 subjects were screened, and of these, 406 subjects were randomized into the study. Among the 281 randomized subjects included in the IAS, a greater proportion of subjects in the irbesartan arm as compared to the sparsentan arm discontinued study drug before Week 36 (11% versus 5%, respectively). The proportion of subjects who discontinued the study before Week 36 was low (1%) in both groups. As of the data cuff, 19% of subjects in the IAS randomized to irbesartan had discontinued treatment as compared to 13% of subjects in the sparsentan and 7% of subjects in the irbesartan group as compared to 3% in the sparsentan group had discontinued from the study.

Table 9. Subject Disposition, Study PROTECT, IAS

Disposition Category	Irbesartan n (%)	Sparsentan n (%)	Total n (%)
No. subjects screened			671
No. screening failures			265
No. subjects randomized			406
No. subjects in IAS			281
Subjects randomized, IAS	140 (100)	141 (100)	281(100)
ITT/mITT population	139 (99)	140 (99)	279 (99)
Per protocol population	128 (91)	133 (94)	261 (93)
Safety population	139 (99)	140 (99)	279 (99)
Discontinued study drug (before data cutoff)	27 (19)	18 (13)	45 (16)
Adverse event	8 (6)	13 (9)	21 (7)
Patient decision	11 (8)	3 (2)	14 (5)
Physician decision	5 (4)	0 (0)	5 (2)
Other	1 (1)	2 (1)	3 (1)
Discontinued study drug (before Week 36)	16 (11)	7 (5)	23 (8)
Discontinued study (before data cutoff)	10 (7)	4 (3)	14 (5)
Lost to follow-up	1 (1)	0 (0)	1 (<1)
Physician decision	2 (1)	0 (0)	2 (1)
Withdrawal of consent	7 (5)	4 (3)	11 (4)
Death	0 (0)	0 (0)	0 (0)
Discontinued study (before Week 36)	1 (1)	1 (1)	2 (1)

Source: Statistical Reviewer
Abbreviation: IAS, interim analysis set; mITT, modified intention-to-treat; N, number of subjects; n, number of subjects with at least one event

Of the randomized subjects in the IAS, the proportion of subjects with available Week 36 UP/C data was slightly lower in irbesartan group (91%) as compared to sparsentan group (96%) (Table 10). The majority of subjects with missing Week 36 UP/C data had discontinued treatment early.

Table 10. Subjects With Urine Protein to Creatinine Ratio Data at Week 36, Study PROTECT, IAS

Disposition Category	Irbesartan N=140 n (%)	Sparsentan N=141 n (%)
Valid 36-week value	128 (91.4)	135 (95.7)
Received 36 weeks of treatment	119 (85.0)	131 (92.9)
Discontinued treatment early	9 (6.4)	4 (2.8)
Missing 36-week value	12 (8.6)	6 (4.3)
Received 36 weeks of treatment	4 (2.9)	2 (1.4)
Discontinued treatment early	8 (5.7)	4 (2.8)

Source: Statistical Reviewer
Abbreviation: IAS, interim analysis set; mITT, modified intention-to-treat; N, number of subject; UP/C, urine protein to creatinine ratio

Baseline Demographics and Disease Characteristics

Among the subjects included in the IAS, there were some differences between the groups in baseline demographics (Table 11). Specifically, the proportion of females, Asians and subjects enrolled at sites in the Asia Pacific area was numerically greater in the sparsentan as compared to irbesartan arm. These imbalances do not raise concerns about the interpretability of the study results. To date, gender has not been identified as an important risk factor for disease progression, and, if anything, the imbalance in race might be expected to bias the results in favor

of the control arm (Barbour et al. 2013). The mean age (46 years) was similar in the two groups. Most subjects were white or Asian, reflecting the epidemiology of IgAN (Galla 1995). Approximately 16% of subjects included in the IAS were enrolled at sites in the United States.

Table 11. Baseline Demographics (ITT Population), Study PROTECT, IAS

Demographics	Irbesartan N=140	Sparsentan N=141	Total N=281
Sex, n (%)			
Female	37 (26.4)	50 (35.5)	87 (31.0)
Male	103 (73.6)	91 (64.5)	194 (69.0)
Age, years			
Mean (SD)	45.5 (11.75)	46.8 (13.08)	46.1 (12.43)
Median	45.0	48.0	46.0
IQR	36.5, 54.5	38.0, 57.0	37.0, 56.0
Min, max	19.0, 76.0	18.0, 73.0	18.0, 76.0
Age categories, n (%)			
<=45 year	72 (51.4)	67 (47.5)	139 (49.5)
>45 years	68 (48.6)	74 (52.5)	142 (50.5)
Race, n (%)			
Asian	38 (27.1)	59 (41.8)	97 (34.5)
Black or African American	3 (2.1)	1 (<1)	4 (1.4)
White	94 (67.1)	79 (56.0)	173 (61.6)
Other	5 (3.6)	2 (1.4)	7 (2.5)
Ethnicity, n (%)			
Hispanic or Latino	9 (6.4)	10 (7.1)	19 (6.8)
Not Hispanic or Latino	128 (91.4)	131 (92.9)	259 (92.2)
Not reported	3 (2.1)	0	3 (1.1)
Geographic region, n (%)			
Asia Pacific	31 (22.1)	63 (44.7)	94 (33.5)
Europe	82 (58.6)	59 (41.8)	141 (50.2)
North America	27 (19.3)	19 (13.5)	46 (16.4)

Source: Statistical Reviewer Analysis; adslir11.xpt
Abbreviations: IQR, interquartile range; IAS, interim analysis set; ITT, Intention-to-treat population; SD, standard deviation

Baseline clinical characteristics were, as a whole, similar between the two groups (Table 12). The mean eGFR was 56 mL/min/1.73 m^2 and mean UP/C was 1.4 g/g.

Table 12. Baseline Clinical Characteristics, Study PROTECT, IAS

Characteristic	Irbesartan N=140	Sparsentan N=141	Total N=281
eGFR			
Mean (SD)	55.6 (22.6)	57.1 (24.7)	56.4 (23.7)
Median	50.0	50.0	50.0
IQR	38.0, 68.5	38.0, 71.0	38.0, 70.0
Min, max	26.0, 123.0	24.0, 128.0	24.0, 128.0
eGFR Category, n (%)			
<30	4 (2.9)	9 (6.4)	13 (4.6)
>=30 to <45	55 (39.3)	48 (34.0)	103 (36.7)
>=45 to <60	31 (22.1)	34 (24.1)	65 (23.1)
>=60 to <90	36 (25.7)	31 (22.0)	67 (23.8)
>=90	14 (10.0)	19 (13.5)	33 (11.7)
UP/C (g/g)			
Mean (SD)	1.5 (0.94)	1.4 (0.83)	1.4 (0.89)
Median	1.2	1.3	1.2
IQR	0.9, 1.7	0.8, 1.8	0.8, 1.8

Characteristic	Irbesartan N=140	Sparsentan N=141	Total N=281
Min, max	0.2, 6.9	0.2, 4.2	0.2, 6.9
Urinary protein excretion (g/day)			
Mean (SD)	2.2 (1.23)	2.1 (1.37)	2.2 (1.30)
Median	1.8	1.7	1.8
IQR	1.4, 2.6	1.2, 2.7	1.3, 2.6
Min, max	0.5, 7.5	0.4, 7.2	0.4, 7.5
Urinary protein excretion category, n (%)			
<=1.75 g/day	62 (44.3)	73 (51.8)	135 (48.0)
>1.75 g/day	78 (55.7)	68 (48.2)	146 (52.0)

Source: Statistical Reviewer Analysis; adslir11.xpt
Abbreviations: IAS, interim analysis set; IQR, interquartile range; ITT, Intention-to-treat population; SD, standard deviation; UP/C, urine protein to creatinine ratio

Primary Efficacy Endpoint, IAS

The primary endpoint at the interim analysis was the geometric mean ratio of UP/C at Week 36 relative to baseline. At Week 36, the UP/C geometric mean was 85.1% and 55.2% of the baseline value for subjects in the irbesartan and sparsentan groups, respectively. The ratio of the geometric mean relative to baseline between the two groups was 0.65 (95%CI: 0.55, 0.77), i.e., the geometric mean relative to baseline at Week 36 was 35% lower (95%CI: 23% lower to 45% lower; p-value<0.0001) for the sparsentan arm compared to the irbesartan arm (Table 13).

Table 13. MMRM Results of Geometric Mean Ratio of Urine Protein to Creatinine Ratio at Week 36 Relative to Baseline, Study PROTECT, IAS

Variable	Irbesartan N=140	Sparsentan N=141
Adjusted geometric mean of UP/C		
Baseline	1.24	1.22
Week 36	1.04	0.68
Adjusted GMPC from baseline in UP/C at Week 36	-14.9 (-24.4, -4.1)	-44.8 (-50.9, -37.9)
Adjusted GM relative to baseline at Week 36	85.1 (75.6, 95.9)	55.2 (49.1, 62.1)
Ratio of geometric mean relative to baseline at Week 36 (95% CI)	0.65 (0.55, 0.77)	
p-value	<0.0001	

Source: Statistical Reviewer.
Note 1: MMRM was used to calculate adjusted geometric means, 95%CI and p-values. The MMRM model used analysis visits up to Week 36, and included treatment, baseline log (UP/C), analysis visit, treatment-by-analysis interaction, and randomization stratification factors as fixed effects, and patient as random effect. UP/C data were analyzed on natural log scale. Estimated LS mean and 95% CIs are converted to percentages as follows: [exp (least squares mean change from baseline in natural log (UP/C)) – 1] × 100. An unstructured covariance structure was used.
Note 2: Missing data were imputed using multiple imputation under the missing at random assumption and combined using Rubin's rule. Data observed after treatment discontinuation and initiation of rescue therapy were used in the analysis (treatment policy strategy).
Abbreviations: CI, confidence interval; GMR, geometric mean ratio; GMPC, geometric mean percentage change; IAS, interim analysis set; MMRM, mixed model repeated measures; N, number of subjects in each group; UP/C, urine protein to creatinine ratio

The geometric mean ratio of UP/C at each visit up to Week 36 relative to baseline between the two arms was nominally significant by Week 4 and the treatment effect on proteinuria appeared to be maintained over time (Table 14).

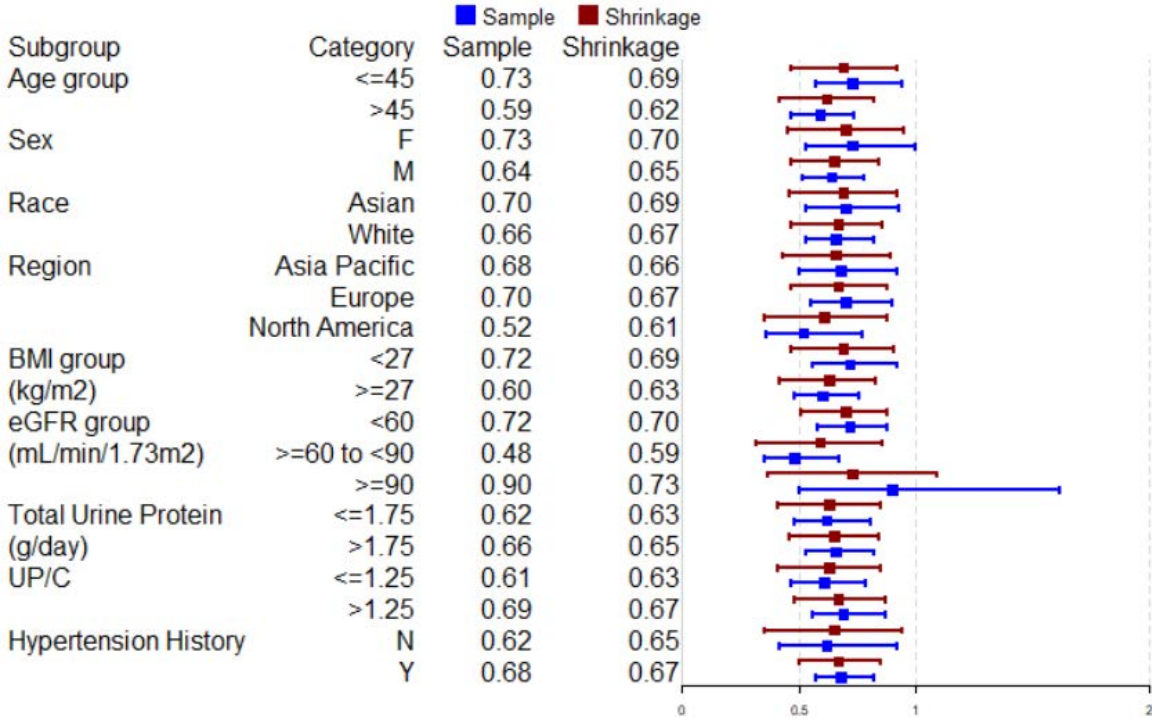
Table 14. Geometric Mean Ratio of Urine Protein to Creatinine Ratio at Different Visit Week Relative to Baseline, Study PROTECT, IAS

	Irbesartan N=140		Sparsentan N=141		
Adjusted GMR	**n**	**GMPC (95%CI)**	**n**	**GMPC (95%CI)**	**GMR (95%CI)**
Week 4	131	-4.6 (-11.8, 3.3)	137	-34.8 (-39.7, -29.5)	0.68 (0.61, 0.76)
Week 6	133	-5.5 (-13.8, 3.6)	138	-36.2 (-41.8, -30.1)	0.68 (0.59, 0.77)
Week 12	128	-8.8 (-17.7, 1.0)	137	-40.3 (-45.9, -34.1)	0.65 (0.57, 0.75)
Week 24	122	-14.3 (-23.2, -4.3)	137	-42.3 (-48.2, -35.7)	0.67 (0.58, 0.78)
Week 36	128	-14.9 (-24.4, -4.1)	135	-44.8 (-50.9, -37.9)	0.65 (0.55, 0.77)

Source: Statistical Reviewer.
Note: results were from the same MMRM analysis as primary analysis.
Abbreviations: CI, confidence interval; GMR, geometric mean ratio; GMPC, geometric mean percentage change; IAS, interim analysis set; MMRM, mixed model repeated measures; N, number of subjects in each group; UP/C, urine protein to creatinine ratio

Results were consistent across demographic subgroups (age, sex, race, region) and baseline disease characteristics subgroups (BMI, eGFR, total urine protein, UP/C, and hypertension history) for the primary endpoint.

Figure 2. Subgroup Analysis for the Geometric Mean Ratio of Urine Protein to Creatinine Ratio at Week 36 Relative to Baseline, Study PROTECT, IAS



Source: Statistical Reviewer.
Note1: The subgroup analyses used the same MMRM analysis as was used for the primary analysis. No imputation was performed for subgroup analyses; data observed after treatment discontinuation and initiation of rescue therapy were used in the analyses (treatment policy strategy).
Note2: There are typically some random highs and random lows in sample estimates of subgroup treatment effects due to small sample sizes and large variability for some subgroups. Therefore, we also derived shrinkage estimates of subgroup treatment effects using a Bayesian hierarchical model based on summary sample estimates. This approach leads to improved precision and lower variability, shown with narrower confidence intervals, most notably in subgroups with a smaller sample size. The total variability in the sample estimates is the sum of the within subgroup variability of the sample estimator and the across subgroups variability in the underlying/true parameter values. A shrinkage estimate of the subgroup treatment effect, which borrows information from the other subgroups while estimating the treatment effect for a specific subgroup, is a "weighted" average of the sample estimate and overall estimate. A set of fairly noninfluential priors $\mu \sim normal(0, 100), \tau^2 \sim invers\_gamma(0.001, 0.001)$]were used to derive shrinkage estimates for all subgroups.
Abbreviations: BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate; F, female; IAS, interim analysis set; M, male; MMRM, mixed model repeated measures; N, number of subjects in each group; UP/C, urine protein to creatinine ratio.

(b) (4)

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(b) (4)

Additional Subgroup Analyses

Given the available data on the efficacy and safety of the product and the intent of the accelerated approval program, the indicated population should be limited to patients at risk of rapid disease progression over a relatively short time frame. For the purpose of accelerated approval, regulatory precedent has been to use a UP/C >1.5 g/g to define this population. Exploratory analyses of the treatment effect on the primary endpoint and (b) (4) were conducted in the subgroup of patients with baseline UP/C <1.5 g/g and >1.5 g/g. These analyses were based on the IAS and used methods similar to those used in the subgroup analyses of the primary endpoint and key secondary endpoints.

For the primary endpoint, the findings in these subgroups were consistent with those seen in the general study population. In the subgroup with a UP/C < 1.5 g/g, the ratio of the geometric mean relative to baseline at Week 36 in UP/C was 0.62 (95% CI 0.50, 0.77), while in the subgroup with a UP/C ≥1.5 g/g, it was 0.71 (95% CI 0.54, 0.93). (b) (4)

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7. Safety (Risk and Risk Management)

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

Nonclinical studies, including safety pharmacology studies, general toxicology studies, a full battery of genetic toxicology, reproductive and developmental toxicology studies, and carcinogenicity studies, were conducted to assess the safety of sparsentan. The key safety findings from these studies and their clinical relevance are discussed below. Exposure multiples with respect to the maximum recommended human dose (MRHD) of 400 mg per day are listed in the table below. (See detailed nonclinical study summaries in Section 13.1).

Embryo-Fetal Toxicities

In embryo-fetal developmental toxicity studies in pregnant rats and rabbits, teratogenicity and developmental toxicity were observed. The findings were consistent with the pharmacologic class of compounds that inhibit ET_A and angiotensin II type 1 (AT_1) receptors. The relevant animal data are summarized below.

In pregnant rats treated with sparsentan at doses of 80, 160, and 240 mg/kg/day, dose-dependent toxicity and teratogenicity marked by craniofacial malformations, skeletal abnormalities, embryo-fetal lethality, and reduced fetal weight were observed at all doses. The lowest dose tested (80 mg/kg/day) provided an exposure approximately 10 times the AUC at the MRHD. Although maternal toxicity was also observed at all doses tested, the fetal toxicity and teratogenicity can be attributed to the known effects of pharmacological inhibition of angiotensin II and endothelin receptors in the developing fetus. The no observed adverse effect level (NOAEL) for embryo fetal toxicity in the rat could not be established as it was presumptively <80 mg/kg/day and <10-times the MRHD.

In pregnant rabbits, oral administration of sparsentan at doses of 2.5, 10, and 40 mg/kg/day resulted in maternal mortality and abortions at doses ≥10 mg/kg/day. Two litters each from dams at doses of 10 and 40 mg/kg/day had all dead or resorbed conceptuses. The number of surviving fetuses was sufficient for evaluation. An increase in fetal variation (supernumerary cervical ribs) was observed at 40 mg/kg/day but there were no fetal malformations or changes in fetal viability and growth of surviving fetuses. Exposure at the highest dose tested was 5-times lower than clinical exposure due to the inability of pregnant rabbits to tolerate higher exposures. As a result, the rabbit study is of little value in the assessment of fetal risk.

In the pre- and postnatal developmental toxicity study, administration of sparsentan at doses of 5, 20 and 80 mg/kg/day to pregnant rats caused toxicity in offspring marked by increased pup mortality during the neonatal period through weaning at 80 mg/kg/day, and decreased growth (decrease in pup body weights) after weaning at ≥20 mg/kg/day. These 80 and 20 mg/kg/day doses provide an exposure approximately 10 times and 3 times the MRHD based on AUC, respectively. The NOAEL for pre- and postnatal development was the low dose of 5 mg/kg/day.

Fertility

In a fertility and early embryonic development study, no adverse effects were observed on male or female rats administered sparsentan at doses up to 320 mg/kg/day, which provided approximately 10 and 14-times the AUC at the MRHD for males and females, respectively. Key endpoints measured in this study included estrous cycles, mating, fertility, pregnancy incidence, and a spermatogenesis evaluation in males. Adverse effects on male reproductive tissues were also not evident in a 6-month study in rats at doses up to 320 mg/kg/day (approximately 10 times the AUC at the MRHD) and a 9-month study in monkeys at doses up to 200 mg/kg/day (approximately 1.3 times the AUC at the MRHD). A 2-year study in rats did not identify any adverse histopathological change to epididymal or testicular tissues; however, exposures were limited by tolerability and fell below clinical levels (~0.7 times MRHD).

Bosentan, ambrisentan and macitentan are three approved ERAs in the United States. Decreased sperm counts have been reported in patients receiving bosentan. Human sperm evaluation has not been reported with ambrisentan and macitentan, and it was not conducted with sparsentan. In animal studies, testicular tubular atrophy in rodents has been reported with these approved ET_A receptor antagonists. Effects on sperm counts and male fertility were reported in male rats treated with ambrisentan at high exposures. These animal findings were not observed in the rat studies conducted with sparsentan at adequate exposures, suggesting a minimal concern for this potential human risk. However, the risk for human spermatogenesis cannot be fully excluded based on the previous findings with the approved ERAs.

General Toxicology (Pivotal) Studies

General toxicity studies were conducted for durations up to 13 weeks in mice, 26 weeks in rats, and 39 weeks in monkeys. Key findings in the pivotal toxicology studies and their clinical relevance are discussed below.

Kidney

Adverse histological changes to the kidneys were observed in repeated dose toxicology studies in mice, rats, and monkeys. Minimal to moderate hypertrophy/hyperplasia of the juxtaglomerular apparatus (JGA) were noted in mice at doses ≥50 mg/kg/day following 13 weeks of treatment, in rats at all dose levels (≥15 mg/kg/day) following 13- and 26-week treatment, and in monkeys at all dose levels (≥10 mg/kg/day) following 13- and 39-week treatment. The incidence/severity of this finding was dose-dependent and partially reversible (lower severity) at the end of the recovery periods in the rat and monkey. Following 26 weeks of treatment in rats, minimal to mild renal tubular degeneration was noted at the 320 mg/kg/day dose with increased incidence/severity compared to the control, and it was fully reversible. Minimal to moderate interstitial fibrosis was observed at doses ≥80 mg/kg/day with a much higher incidence/severity at 320 mg/kg/day. At the end of the recovery period, interstitial fibrosis of minimal severity was still present in one 320 mg/kg/day male, indicative of reversibility. Partially reversible minimal to mild renal cortical interstitial fibrosis was observed in monkeys at doses ≥10/125 mg/kg/day following 39 weeks of treatment.

In addition to the kidney histopathologic observations, increased kidney weights, which reversed at the end of the recovery period, were observed in rats at higher doses in a less than dose-dependent pattern. There was no treatment related change in kidney weights in mice and

monkeys. Increases in blood urea nitrogen and creatinine (Cr) levels, which resolved by the end of the recovery period, were observed at high doses in both rats and monkeys.

Most of the kidney findings and related clinical chemistry were reversible or reversing following a recovery period. Based on the incidence/severity and reversibility, the key kidney findings were mainly related to the high dose of 320 mg/kg/day in rats (approximately 10.4 to 18.8 times the AUC at the MRHD), and the doses of ≥10/125 mg/kg/day (approximately 0.6 times the AUC at the MRHD) in monkeys. These findings were considered related to pharmacological actions, and consistent with known effects of ARBs and ACE inhibitors. The finding of juxtaglomerular apparatus hypertrophy/hyperplasia is thought to be a consequence of long-term inhibition of the renin-angiotensin system, as reported for ACE inhibitors and ARBs. Increases in blood urea nitrogen and/or Cr have also been observed with ACE inhibitors and ARBs. Although the renal findings were observed at relevant clinical exposures in healthy animals, its significance for potential human risk would not be different from the other ARBs based on mechanism of action, and its translatability to the intended patient population should be aligned with the clinical safety profile.

Hematology/Red Cell Mass:

A reversible decrease in red cell mass (red blood cell count, hemoglobin concentration, and hematocrit) about 6 to 7% and 9 to 12% was noted at 80 and 320 mg/kg/day, respectively, in rats following 26-week treatment. In monkeys, following 39 weeks of treatment, a decrease (16 to 24%) in red cell mass associated with minimal hypoplasia in bone marrow was observed at a high dose of 200 mg/kg/day (approximately 1.3 to 1.7 times the AUC at the MRHD), which was fully resolved at the end of the recovery period.

The finding was consistent with the effects of the other ARBs and could be attributed to reduced levels of erythropoietin and/or reduced sensitivity to erythropoietin, which are dependent on the renin-angiotensin system (Kim et al. 2017). Decreases in hemoglobin concentration and hematocrit have also been reported with other endothelin receptor antagonists in clinical studies. Although the decrease in red cell mass occurred at relevant clinical exposures in monkeys, it was of a relatively small magnitude and reversible, and such effects can be assessed in clinical studies; hence, the clinical hematology data is the most appropriate data set to interrogate this nonclinical signal further.

Other

Treatment-related decreases in body weight were observed in rats and monkeys after exposure to high doses, which partially resolved at the end of the recovery period. Single cell necrosis in the liver was observed in the 13-week mouse study only at a high dose of 750 mg/kg/day. Reversible minimal to mild liver hypertrophy was observed only in rodents and is considered an adaptive metabolic response to high drug load and was not considered adverse in the absence of degenerative findings. Other hematology changes, including increased platelet counts and decreased white blood cell counts at high doses in rats, were of small magnitude and fully reversible. These nonclinical findings were generally not significant and raise little concern for human risk.

Genetic Toxicology

There was no evidence of mutagenicity or clastogenicity for sparsentan in in vitro bacteria reverse mutation and chromosomal aberration assays or in an in vivo rat micronucleus study.

Carcinogenicity

In the 2-year rat carcinogenicity study, there was no evidence of an increased incidence of neoplasia in male rats orally administered 15 mg/kg/day (dose limited by tolerability) and in female rats orally administered up to 240 mg/kg/day, which provides an exposure approximately 0.7 times and 26 times the AUC at the MRHD, respectively. In the 26-week transgenic mouse study, there was no evidence of an increased incidence of neoplasia in male and female mice orally administered sparsentan doses up to 600 mg/kg/day.

Table 19. Exposure Ratios for Major Toxicology Studies

Study	Sex	Dose[3] (mg/kg/day)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)	Human Exposure Multiples[1, 2]
General Toxicology (pivotal studies) studies				
13-week mouse toxicology	M	**200**	321000	4.18
	F		427000	5.56
26-week rat toxicology	M	**80**	318000	4.14[1]
	F		99700	1.30[1]
	M	320	795000	10.35[1]
	F		1440000	18.75[1]
39-week monkey toxicology	M	**50**	16700	0.22[1]
	F		12700	0.17[1]
	M	125	187000[$]	NA[$]
	F		45700	0.60[1]
	M	200	98500	1.28[1]
	F		134000	1.74[1]
Carcinogenicity				
Carcinogenicity - 26-week mouse Tg.rasH2	M	600	376000	NA
	F		549000	NA
Carcinogenicity - 2-year rat	M	15	53700	0.70[1]

Study	Sex	Dose[3] (mg/kg/day)	$AUC_{0\text{-}24hr}$ (ng·hr/mL)	Human Exposure Multiples[1, 2]
	F	240	2030000	26.43[1]
Reproductive toxicology				
Rat - Fertility and early development	M	**320**	754000*	9.82[1]
			HED 3114 mg/kg	*7.8*[2]
	F		1110000*	14.45[1]
			HED 3114 mg/kg	*7.8*[2]
Rat - Embryo-fetal development	F	<80	805000	10.48[1]
Rabbit - Embryo-fetal development		2.5	1170	0.02[1]
		10	9550	0.12[1]
	F	40	13500	0.18[1]
Rat - Pre- and postnatal development		**5**	50313#	0.66[1]
			HED 49 mg/kg	*0.1*[2]
		20	201250#	2.62[1]
			HED 195 mg/kg	*0.5*[2]
	F	80	805000#	10.48[1]
			HED 778 mg/kg	*1.9*[2]

Source: Pharmacology/toxicology reviewer
1.Human exposure multiples are estimated based on steady-state geometric mean $AUC_{0\text{-}24hr}$ of 76800 µg·hr/mL on Day 57 following oral administration of 400 mg sparsentan to adult focal segmental glomerulosclerosis (FSGS) patients with a body weight of >50 kg (Final pharmacokinetic report (TVTX-RE021-202) of Study no RET-D-001;
2.Human exposure multiples are estimated based on body surface area (mg/m^2) for 60 kg body weight and human dose of 400 mg/kg when animal AUC data is not available;
3. Dose in bold font means NOAEL (No Observed Adverse Effect Level);
* - $AUC_{0\text{-}24hr}$ inferred from the exposure at dose of 320 mg/kg in rat 13-week oral toxicity study;
# - $AUC_{0\text{-}24hr}$ inferred from the exposure at dose of 80 mg/kg/day in the pregnant rats from the rat EFD study; the $AUC_{0\text{-}24hr}$ values at lower doses of 20 and 5 mg/kg/day were estimated as 1/4 and 1/16 of the $AUC_{0\text{-}24hr}$ values at 80 mg/kg/day, respectively.
$ -The exposure at this dose level had large variation and was unreasonably high. It is considered unreliable for exposure multiple calculation.
Abbreviations: AUC, area under the concentration-time curve; HED, human equivalent dose

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Sparsentan is an endothelin and angiotensin II receptor antagonist. FDA-approved ERAs include ambrisentan, bosentan, and macitentan, which are each indicated for the treatment of pulmonary arterial hypertension. FDA-approved labeling for all three agents includes a boxed warning for embryo-fetal toxicity and bosentan also has a boxed warning for hepatotoxicity. The Warnings and Precautions section of FDA-approved labeling for these agents include the following: embryo-fetal toxicity, pulmonary edema with pulmonary veno-occlusive disease, decreased sperm counts, decreases in hemoglobin and hematocrit, and fluid retention. Both bosentan and macitentan also contain a Warning and Precaution for hepatotoxicity. All three agents are only available through a risk evaluation and mitigation strategy (REMS) program. All three agents have a REMS for the risk of embryo-fetal toxicity and bosentan also has a REMS for the risk of hepatotoxicity.

The Warnings and Precautions section of FDA-approved labeling for ARBs includes the following: fetal toxicity (boxed warning), hypotension (in volume- or salt-depleted patients), impaired renal function, and hyperkalemia. Olmesartan also contains a Warning and Precaution for sprue-like enteropathy, which is not thought to be a class effect.

7.3. Potential Risks or Safety Concerns Identified Through Postmarket Experience

This drug has not yet been marketed.

7.4. FDA Approach to the Safety Review

The safety review included a review of data quality and integrity, as well as adverse event (AE) and laboratory datasets. There were no concerns regarding submission quality, conduct of the studies with respect to assessment of safety, or the Applicant's characterization of adverse events. Data issues identified had low impact due to the low number of records affected (<1%).

The safety review was mainly based on data collected from the phase 3 study 021IGAN17001 (PROTECT) by the interim data lock date of July 30, 2021. Data from two FSGS studies (DUET and DUPLEX) were also used to assess for a signal for hepatotoxicity. The safety review focused on the safety population (all enrolled subjects who received at least one dose of study treatment up to the data cutoff date) in the PROTECT study. Results are presented for this population (404 subjects) unless otherwise specified. Safety results for the subpopulation of subjects who completed at least 36 weeks of treatment (i.e., ≥252 days) by the data lock date (295 subjects) and for the subpopulation of patients with baseline UP/C ≥1.5 g/g (142 subjects) were also conducted and compared with the safety population. In general, the results from these two subgroup analyses were consistent with the results for the safety population. In the sections that follow, adverse events are generally presented as a risk difference (RD), which was calculated as the difference in the percentage of subjects with adverse events between the sparsentan and the irbesartan group: a negative RD favors sparsentan and a positive RD favors irbesartan.

The evaluation of safety signals was based on the FDA Medical Dictionary for Regulatory Activities queries (FMQs, version 2.0), Standard Medical Dictionary for Regulatory Activities queries (SMQs, version 23.0), and the Applicant's predefined queries. In general, broad FMQs and SMQs were used to detect general imbalances in adverse events between groups and narrow FMQs and SMQs were used to further analyze adverse events of special interest (AESIs). Applicant defined terms were provided in the interim Clinical Study Report and the Appendix of the Analysis Data Reviewer's Guide.

Laboratory and vital signs relevant to AESIs are summarized in Section 7.6.6 under each AESI. Other labs and vital signs are summarized in Sections 7.6.7 and 7.6.8.

7.5. Adequacy of the Clinical Safety Database

The mean and median exposure duration was balanced between the sparsentan and the irbesartan groups (Table 20). Over 120 subjects were exposed to sparsentan for longer than 50 weeks. The submitted safety data is considered adequate to assess safety for the purposes of approval, with an additional requirement to further assess liver safety in the postmarketing setting.

Table 20. Duration of Exposure, Safety Population and 36-Week Subpopulation, Study PROTECT

Parameter	SPA 400 mg N=202 n (%)	IRB 300 mg N=202 n (%)	SPA 400 mg 36 Week N=148 n (%)	IRB 300 mg 36 Week N=147 n (%)
Duration of treatment, weeks				
Mean (SD)	64.3 (35)	61.3 (35.6)	80.3 (26.3)	76.7 (28.9)
Median (Q1, Q3)	73.4 (32.6, 95.5)	60.9 (27, 93.4)	84.2 (60.4, 103.6)	81.4 (54.7, 100.8)
Min, Max	0.1, 115.9	0.1, 114.9	0.1, 115.9	2.6, 114.9
Total exposure (person years)	249	237	228	216
Patients treated, by duration, n (%)				
<12 weeks	14 (6.9)	17 (8.4)	3 (2.0)	5 (3.4)
≥12 weeks	188 (93.1)	185 (91.6)	145 (98.0)	142 (96.6)
≥26 weeks	156 (77.2)	154 (76.2)	143 (96.6)	139 (94.6)
≥50 weeks	127 (62.9)	116 (57.4)	127 (85.8)	116 (78.9)
≥100 weeks	43 (21.3)	42 (20.8)	43 (29.1)	42 (28.6)

Source: adex.xpt and adsl.xpt; Software: R
The PROTECT 36-week subpopulation is defined as subjects who completed at least 36 weeks of treatment by the interim data lock (CUTOFFDT-RANDDT +1≥252).
Abbreviations: IRB, Irbesartan; N, number of subjects in treatment arm; n, number of subjects with given treatment duration; Q1, first quartile; Q3, third quartile; SD, standard deviation; SPA, sparsentan

7.6. Safety Results

7.6.1. Overview of Treatment-Emergent Adverse Events

In the PROTECT study, treatment-emergent adverse events (TEAEs) were common in both arms, though the proportion was numerically greater in the sparsentan as compared to the irbesartan group (Table 21). Serious adverse events (SAEs) and severe adverse events were balanced between the two treatment groups. More subjects on sparsentan discontinued treatment permanently or had study drug dose modification due to TEAEs compared to those on irbesartan.

Table 21. Overview of Adverse Events, Safety Population, Study PROTECT

Event Category	SPA 400 mg N=202 n (%)	IRB 300 mg N=202 n (%)	SPA 400 mg vs. IRB 300 mg Risk Difference (%) (95% CI)
SAE	28 (13.9)	27 (13.4)	0.5 (-6.2, 7.2)
SAEs with fatal outcome	0	0	0 (0, 0)
Life-threatening SAEs	3 (1.5)	2 (1.0)	0.5 (-1.7, 2.7)
AE leading to permanent discontinuation of study drug	16 (7.9)	9 (4.5)	3.5 (-1.2, 8.2)
AE leading to dose modification of study drug	48 (23.8)	30 (14.9)	8.9 (1.3, 16.6)
AE leading to interruption of study drug	29 (14.4)	21 (10.4)	4.0 (-2.5, 10.4)
AE leading to reduction of study drug	22 (10.9)	12 (5.9)	5.0 (-0.4, 10.3)

Event Category	SPA 400 mg N=202 n (%)	IRB 300 mg N=202 n (%)	SPA 400 mg vs. IRB 300 mg Risk Difference (%) (95% CI)
Any AE	166 (82.2)	147 (72.8)	9.4 (1.3, 17.5)
Severe and worse	14 (6.9)	11 (5.4)	1.5 (-3.2, 6.2)
Moderate	75 (37.1)	67 (33.2)	4.0 (-5.3, 13.3)
Mild	77 (38.1)	69 (34.2)	4.0 (-5.4, 13.3)

Source: adae.xpt; Software: R
TEAE defined as any AE that newly appears, increases in frequency, or worsens in severity following initiation of study medication, but within 30 days after the last dose.
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Severity as assessed by the investigator.
Abbreviations: AE, adverse event; CI, confidence interval; IRB, irbesartan; N, number of patients in treatment arm; n, number of patients with at least one event; SAE, serious adverse event; SPA, sparsentan; TEAE, treatment-emergent adverse event

7.6.2. Deaths

There were no deaths in the PROTECT study by the data cutoff date.

7.6.3. Serious Treatment-Emergent Adverse Events

The proportion of subjects who experienced an SAE in the PROTECT study was similar in the sparsentan (13.9%) and irbesartan (13.4%) groups. In general, any imbalances observed for individual preferred terms (PTs) between sparsentan versus irbesartan in an evaluation of SAEs under the broad FMQs were consistent with sparsentan's mechanism of action (Table 22). The SAE PTs with a RD >0.5% for sparsentan versus irbesartan were acute kidney injury (RD 1.5%) and chronic kidney disease (RD 1.0%). Events of chronic kidney disease are not thought to be due to treatment and are likely reflective of worsening underlying kidney disease. Narratives for SAEs associated with AESIs are provided in Section 7.6.6 and additional narratives for key SAEs are provided in Section 17.1.

Table 22. Subjects With Serious Treatment-Emergent Adverse Events by System Organ Class and Broad FDA Medical Query, Safety Population, Study PROTECT

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
Gastrointestinal disorders			
Nausea	2 (1.0%)	0 (0.0%)	1.0 (-0.4, 2.4)
Small intestinal obstruction	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Vomiting	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Renal and urinary disorders			
Acute kidney injury	5 (2.5%)	3 (1.5%)	1.0 (-1.7, 3.7)
Acute kidney injury	4 (2.0%)	1 (0.5%)	1.5 (-0.7, 3.6)
General disorders and administration site conditions			
Fall	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Dizziness	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Hypotension	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
Gastrointestinal Disorders			
Vomiting	2 (1.0%)	0 (0.0%)	1.0 (-0.4, 2.4)
Small intestinal obstruction	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Vomiting	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Respiratory, thoracic, and mediastinal disorders			
Pneumonitis	2 (1.0%)	0 (0.0%)	1.0 (-0.4, 2.4)
Pleural effusion	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Pneumonitis	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Nervous system disorders			
Syncope	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Dizziness	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Hypotension	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)

Source: adslir8, adaeir8; Software: R
FMQ RD >0.5%
PT RD >0.2%
TEAE defined as any AE that newly appears, increases in frequency, or worsens in severity following initiation of study medication, but within 30 days after the last dose.
FMQ version v2
Difference is shown between sparsentan and irbesartan
Abbreviations: N, number of patients in treatment arm; n, Number of patients with an event; CI, confidence interval; AE, adverse event; TEAE, treatment-emergent adverse event

7.6.4. Adverse Events Leading to Treatment Discontinuation

In the PROTECT study, more subjects in the sparsentan group (7.9%) experienced TEAEs leading to permanent discontinuation of study drug than in the irbesartan group (4.5%). PTs with a RD >0.5% for sparsentan versus irbesartan were acute kidney injury (RD 1.5%), chronic kidney disease (RD 1.0%), and alanine aminotransferase increased (RD 1.0%). In general, the imbalances for drug discontinuation observed in an evaluation of broad FMQs for sparsentan versus irbesartan were not unexpected (Table 23).

Table 23. Subjects With Broad FDA Medical Query Leading to Treatment Discontinuation by Treatment Arm, Safety Population, Study PROTECT

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
Renal and urinary disorders			
Acute kidney injury	5 (2.5%)	2 (1.0%)	1.5 (-1.1, 4.0)
Acute kidney injury	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Blood creatinine increased	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
General disorders and administration site conditions			
Fall	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Dizziness	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Orthostatic hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
Nervous system disorders			
Syncope	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Dizziness	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Orthostatic hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hepatobiliary disorders			
Hepatic Injury	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Alanine aminotransferase increased	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Aspartate aminotransferase increased	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Vascular disorders			
Hypotension	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Orthostatic hypotension	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)

Source: adslir8, adaeir8; Software: R
FMQ RD >0.5%
PT RD >0.2%
TEAE defined as any AE that newly appears, increases in frequency, or worsens in severity following initiation of study medication, but within 30 days after the last dose.
FMQ version v2
Difference is shown between sparsentan and irbesartan
Abbreviations: N, number of patients in treatment arm; n, Number of patients with an event; CI, confidence interval; AE, adverse event; TEAE, treatment-emergent adverse event

7.6.5. Treatment-Emergent Adverse Events

In general, for the PROTECT study, analyses of the sparsentan versus irbesartan groups among queries of TEAEs (individual PTs, broad FMQs, and broad SMQs) were consistent with each other. An analysis of broad FMQs (Table 24) showed that most of the observed imbalances for the sparsentan versus irbesartan groups were for AESIs, which was consistent with the mechanism of action of sparsentan (see Section 7.6.6 for details).

More subjects had the PT of fatigue in the sparsentan (7.9%) than the irbesartan (3.0%) group (Table 24). There were no fatigue SAEs, severe AEs, or AEs leading to treatment discontinuation. Most of the events were mild to moderate in severity. Among the four subjects on sparsentan with moderate fatigue, two subjects experienced fatigue on the same day as other AEs: one subject also had cough and abdominal discomfort, and the other subject also had dizziness, malaise, and nausea.

An analysis of broad FMQs revealed an imbalance for the sparsentan versus irbesartan groups for the following TEAEs that were not AESIs: nausea, malignancy, diabetic ketoacidosis, and hypersensitivity. There was no clear mechanistic basis for these findings, and overall, the findings were not concerning. Further details for these TEAEs, including narrative summaries, are provided in Section 17.1.

Table 24. Broad FMQ With Risk Difference >2%, Safety Population, Study PROTECT

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
General disorders and administration site conditions			
Fall	54 (26.7%)	26 (12.9%)	13.9 (6.2, 21.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Dizziness	31 (15.3%)	14 (6.9%)	8.4 (2.3, 14.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Peripheral edema	26 (12.9%)	13 (6.4%)	6.4 (0.7, 12.2)
Edema peripheral	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Peripheral swelling	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Fatigue	22 (10.9%)	12 (5.9%)	5.0 (-0.4, 10.3)
Fatigue	16 (7.9%)	6 (3.0%)	5.0 (0.6, 9.3)
Nervous system disorders			
Syncope	47 (23.3%)	20 (9.9%)	13.4 (6.2, 20.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Somnolence	18 (8.9%)	6 (3.0%)	5.9 (1.4, 10.5)
Fatigue	16 (7.9%)	6 (3.0%)	5.0 (0.6, 9.3)
Cardiac disorders			
Arrhythmia	32 (15.8%)	22 (10.9%)	5.0 (-1.7, 11.6)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Heart failure	32 (15.8%)	19 (9.4%)	6.4 (-0.0, 12.9)
Edema peripheral	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Peripheral swelling	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Ear and labyrinth disorders			
Vertigo	28 (13.9%)	13 (6.4%)	7.4 (1.6, 13.3)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Vascular disorders			
Hypotension	26 (12.9%)	14 (6.9%)	5.9 (0.1, 11.7)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Gastrointestinal Disorders			
Pancreatitis	14 (6.9%)	7 (3.5%)	3.5 (-0.9, 7.8)
Lipase increased	10 (5.0%)	4 (2.0%)	3.0 (-0.6, 6.5)
Vomiting	14 (6.9%)	7 (3.5%)	3.5 (-0.9, 7.8)
Nausea	8 (4.0%)	4 (2.0%)	2.0 (-1.3, 5.3)
Renal and urinary disorders			
Acute kidney injury	24 (11.9%)	14 (6.9%)	5.0 (-0.7, 10.6)
Acute kidney injury	8 (4.0%)	2 (1.0%)	3.0 (-0.0, 6.0)
Gastrointestinal disorders			
Nausea	13 (6.4%)	6 (3.0%)	3.5 (-0.6, 7.6)
Nausea	8 (4.0%)	4 (2.0%)	2.0 (-1.3, 5.3)
Hepatobiliary disorders			
Hepatic Injury	13 (6.4%)	7 (3.5%)	3.0 (-1.3, 7.2)
Gamma-glutamyltransferase increased	6 (3.0%)	3 (1.5%)	1.5 (-1.4, 4.4)
Neoplasms benign, malignant, and unspecified (incl cysts and polyps)			
Malignancy	6 (3.0%)	0 (0.0%)	3.0 (0.6, 5.3)
Basal cell carcinoma	3 (1.5%)	0 (0.0%)	1.5 (-0.2, 3.2)

System Organ Class *FDA Medical Query (Broad)* Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)
Blood and lymphatic system disorders			
Anemia	11 (5.4%)	6 (3.0%)	2.5 (-1.4, 6.4)
Anemia	8 (4.0%)	5 (2.5%)	1.5 (-2.0, 4.9)
Endocrine disorders			
Diabetic ketoacidosis	7 (3.5%)	2 (1.0%)	2.5 (-0.4, 5.3)
Immune system disorders			
Hypersensitivity	22 (10.9%)	17 (8.4%)	2.5 (-3.3, 8.2)
Pruritus	9 (4.5%)	4 (2.0%)	2.5 (-1.0, 5.9)
Asthma	4 (2.0%)	0 (0.0%)	2.0 (0.1, 3.9)

Source: adslir8, adaeir8; Software: R
FMQ RD >2%
PT RD >1%
TEAE defined as any AE that newly appears, increases in frequency, or worsens in severity following initiation of study medication, but within 30 days after the last dose
FMQ version v2
Difference is shown between sparsentan and irbesartan
Abbreviations: AE, adverse event; CI, confidence interval; N, number of patients in treatment arm; n, Number of patients with an event; TEAE, treatment-emergent adverse event

7.6.6. Adverse Events of Special Interest

Adverse events of special interest (AESI) for sparsentan were based on the known and potential risks of sparsentan, and included hepatotoxicity, fluid retention, hypotension, acute kidney injury, hyperkalemia, tachycardia, and anemia. The initial broad FMQ analysis revealed an unexpected imbalance for pancreatic-associated AEs, and these events were analyzed further (see below).

7.6.6.1. Hepatotoxicity

Adverse Events

Hepatoxicity is an AESI for sparsentan given its mechanism of action (i.e., endothelin receptor antagonist). Bosentan and macitentan are both FDA-approved ERAs for the treatment of PAH and have a known risk of hepatotoxicity that is described in labeling. In the PROTECT study, there was an imbalance between the sparsentan (6.4%) and irbesartan (3.5%) groups for the broad SMQ of hepatic disorders (Table 25). Analyses using the narrow SMQ of hepatic disorders and the broad and narrow FMQs of hepatic injury were consistent with the results of the analysis of the broad SMQ of hepatic disorders.

In the PROTECT study, there were no hepatic injury-related SAEs in the sparsentan group. Two subjects (Subjects (b) (6) and (b) (6)) in the sparsentan group discontinued study treatment due to a hepatic injury-related AE. Both events were considered potentially related to sparsentan by the investigator. One subject (Subject (b) (6)) in the sparsentan group had a severe AE of ALT increased, which was considered possibly related to the study medication by the investigator. See the case level analysis subsection below for more details.

Table 25. Broad SMQ of Hepatic Disorders, Safety Population, Study PROTECT

Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	13 (6.4%)	7 (3.5%)	3.0 (-1.3, 7.2)
Gamma-glutamyltransferase increased	6 (3.0%)	3 (1.5%)	1.5 (-1.4, 4.4)
Alanine aminotransferase increased	8 (4.0%)	6 (3.0%)	1.0 (-2.6, 4.6)
Blood alkaline phosphatase abnormal	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Cholestasis	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hepatic steatosis	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hepatitis	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Hypoalbuminemia	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Aspartate aminotransferase increased	4 (2.0%)	5 (2.5%)	-0.5 (-3.4, 2.4)
Serious	0 (0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Other	0 (0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Resulting in discontinuation	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Relatedness			
Related	2 (1.0%)	0 (0.0%)	1.0 (-0.4, 2.4)
Possibly related	5 (2.5%)	3 (1.5%)	1.0 (-1.7, 3.7)
Unlikely related	4 (2.0%)	4 (2.0%)	0.0 (-2.7, 2.7)
Not related	5 (2.5%)	1 (0.5%)	2.0 (-0.4, 4.3)
Maximum severity			
Mild	10 (5.0%)	5 (2.5%)	2.5 (-1.2, 6.2)
Moderate	2 (1.0%)	2 (1.0%)	0.0 (-1.9, 1.9)
Severe	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Outcome			
Recovered	8 (4.0%)	5 (2.5%)	1.5 (-2.0, 4.9)
Recovering	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Sequelae	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Not recovered	3 (1.5%)	2 (1.0%)	0.5 (-1.7, 2.7)

Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest; SMQ, standardized medical dictionary for regulatory query

Analysis of Liver-Related Laboratory Parameters

In the PROTECT study, there was no significant difference in mean change from baseline over time for most liver-related laboratory parameters between the sparsentan and irbesartan groups, except for alkaline phosphatase (ALP) and bilirubin (Figure 5 and Figure 6). Elevations in ALT ≥3x ULN during the treatment period (regardless of baseline level) occurred in 2.5% of subjects in the sparsentan group compared to 2% of subjects in the irbesartan group. The proportion of patients who shifted from < upper limit of normal (ULN) at baseline to >3× ULN at any time postbaseline for liver-related laboratory parameters was similar in the two groups (Table 26).

Figure 5. Mean Alkaline Phosphatase Change From Baseline Over Time, Safety Population, Study PROTECT

Source: FDA
Abbreviations: IRB, irbesartan; SPA, sparsentan

Figure 6. Mean Total Bilirubin Change From Baseline Over Time, Safety Population, Trial Study PROTECT

Source: FDA
Abbreviations: IRB, irbesartan; SPA, sparsentan

Table 26. Shift From Less than ULN at Baseline to >3× ULN at Any Time Postbaseline, Safety Population, Study PROTECT

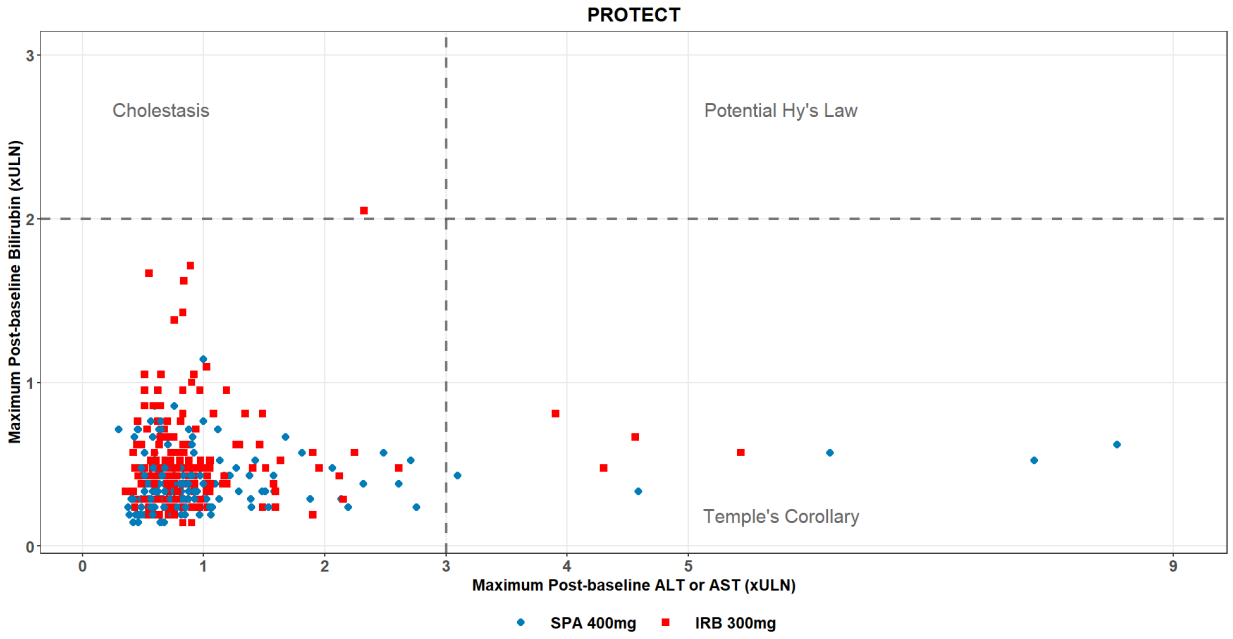
Laboratory Parameter	Sparsentan n/N (%)	Irbesartan n/N (%)	Absolute Risk Difference (95.0% CI)[1]
Alkaline phosphatase (IU/L)	1 / 202 (0.5%)	0 / 202 (0%)	0.5 (-0.5, 1.5)
Alanine aminotransferase (U/L)	3 / 202 (1.5%)	2 / 202 (1.0%)	0.5 (-1.7, 2.7)
Aspartate aminotransferase (U/L)	2 / 202 (1.0%)	1 / 202 (0.5%)	0.5 (-1.2, 2.2)
Bilirubin (umol/L)	0 / 202 (0%)	0 / 202 (0%)	0 (0, 0)

Source: adslir8, adlb; Software: R
1Difference is shown between sparsentan and irbesartan
Abbreviations: CI, Confidence Interval; N, number of patients in treatment arm; n, Number of patients with an event; ULN, upper limit of normal

Cholestatic Drug-Induced Liver Injury Screening Plot

There were no potential Hy's law cases or subjects with jaundice (i.e., serum total bilirubin >2× ULN) in the sparsentan group in the PROTECT study (Figure 7). Although the total number of cases meeting Temple's Corollary was the same for both the sparsentan and irbesartan groups (three subjects each), there were more subjects in the sparsentan group who had ALT or AST >5× ULN compared to irbesartan (Figure 7).

Figure 7. Cholestatic Drug-Induced Liver Injury Screening Plot, Safety Population, Study PROTECT



Source: adlb.xpt; Software: R
Each data point represents a patient plotted by their maximum ALT or AST versus their maximum total bilirubin values in the postbaseline period. All patients with at least one postbaseline ALT or AST and bilirubin are plotted.
A potential Hy's Law case was defined as having any postbaseline total bilirubin equal to or exceeding 2× ULN within 30 days after a postbaseline ALT or AST equal to or exceeding 3× ULN, and ALP less than 2× ULN
Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; IRB, irbesartan; SPA, sparsentan; ULN, upper limit of normal

Case Level Analysis

To further evaluate the signal for hepatotoxicity, potential drug-induced liver injury (DILI) cases were identified from the PROTECT, DUET,[5] and DUPLEX[6] studies. Ten potential DILI cases were reviewed by a hepatologist in the Division of Hepatology and Nutrition (DHN). These cases either met the Temple's corollary criteria and/or were associated with significant liver-related SAEs. As discussed above, no subjects had serum liver test elevations that met Hy's Law.

Of the 10 DILI cases, five were considered as probably related (rows 1 through 5 in Table 27), three as possibly related (rows 6 through 8 in Table 27), and two as unlikely related to sparsentan by DHN. Both of the unlikely related cases (Subjects (b) (6) and (b) (6); DUET) had only modest ALT elevations (3 to 5×ULN) and the elevations were resolving while the subjects remained on sparsentan. The cause of these elevations is unknown.

For subjects with DILI events that were thought to be probably or possibly related to sparsentan, the pattern of injury was largely hepatocellular. There were two cases of mixed or cholestatic injuries, both of which were thought by DHN to be possibly related to sparsentan. The latency period for each probable or possible DILI event after initiation of sparsentan was long. The mean latency period was 216 days (approximately 7 months) and was skewed by one probable DILI case with a latency period of 406 days (approximately 13 months). The median latency period was 168 days (approximately 5 months). DILI with a long latency period was also observed for other ERAs (bosentan, macitentan, ambrisentan), although the period observed for sparsentan was longer than for these other agents. In general, the elevations in liver enzymes resolved/were resolving with discontinuation of sparsentan but reoccurred with sparsentan rechallenge.

[5] The DUET study is a randomized, double-blind, multicenter, phase 2 study in patients with FSGS. The study evaluated the effect of sparsentan (200, 400, or 800 mg once daily) compared with irbesartan (300 mg once daily) to lower the UP/C after 8 weeks of treatment.

[6] The DUPLEX study is an ongoing global phase 3, multicenter, randomized, double-blind, active-controlled study to evaluate the efficacy and safety of sparsentan (800 mg once daily) versus irbesartan (300 mg once daily) in patients with primary or genetic FSGS. As of the data cutoff date (January 22, 2021), the study is fully enrolled, with 371 subjects randomized and having received at least one dose of double-blind treatment (irbesartan or sparsentan). Safety data is available from the pre-specified interim analysis at Week 36.

Table 27. Summary Demographics and Serum Liver Tests for Cases With Probable or Possible DILI Cases Due to Sparsentan, Studies PROTECT, DUET, and DUPLEX

#	ID	Causality Score*	Alternate diagnosis	Study	Age (yr)	Sex	Race	Hy's Law	Latency from start drug (da)	Latency from stop drug (da)	ALT peak (U/L)	AST peak (U/L)	ALP peak (U/L)^	Bilirubin peak (mg/dL)	R value peak (ALT)	R value peak (AST)
1	(b) (6)	3	NA	021IGAN17001 PROTECT	47	M	White	No	168	-85	805	480	104	1.6	23.7	14.1
2		3	NA	021IGAN17001 PROTECT	27	M	White	No	257	-118	277	89	104	0.6	8.1	2.6
3		3	NA	021IGAN17001 PROTECT	55	M	White	No	166	-2	350	144	113	0.8	9.5	3.9
4		3	NA	021IGAN17001 PROTECT	54	M	Latinx	No	406	-6	188	76	104	0.3	5.5	2.2
5		3	NA	021FSGS16010	42	M	Latinx	No	82	-22	759	504	104	0.6	22.3	14.8
6		4	Gallstone disease	021IGAN17001 PROTECT	72	M	White	No	174	-3	322	177	104	0.6	9.5	5.2
7		4	Unknown	RET-D-001 DUET	65	F	White	No	28	0	179	103	191	0.5	2.9	1.6
8		4	Acute hepatitis C	RET-D-001 DUET	38	M	White	No	319	-30	1288	420	155	0.6	25.4	8.3
				Mean	*50*				*200*	*-33*	*521*	*249*	*122*	*0.7*	*13.4*	*6.6*
				Std dev	*13.7*				*115*	*41*	*368*	*173*	*31*	*0.4*	*8.4*	*4.9*
				Median	*50.5*				*171*	*-14*	*336*	*161*	*104*	*0.6*	*9.5*	*4.6*
				Min	*27*				*28*	*-118*	*179*	*76*	*104*	*0.3*	*2.9*	*1.6*
				Max	*72*				*406*	*0*	*1288*	*504*	*191*	*1.6*	*25.4*	*14.8*

*1=definite, 2=highly likely, 3=probable, 4=possible, 5=unlikely, 6=indeterminate
^For R-value calculations, ULN of 104 U/L for ALP imputed when peak ALP remained normal
NA = not applicable
Source: FDA
Abbreviations: ALP, alkaline phosphatase; DILI, drug-induced liver injury; ULN, upper limit of normal

The narratives for the five probable and one possible DILI case are summarized below.

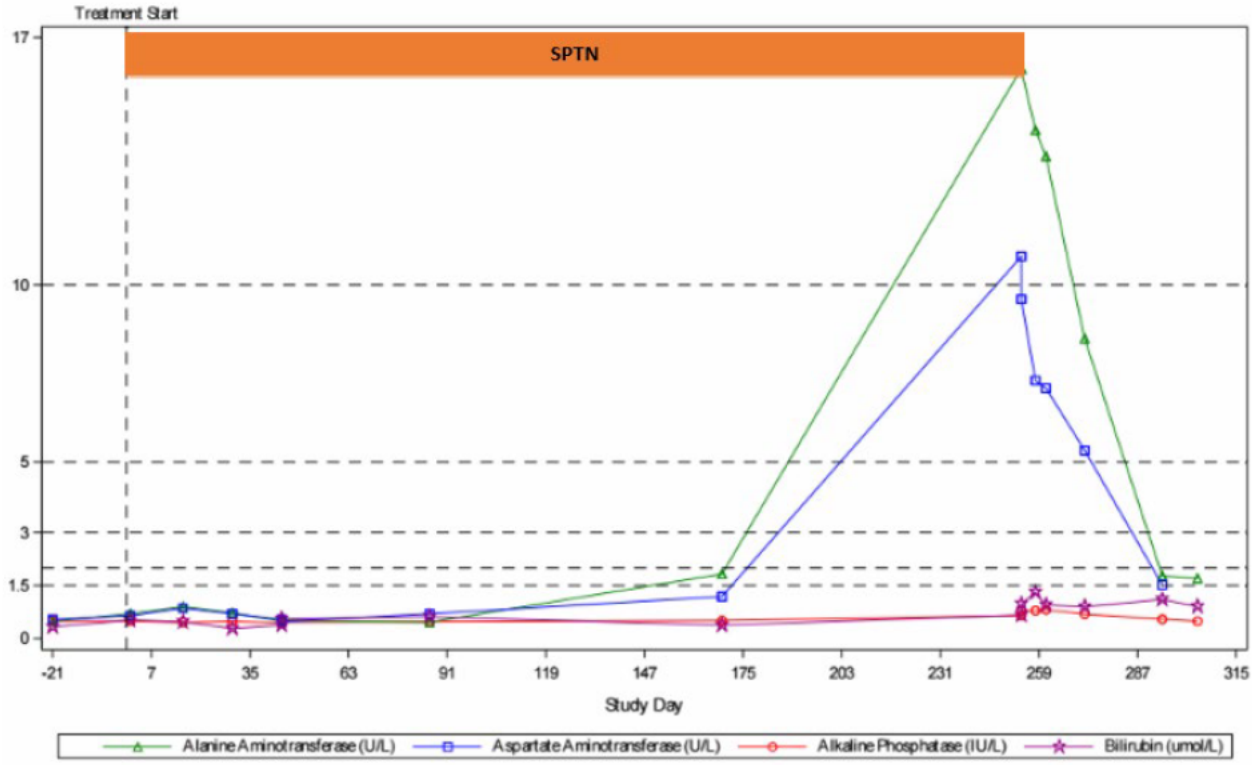
Subject (b) (6) (PROTECT)

The subject was a 47-year-old white male with IgAN. The subject's baseline medication history included irbesartan, beclomethasone-formoterolo (inhaler), ramipril, and ursodeoxycholic acid. He had received an mRNA COVID-19 vaccine (date unknown). At baseline, his ALT was 29 U/L, AST was 24 U/L, ALP was 64 IU/L, and total bilirubin (TB) was 0.64 mg/dL. He started sparsentan on (b) (6). On (b) (6) (Day 169), his ALT increased to 75 U/L, AST increased to 44 U/L, and ALP and TB remained within normal limits. He had no associated symptoms and his sparsentan was continued. At the next laboratory check on (b) (6) (Day 254), ALT was 661 U/L and subsequently increased to 806 U/L on recheck later that same day. His AST was 480 U/L and his ALP and TB remained within normal limits. The subject denied initiation of new medications (prescription or over the counter) or herbal supplements and denied recent alcohol intake. His sparsentan was permanently discontinued. After discontinuation of sparsentan, his TB later peaked at 1.58 mg/dL (reference ULN =1.2 mg/dL) but did not reach jaundice level (i.e., 2×ULN). His direct bilirubin also increased from <0.18 mg/dL to 0.41 mg/dL (reference ULN =0.3 mg/dL). While remaining off of sparsentan, his liver enzyme levels decreased by 50% within 40 days but did not return to normal by the last available follow-up data (Figure 8). His HAV IgM, HBsAg, anti-HBc IgM, anti-HCV antibody and anti-EBV IgM were negative. His liver ultrasound was normal. No other liver evaluation tests were available.

DHN Assessment

This case is assessed as probably due to sparsentan based on the partial resolution after sparsentan was discontinued. The DILI onset is thought to be when the ALT increased to 75 U/L from his baseline of 29 U/L. This was the only observed case with an associated TB elevation to > ULN.

Figure 8. Serum Liver Test Over Time for Subject [redacted] (b) (6), Study PROTECT



Source: Applicant
Abbreviations: SPTN, sparsentan

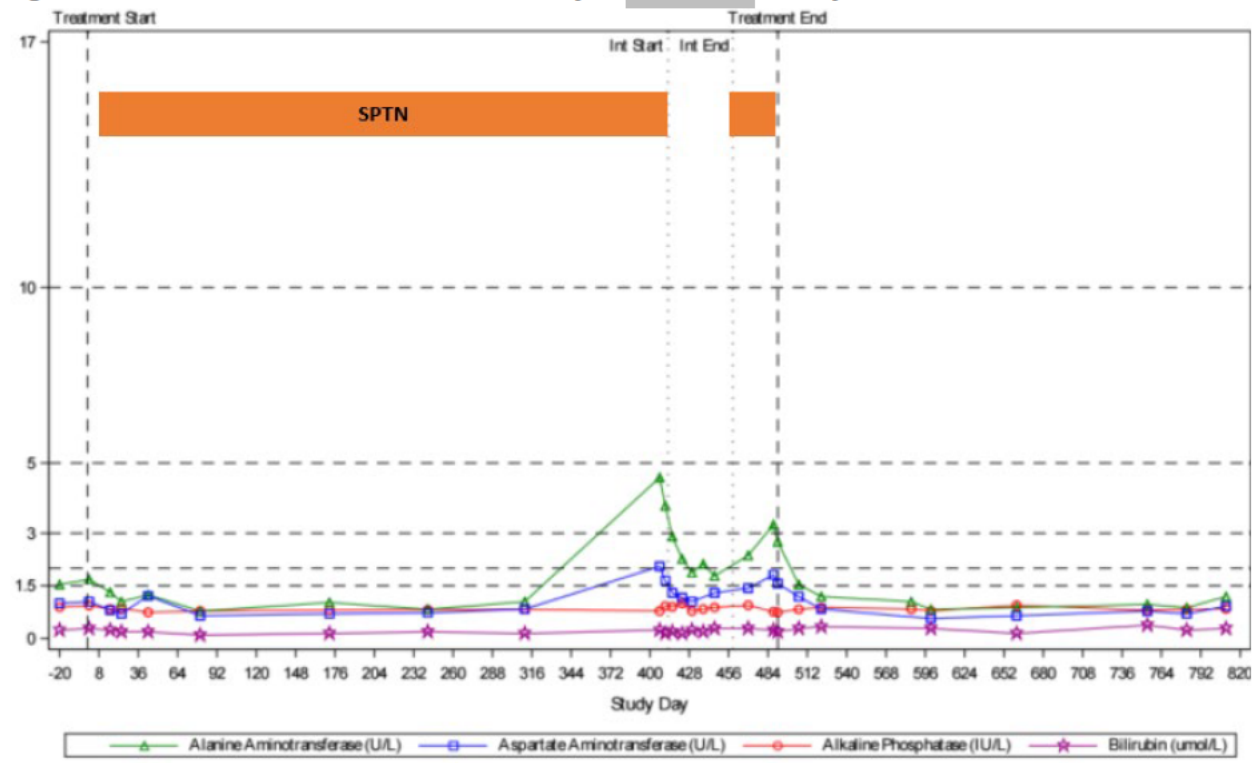
Subject [redacted] (b) (6) (PROTECT)

The subject was a 54-year-old Hispanic male with IgAN. At baseline, he had a relevant medical history of hepatitis A infection (over 40 years prior). At baseline, his ALT was 69 U/L, AST was 39 U/L, ALP was 121 IU/L, TB was 0.3 mg/dL, and BMI was 30.7 kg/m^2. He started sparsentan on [redacted] (b) (6). His ALT decreased from the modestly elevated screening level shortly thereafter. On [redacted] (b) (6) (Day 407), he had an asymptomatic elevation in his transaminases (TAs). Sparsentan was temporarily discontinued on [redacted] (b) (6) (Day 413). The TAs decreased and sparsentan was restarted on [redacted] (b) (6) (Day 470), which was followed by an immediate increase in ALT (133 U/L) and AST (67 U/L) (Figure 9). Sparsentan was permanently discontinued on [redacted] (b) (6) (Day 490). The liver ultrasound showed steatosis, and no other liver-evaluation tests were reported.

DHN Assessment

This case is difficult to assess because of the very long latency period and lack of information on further liver evaluation testing. However, it is assessed as probably due to sparsentan because of the reoccurrence of TA elevation after restarting sparsentan.

Figure 9. Serum Liver Test Over Time for Subject [redacted] (b) (6), Study PROTECT



Source: Applicant
Abbreviations: SPTN, sparsentan

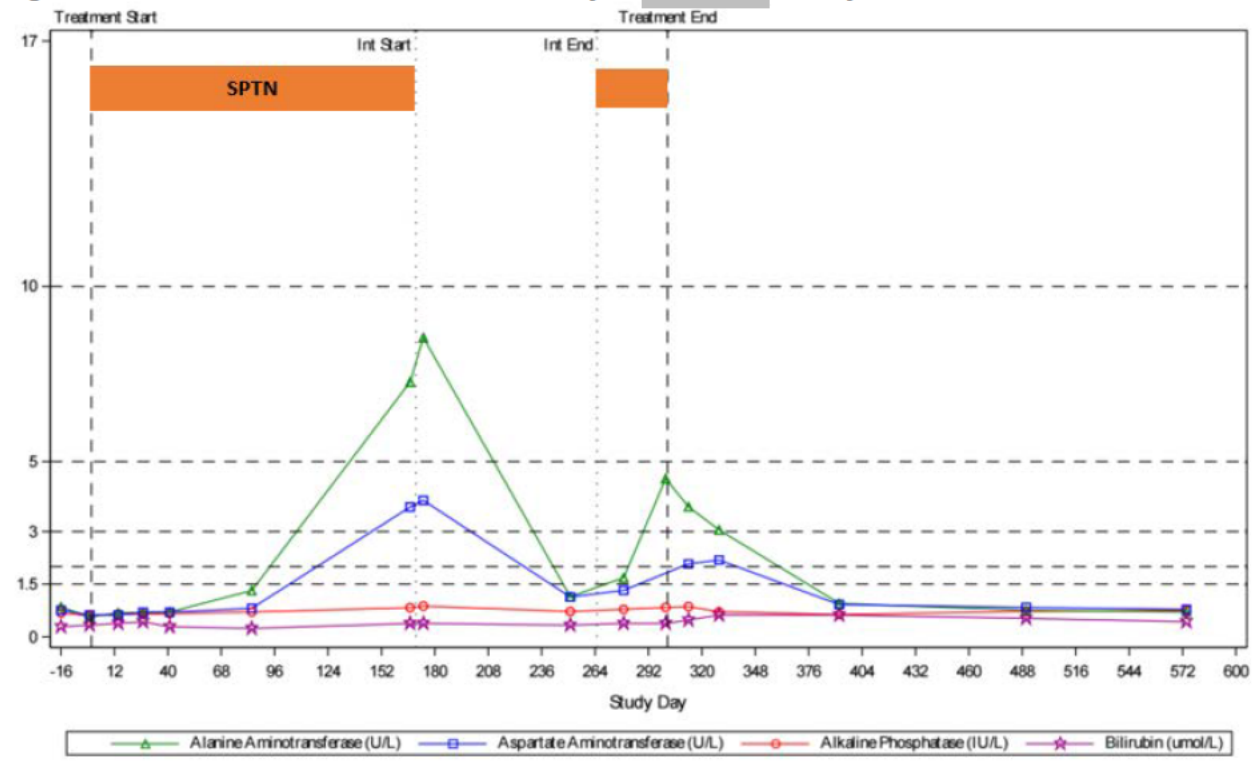
Subject [redacted] (b) (6) (PROTECT)

The subject was a 55-year-old white man with IgAN. His baseline liver tests included ALT 23 U/L, AST 23 U/L, ALP 77 IU/L, and "normal" TB. He started sparsentan on [redacted] (b) (6). On [redacted] (b) (6) (Day 84), his ALT increased from 29 U/L to 54 U/L. Treatment with sparsentan continued. On [redacted] (b) (6) (Day 167), his was noted to have asymptomatic "elevation" of his TAs. Sparsentan was temporarily discontinued on [redacted] (b) (6) (Day 169), and his TAs subsequently decreased. Liver ultrasound imaging was unremarkable. His HAV IgM, anti-HBc IgM and anti-HCV antibodies were negative. No other evaluation tests were noted. Sparsentan was restarted on [redacted] (b) (6) (Day 266), and his liver enzymes increased again. Sparsentan was discontinued permanently on [redacted] (b) (6) (Day 302), after which the enzymes fell back to normal range (Figure 10).

DHN Assessment

This case is thought to be probably due to sparsentan because of the positive rechallenge.

Figure 10. Serum Liver Test Over Time for Subject [redacted] (b) (6), Study PROTECT



Source: Applicant
Abbreviations: SPTN, sparsentan; ULN, upper level of normal

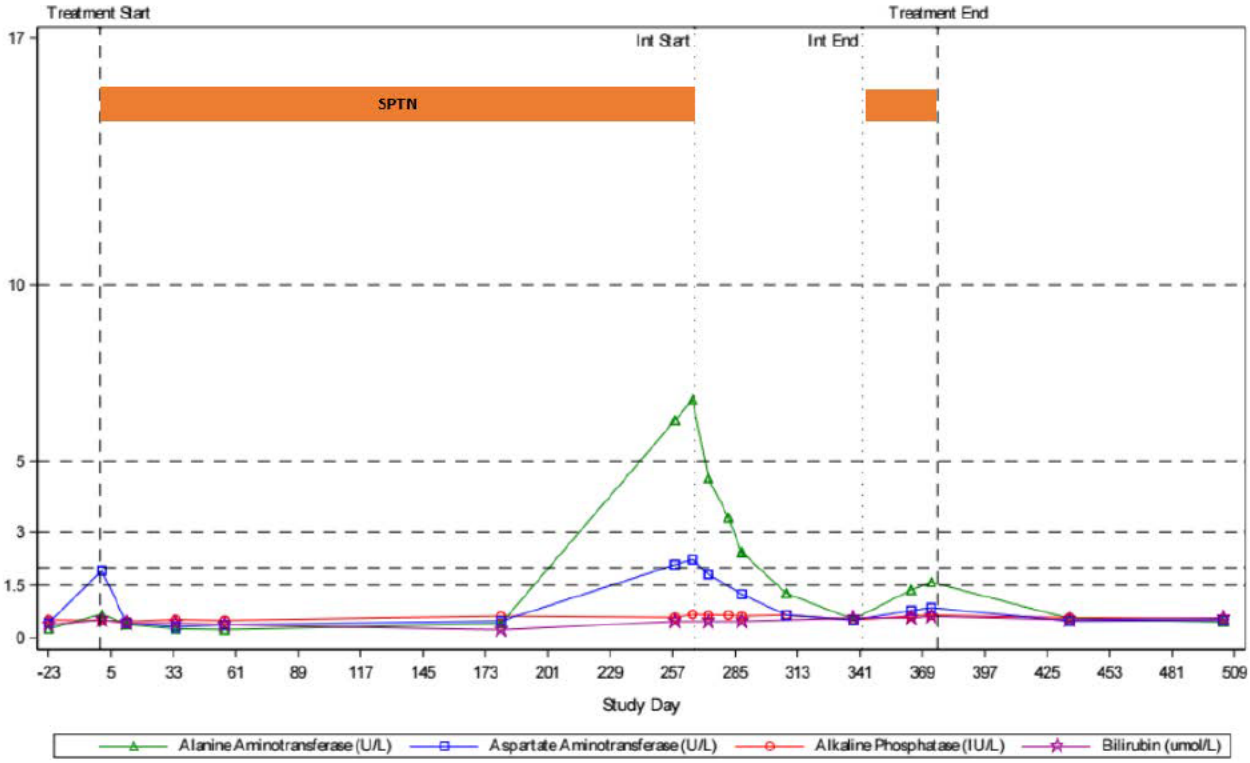
Subject [redacted] (b) (6) (PROTECT)

The subject was a 27-year-old white male with IgAN. At baseline, his ALT was 28 U/L, AST was 71 U/L, ALP was 65 IU/L, and TB was "normal." On [redacted] (b) (6), he started sparsentan. On [redacted] (b) (6) (Day 258), his ALT was 253 U/L, AST 77 U/L, ALP 104 U/L, and TB 0.6 mg/dL. His sparsentan was continued; however, on [redacted] (b) (6) (Day 266), his ALT and AST peaked at 277 U/L and 89 U/L respectively, and his sparsentan was temporarily discontinued. Thereafter, his liver enzymes fell back to normal range. His sparsentan was restarted on [redacted] (b) (6) (Day 343). Shortly after, his liver enzymes began to increase again, and sparsentan was permanently discontinued on [redacted] (b) (6) (Day 376) (Figure 11). No other liver evaluation tests were performed.

DHN Assessment

This case is thought to be probably due to sparsentan because of the reoccurrence of elevated TAs after restarting sparsentan.

Figure 11. Serum Liver Test Over Time for Subject [redacted] (b) (6), Study PROTECT



Source: Applicant
Abbreviations: SPTN, sparsentan

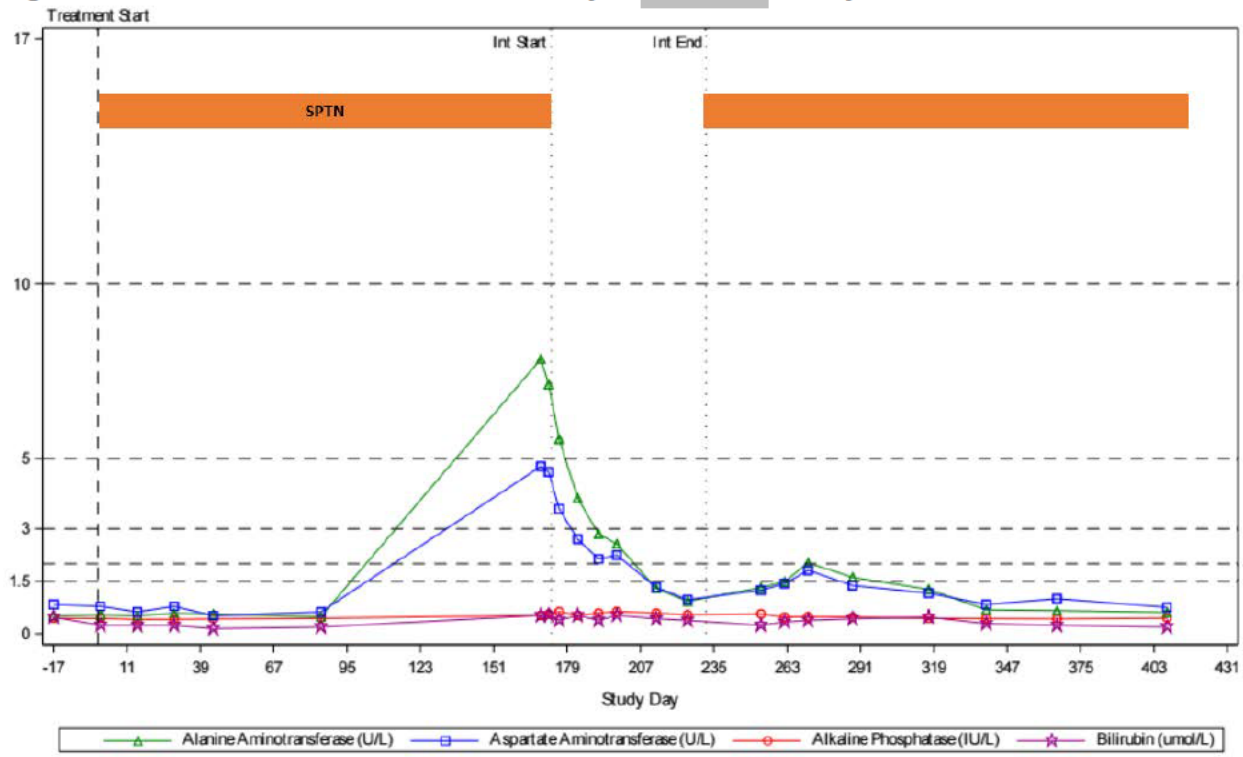
Subject [redacted] (b) (6) (PROTECT)

The subject was a 72-year-old white male with IgAN. At baseline his liver tests were all "normal." He started sparsentan on [redacted] (b) (6). On [redacted] (b) (6), (Day 175), ALT and AST levels increased to 322 U/L and 177 U/L without symptoms. Sparsentan was temporarily discontinued on [redacted] (b) (6), (Day 178). His liver enzymes fell to back to normal range on [redacted] (b) (6) (Day 231), and sparsentan was restarted on [redacted] (b) (6) (Day 239). His liver enzymes subsequently increased and then stabilized despite continued exposure to sparsentan (Figure 12). No further evaluation testing was done.

DHN Assessment

The pattern of liver enzyme elevation after restarting sparsentan appears to be consistent with a positive rechallenge followed by the development of tolerance. Figure 12 shows that the liver enzymes appeared to modestly decrease even before sparsentan was initially discontinued. This case is notable for possible tolerance to the event of DILI with sparsentan.

Figure 12. Serum Liver Test Over Time for Subject [redacted] (b) (6), Study PROTECT



Source: Applicant
Abbreviations: SPTN, sparsentan

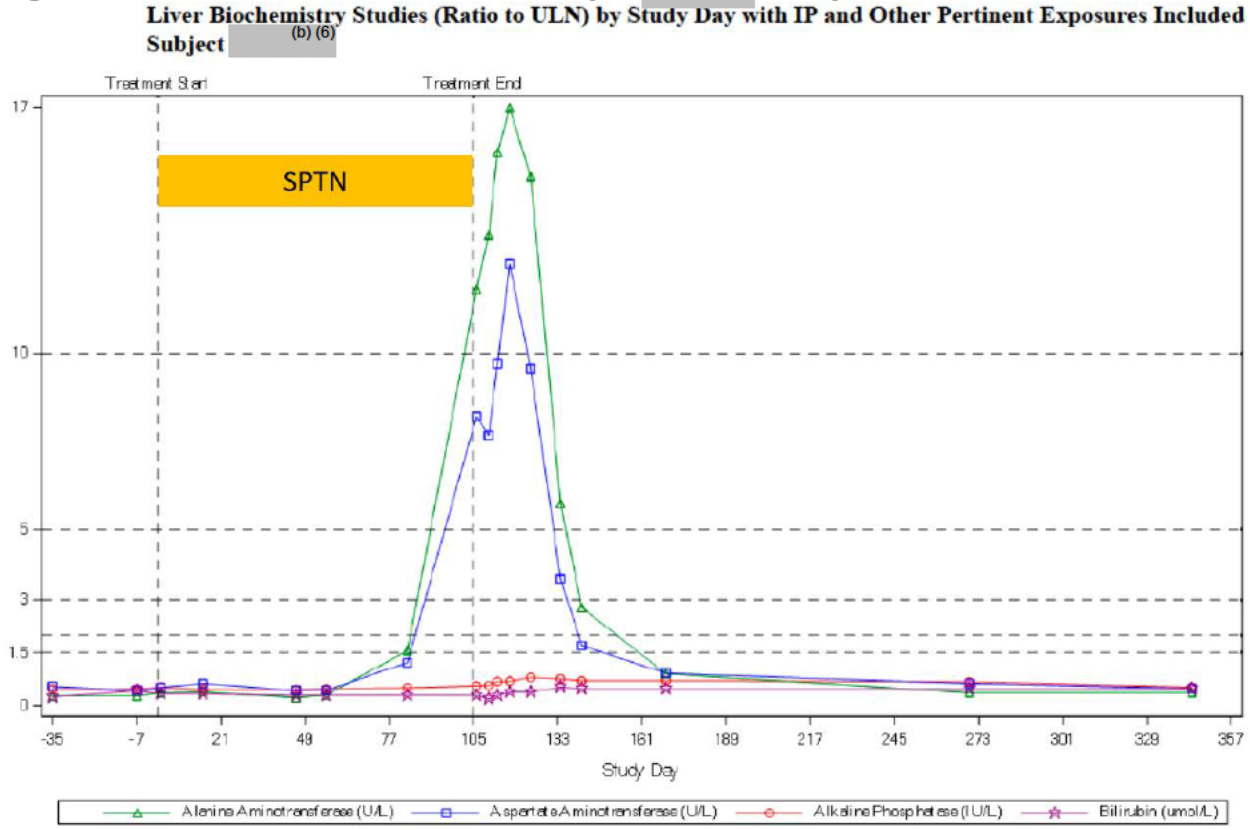
Subject [redacted] (b) (6) (DUPLEX)

The subject was a 42-year-old white male with FSGS. At baseline, his ALT was 11 U/L, AST was 20 U/L, and ALP and TB were "normal." He started sparsentan on [redacted] (b) (6). On [redacted] (b) (6) (Day 83), his ALT increased to 64 U/L and AST increased to 45 U/L. ALP and TB remained within normal range. His sparsentan was discontinued on Day 105. However, his liver tests continued to increase, peaking on [redacted] (b) (6) (Day 120) before falling back to baseline. Liver evaluation tests included unremarkable liver ultrasound, negative acute hepatitis A, B and C serologies, and negative ANA. The patient reported no new medications. Information on alcohol use was not provided. Assessment of CMV, EBV, or HEV was not performed. The patient remained asymptomatic throughout.

DHN Assessment

This case is thought to be probably due to sparsentan given the resolution after discontinuation of sparsentan and no obvious alternate etiology.

Figure 13. Serum Liver Test Over Time for Subject (b) (6), Study DUPLEX



Source: Applicant
Abbreviations: SPTN, sparsentan; ULN, upper level of normal

7.6.6.2. Fluid Retention

Adverse Events

Fluid retention is a known risk of ERAs. In the PROTECT study, there was an imbalance in the narrow FMQ of "peripheral edema" between the sparsentan (12%) and irbesartan (6%) groups (Table 28). There were no associated SAEs, severe AEs, or AEs leading to discontinuation. Most subjects were assessed as recovered or were recovering by the interim data lock. An analysis by baseline eGFR level did not suggest a greater risk in subjects with lower levels of kidney function.

Analyses of the narrow SMQ of "haemodynamic oedema, effusions and fluid overload" showed similar results as the analysis of the narrow FMQ of "peripheral edema." An additional analysis of the narrow FMQ of "dyspnea" (PTs: dyspnoea and dyspnoea exertional) showed no imbalance between the sparsentan and irbesartan arms (incidence of 2.5% in both arms).

Table 28. Narrow FMQ of Peripheral Edema, Safety Population, Study PROTECT

Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Edema peripheral	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Peripheral swelling	3 (1.5%)	0 (0.0%)	1.5 (-0.2, 3.2)
Serious	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Resulting in discontinuation	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Maximum severity			
Mild	20 (9.9%)	9 (4.5%)	5.4 (0.4, 10.5)
Moderate	5 (2.5%)	4 (2.0%)	0.5 (-2.4, 3.4)
Severe	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Outcome	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Recovered	20 (9.9%)	10 (5.0%)	5.0 (-0.1, 10.0)
Recovering	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Not recovered	2 (1.0%)	2 (1.0%)	0.0 (-1.9, 1.9)
Baseline eGFR level (mL/min/1.73 m^2)			
n/N (%)			5.9(0.3, 11.6)
<45	8/82 (9.8%)	7/80 (8.8%)	1.0(-7.9, 9.9)
>=45 to <60	8/45 (17.8%)	2/49 (4.1%)	13.7(1.2, 26.2)
>=60	9/75 (12.0%)	4/73 (5.5%)	6.5(-2.5, 15.5)

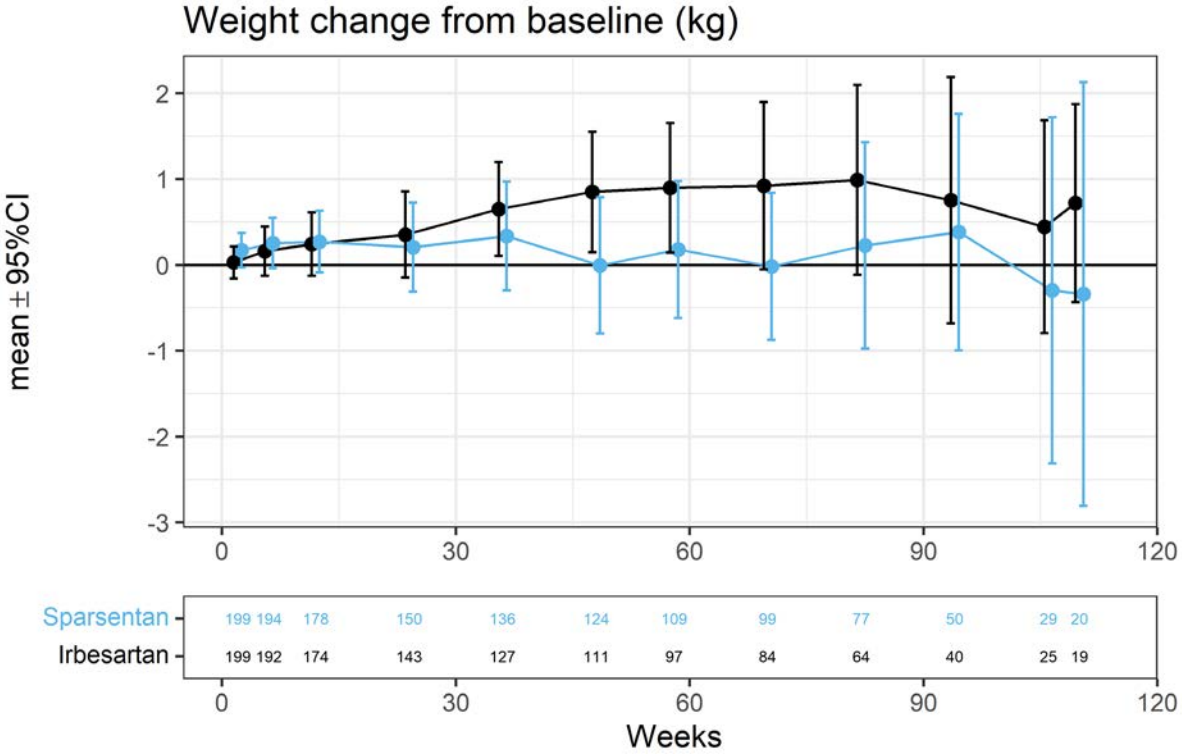
Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest; eGFR, estimated glomerular filtration rate; FMQ, FDA medical query

Weight Gain

The mean weight change from baseline was similar between the two treatment groups (Figure 14) in the PROTECT study. The distributions of maximum weight change from baseline for sparsentan and irbesartan were, for the most part, overlapping (Figure 15). Clinically significant weight gain (i.e., >5 kg) from baseline was observed in 30 (15%) versus 26 (13%) subjects in the sparsentan versus irbesartan arms, respectively.

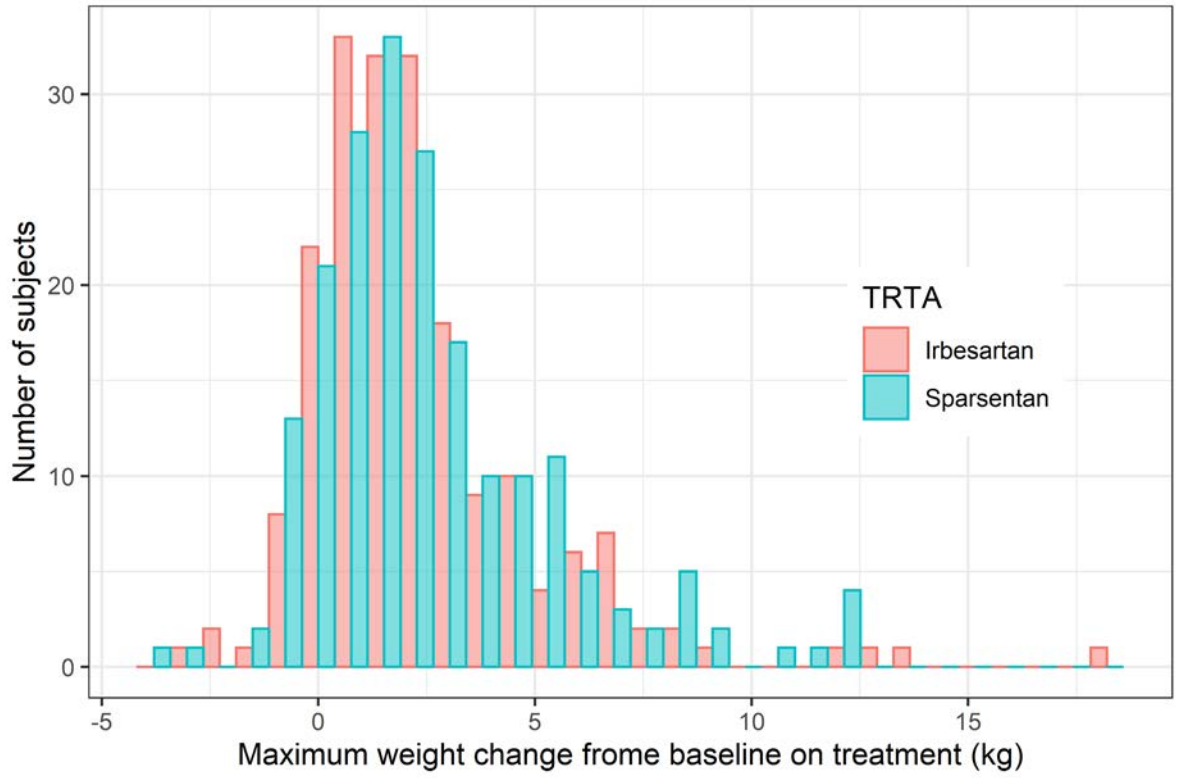
Two subjects had nonserious “weight increased” TEAEs, both in the sparsentan arm. One event was graded as mild and one was reported to be moderate in severity.

Figure 14. Mean Weight Change From Baseline Over Time. Safety Population, Study PROTECT



Source: FDA

Figure 15. Distribution of Maximum Weight Change From Baseline. Safety Population, Study PROTECT



Source: FDA

7.6.6.3. Symptomatic Hypotension

Adverse Events

Hypotension is a known risk of ERAs and ARBs. In the PROTECT study, there was an imbalance in TEAEs related to symptomatic hypotension (Applicant-defined grouped query) for the sparsentan (24%) versus irbesartan (10%) group, which was driven by the PTs of dizziness and hypotension (Table 29). An analysis of the narrow FMQ of hypotension, which includes a subset of the PTs in the Applicant-defined grouped query (PTs: hypotension, orthostatic hypotension, and systolic blood pressure decreased), showed a similar imbalance (13% sparsentan versus 5% irbesartan).

There were three SAEs in the sparsentan group for the Applicant-defined grouped query of symptomatic hypotension: one event of hypotension, one event of moderate dizziness, and one event of mild dizziness. For the event of hypotension, study drug was discontinued (Subject (b) (6), narrative summary below). The two AEs of dizziness were not considered related to study drug by the investigators and no action was taken with respect to study drug. The event of moderate dizziness was confounded by underlying anemia (attributed to chronic disease), hypoglycemia, and weakness (from prolonged fasting for a scheduled procedure) (Subject (b) (6); see Section 7.6.6.7 Anemia for the narrative). The event of mild dizziness was thought by the investigator to be due to blood pressure fluctuations from emotional stress.

There were three symptomatic hypotension (grouped query) events for patients in the sparsentan group that led to treatment discontinuation (see narrative summaries below). Two of these subjects had moderate AEs of symptomatic hypotension (PTs: orthostatic hypotension and dizziness). The investigators considered the events to be related/possibly related to study drug.

- Subject (b) (6): A 68-year-old Asian female had a severe SAE of hypotension. She had a home systolic blood pressure reading of 57 mm Hg after she fainted in the bathroom 3 to 6 hours after the first dose of sparsentan (baseline BP: 140/80 mm Hg). She had been experiencing diarrhea, vomiting, and abdominal pain prior to the event. She was admitted to the hospital and her blood pressure at the time of admission was 115/75 mm Hg with heart rate of 54 bpm. During hospitalization, she experienced diarrhea, vomiting, and blood in her stool. Digital rectal exam showed a small external hemorrhoid but no evidence of further rectal bleed or melena. She discontinued sparsentan as a result of the event. The investigator assessed this event as possibly related to the study drug.
- Subject (b) (6): A 42-year-old Asian female experienced a mild AE of orthostatic hypotension (per investigator). On Day 169, the patient's sitting BP was 107/75 mm Hg and heart rate (HR) was 74 bpm (baseline BP 123/87 mm Hg; baseline HR 81 bpm). Assessments of blood pressure and heart rate with a change in position were not provided. Sparsentan was reduced to 200 mg as a result of the event. On Day 207, her BP (sitting) was 96/60 mm Hg and HR 81 bpm, and the event was classified as moderate severity. Study treatment was permanently discontinued as a result of the event. On Day 285, the event was considered resolved (BP: 133/90 mm Hg). The investigator considered this event as related to study treatment.

- Subject [redacted] (b) (6): A 35- year-old white male experienced a moderate AE of dizziness (i.e., lightheadedness) that occurred during work from crouching to standing or during climbing ladders and depressed mood (low mood and concentration) on Day 115 after initiation of sparsentan. Sparsentan was permanently discontinued on Day 148 as a result of the events. On Day 219, the events were considered resolved. The investigator considered the events to be possibly related to study treatment.

Table 29. Applicant-Defined Symptomatic Hypotension, Safety Population, Study PROTECT

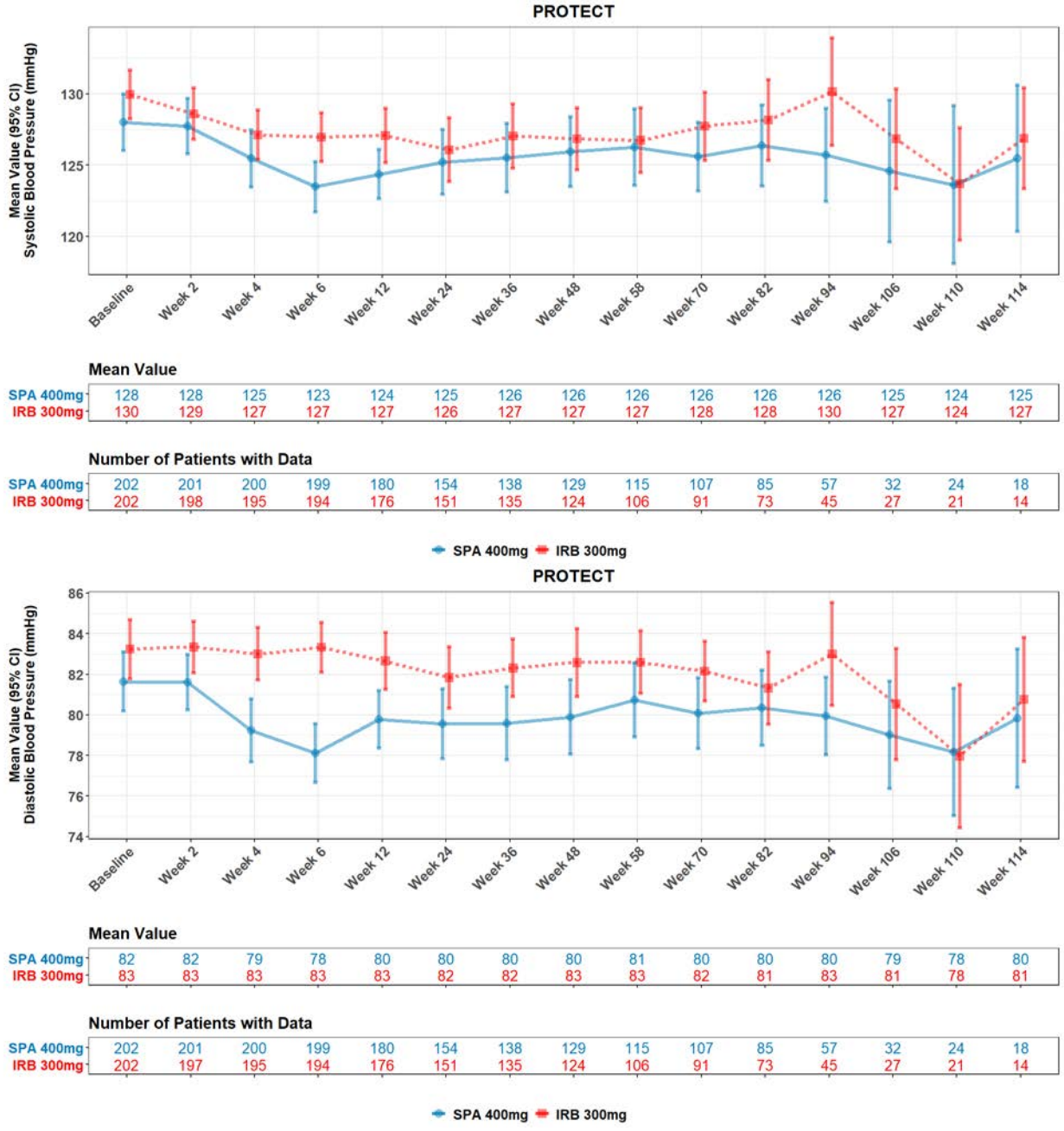
Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	49 (24.3%)	21 (10.4%)	13.9 (6.6, 21.1)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Orthostatic hypotension	7 (3.5%)	5 (2.5%)	1.0 (-2.3, 4.3)
Blood pressure systolic decreased	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)
Dizziness postural	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Syncope	1 (0.5%)	3 (1.5%)	-1.0 (-2.9, 0.9)
Serious	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Requiring hospitalization	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Resulting in discontinuation	3 (1.5%)	0 (0.0%)	1.5 (-0.2, 3.2)
Maximum severity			
Mild	32 (15.8%)	17 (8.4%)	7.4 (1.1, 13.8)
Moderate	16 (7.9%)	4 (2.0%)	5.9 (1.7, 10.1)
Severe	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Outcome	49 (24.3%)	21 (10.4%)	13.9 (6.6, 21.1)
Recovered	41 (20.3%)	21 (10.4%)	9.9 (2.9, 16.9)
Recovering	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Not recovered	5 (2.5%)	0 (0%)	2.5 (0.3, 4.6)

Source: adslir8, adaeir8; Software: R
Abbreviations AE, adverse event; AESI, adverse event of special interest

Blood Pressure

In the PROTECT study, mean systolic blood pressure over time was not substantially different between the sparsentan and the irbesartan groups. However, mean diastolic blood pressure (DBP) over time appeared to be lower in the sparsentan as compared to the irbesartan group (nadir at Week 6) (Figure 16). More patients had a DBP <60 mmHg in the sparsentan arm than the irbesartan arm (Table 30).

Figure 16. Mean and 95% Confidence Interval of Systolic Blood Pressure and Diastolic Blood Pressure Over Time by Treatment Arm, Safety Population, Study PROTECT



Source: advs.xpt; Software: R
Vertical bars show 95% confidence intervals.
Abbreviations: CI, confidence interval; IRB, irbesartan; SPA, sparsentan

Table 30. Percentage of Subjects Meeting Specific Hypotension Levels Postbaseline, Safety Population, Study PROTECT

Blood Pressure (mm Hg)	SPA 400 mg N=202 n/N_w (%)	IRB 300 mg N=202 n/N_w (%)	SPA 400 mg vs. IRB 300 mg Risk Difference (%) (95% CI)
SBP <90	6/202 (3.0)	2/202 (1.0)	2.0 (-0.7, 4.7)
DBP <60	31/202 (15.3)	16/202 (7.9)	7.4 (1.2, 13.6)

Source: advs.xpt; Software: R
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; DBP, diastolic blood pressure; IRB, irbesartan; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; SBP, systolic blood pressure; SPA, sparsentan

7.6.6.4. Acute Kidney Injury

Adverse Events

In the PROTECT study, there was an imbalance for the narrow FMQ of acute kidney injury (AKI) between the sparsentan (4%) and irbesartan (1.5%) groups (Table 31), which was driven by the PT of AKI. The narrow SMQ of acute renal failure, which also included the additional PT of "renal impairment," showed similar results to the narrow AKI FMQ (3% sparsentan versus 2.5% irbesartan). AKI is not an unexpected risk of sparsentan.

In the sparsentan group, four subjects experienced SAEs of AKI (PT) and three subjects experienced AKI (PT) events that led to treatment discontinuation. Below are brief narrative summaries these events. Most of the AKI events included concurrent illness. Given sparsentan's mechanism of action (i.e., angiotensin II receptor antagonist), it is possible that patients are at higher risk of AKI with sparsentan when they are also hypovolemic or have a concurrent illness.

- Subject (b) (6): A 36-year-old white male experienced an SAE of acute kidney injury from study Day 121 to Day 122 that led to sparsentan dose reduction. The patient's baseline creatinine was 1.88 mg/dL. From Day 37 to Day 116, the patient's creatinine measurements ranged from 1.62 mg/dL to 1.96 mg/dL. On Day 120, the patient's creatinine was 2.14 mg/dL (2.29 mg/dL on repeat). The investigator instructed the patient to reduce his sparsentan dosage from 400 mg to 200 mg once daily and to present to the emergency department (ED). On Day 121, the subject presented to the ED (creatinine 2.26 mg/dL). The patient experienced nausea and diarrhea prior to admission (number of days unknown). Treatment included IV sodium chloride. On Day 122, his creatinine decreased to 1.54 mg/dL, which was considered back to baseline, and intravenous fluids were discontinued. The patient was discharged from the hospital and the event was considered resolved. The investigator assessed the serious event of acute kidney as possibly related to study medication. The investigator also noted nausea and vomiting as other causes for the event. The patient experienced another event of AKI (moderate) on Day 665 (creatinine 2.13 mg/dL, eGFR 38 mL/min/1.73 m^2). Sparsentan was discontinued due to the event. The event was considered possibly related to the study drug by the investigator. Additional details regarding the event were not provided.
- Subject (b) (6): A 62-year-old white male experienced an SAE of acute kidney injury on study Day 85 to Day 155 which led to sparsentan dose interruption. On Day 65, the patient developed cough, shortness of breath, fevers, and chills. On Day 85 (narrative incorrectly stated Day 75), a chest x-ray revealed a right-sided basal pneumonia. Laboratory tests were

consistent with AKI (creatinine 2.0 mg/dL, baseline 1.16 mg/dL). The patient was admitted to the hospital for antibiotic treatment and intravenous medications. Some of the patient's antihypertensive medications and diuretics were discontinued and study medication was temporarily discontinued. The patient was discharged 2 days later. Study medication was restarted on Day 107. On Day 155, the event was considered resolved (laboratory results not reported). The investigator assessed the SAE of AKI as possibly related to study drug. The investigator identified pre-existing illness and progression of disease under study as other possible causes of the event.

- Subject (b) (6): An 18-year-old white male experienced an "unconfirmed" SAE of AKI from study Day 30 to Day 34 that led to drug interruption. On Day 30, the patient experienced a high creatinine level of 3.91 mg/dL on routine laboratory tests (baseline 1.24 mg/dL). On Day 33, after the alert was received for the high creatinine level, the patient was immediately contacted and asked to go to the hospital. On Day 34, the patient went to the hospital as requested and had no complaints upon admission. Physical exam was unremarkable. Laboratory tests at that time included serum creatinine 1.13 mg/dL, eGFR 84.5 mL/min/1.73 m^2, and trace amount of proteinuria on urinalysis. The event was considered resolved. Laboratory error was suspected. Study medication was interrupted from Day 34 to Day 36 due to the event.
- Subject (b) (6): A 70-year-old Asian male experienced ongoing severe acute kidney injury that started on study Day 571; sparsentan was permanently discontinued on the same day due to the event. The patient's baseline eGFR was 58 mL/min/1.73 m^2 and his eGFR prior to the event was 45-51 mL/min/1.73 m^2 (dates not provided). Laboratory results on Day 571 included: eGFR 26 mL/min/1.73 m^2, creatinine was 2.41 mg/dL, white blood cell count $133\times10^3/\mu L$, hemoglobin 5.8 g/dL, and platelet count $92\times10^3/\mu L$. On study Day 583, the patient was diagnosed with myelodysplastic syndrome with myeloproliferative disease (confirmed by bone marrow biopsy). The investigator assessed the SAE of acute kidney injury due to myelodysplastic syndrome as unlikely related to study drug.
- Subject (b) (6): A 26-year-old white female experienced an ongoing severe acute kidney injury that started on study Day 183; drug was permanently discontinued on the same day due to the event. The patient's baseline creatinine was 2.09 mg/dL and baseline eGFR was 32 mL/min/1.73 m^2. Laboratory test results on Day 183 included creatinine 4.7 mg/dL and eGFR 12 mL/min. The investigator assessed the event of acute kidney injury as not related to study treatment. Further details of the event were not provided.

Table 31. Narrow FMQ of Acute Kidney Injury, Safety Population, Study PROTECT

Adverse Event	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	8 (4.0%)	3 (1.5%)	2.5 (-0.7, 5.6)
Acute kidney injury	8 (4.0%)	2 (1.0%)	3.0 (-0.0, 6.0)
Prerenal failure	0 (0.0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Serious	4 (2.0%)	2 (1.0%)	1.0 (-1.4, 3.3)
Requiring hospitalization	3 (1.5%)	2 (1.0%)	0.5 (-1.7, 2.7)
Other	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)

Adverse Event	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
Resulting in discontinuation	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Maximum severity			
Mild	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Moderate	3 (1.5%)	2 (1.0%)	0.5 (-1.7, 2.7)
Severe	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Outcome	8 (4.0%)	3 (1.5%)	2.5 (-0.7, 5.6)
Recovered	5 (2.5%)	2 (1.0%)	1.5 (-1.1, 4.0)
Recovering	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Not recovered	1 (0.5%)	1 (0.5%)	0.0 (-1.4, 1.4)
Baseline eGFR level (mL/min/1.73 m^2) n/N (%)			2.5 (-0.7, 5.6)
<45	2 / 82 (2.4%)	3 / 80 (3.8%)	-1.3 (-6.6, 4.0)
>=45 to <60	3 / 45 (6.7%)	0 / 49 (0%)	6.7 (-0.6, 14.0)
>=60	3 / 75 (4.0%)	0 / 73 (0%)	4.0 (-0.4, 8.4)

Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest; eGFR, estimated glomerular filtration rate; FMQ, FDA medical query

Creatinine

An analysis of serum creatinine values exceeding specified levels compared to the last known value was unremarkable for sparsentan compared to irbesartan (Table 32).

Table 32. Subjects with One or More Serum Creatinine Values Exceeding Specified Levels Compared to the Last Known Value, Safety Population, Study PROTECT

Creatinine, high (mg/dL)	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
Level 1 (>1.5× last known value)	4 (2.0%)	9 (4.5%)	-2.5 (-5.9, 1.0)
Level 2 (>2.0× last known value)	1 (0.5%)	2 (1.0%)	-0.5 (-2.2, 1.2)
Level 3 (>3.0× last known value)	1 (0.5%)	0 (0.0%)	0.5 (-0.5, 1.5)

Source: adslir8, adaeir8; Software: R
Difference is shown between sparsentan and irbesartan
Abbreviations: N, number of patients in treatment arm; n, Number of patients with an event; SAE, Serious Adverse Event

7.6.6.5. Hyperkalemia

Given its mechanism of action, hyperkalemia is an expected risk with sparsentan. In the PROTECT study, there was an imbalance in Applicant-defined hyperkalemia events (grouped query) between sparsentan (11%) and irbesartan (9%) (Table 33). There were no SAEs, severe AEs, or AEs leading to discontinuation for either treatment group.

There was not a substantial difference in mean potassium change from baseline between the sparsentan and irbesartan groups over time (Figure 17). There was a shift in potassium from below 6 mmol/L at baseline to above 6 mmol/L at any time postbaseline for six (3%) subjects in the sparsentan group versus three (1.5%) subjects in the irbesartan group.

Table 33. Applicant-Defined Hyperkalemia Events, Safety Population, Study PROTECT

Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	23 (11.4%)	19 (9.4%)	2.0 (-4.0, 7.9)
Hyperkalemia	21 (10.4%)	18 (8.9%)	1.5 (-4.3, 7.2)
Blood potassium increased	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Serious	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Resulting in discontinuation	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Relatedness	23 (11.4%)	19 (9.4%)	2.0 (-4.0, 7.9)
Related	3 (1.5%)	3 (1.5%)	0.0 (-2.4, 2.4)
Possibly related	13 (6.4%)	11 (5.4%)	1.0 (-3.6, 5.6)
Unlikely related	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Not related	8 (4.0%)	5 (2.5%)	1.5 (-2.0, 4.9)
Maximum severity			
Mild	12 (5.9%)	14 (6.9%)	-1.0 (-5.8, 3.8)
Moderate	11 (5.4%)	5 (2.5%)	3.0 (-0.8, 6.8)
Severe	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Outcome	23 (11.4%)	19 (9.4%)	2.0 (-4.0, 7.9)
Recovered	16 (7.9%)	14 (6.9%)	1.0 (-4.1, 6.1)
Recovering	3 (1.5%)	1 (0.5%)	1.0 (-0.9, 2.9)
Sequelae	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Not recovered	3 (1.5%)	4 (2.0%)	-0.5 (-3.0, 2.0)

Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest

Figure 17. Mean Potassium Change From Baseline Over Time, Safety Population, Study PROTECT

Source: adlb.xpt; Software: R
Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.
Abbreviations: CI, confidence interval; IRB, irbesartan; SPA, sparsentan

7.6.6.6. Tachycardia

ERAs have been associated with tachycardia, and therefore, tachycardia was evaluated as an AESI. In the PROTECT study, slightly more subjects had tachycardia-related events in the irbesartan (1.5%) compared to the sparsentan (1%) group for the narrow FMQ of tachycardia (Table 34). Additional analyses of the narrow FMQ of arrhythmia and broad SMQ of cardiac arrhythmias revealed similar findings (i.e., more events for the irbesartan compared to the sparsentan group). There were no tachycardia-related SAEs, severe AEs, or AEs leading to discontinuation.

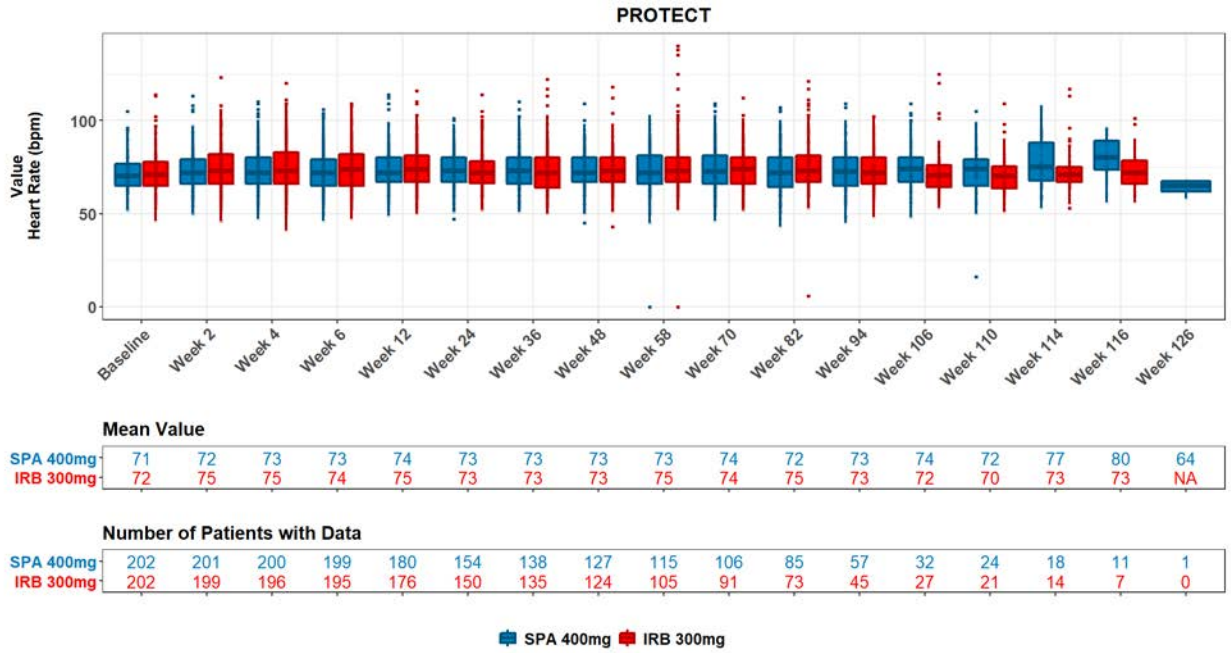
There was no difference in median HR between the two treatment groups over time (Figure 18). More subjects on irbesartan (27%) compared to sparsentan (21%) had a postbaseline HR >100 bpm at some point during the treatment period.

Table 34. Narrow FMQ of Tachycardia, Safety Population, Study PROTECT

Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)
Heart rate increased	2 (1.0%)	0 (0.0%)	1.0 (-0.4, 2.4)
Sinus tachycardia	0 (0.0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Tachycardia	0 (0.0%)	3 (1.5%)	-1.5 (-3.2, 0.2)
Serious	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Resulting in discontinuation	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Relatedness	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)
Related	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Possibly related	2 (1.0%)	2 (1.0%)	0.0 (-1.9, 1.9)
Unlikely related	0 (0.0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Not related	0 (0.0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Maximum severity			
Mild	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)
Moderate	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Severe	0 (0.0%)	0 (0.0%)	0.0 (0.0, 0.0)
Outcome	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)
Recovered	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)

Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest

Figure 18. Median and Interquartile Range of Pulse Rate Over Time by Treatment Arm, Safety Population, Study PROTECT



Source: advs.xpt; Software: R
Boxes span the interquartile range (25th to 75th percentile); horizontal lines indicate median; whiskers indicate 1.5× the interquartile range; individual outliers are those beyond this range.
Abbreviations: IRB, irbesartan; SPA, sparsentan;

7.6.6.7. Anemia

Adverse Events

There was an imbalance between sparsentan (6%) and irbesartan (3%) in Applicant-defined anemia events (grouped query) (Table 35). There were no anemia-related severe AEs or AEs leading to discontinuation of treatment. The anemia narrow FMQ, which was a subset of the Applicant-defined grouped query (excluding the PT of macrocytosis), showed similar results.

One subject in the sparsentan group had an SAE of anemia (PT):

- Subject (b) (6): A 63-year-old Asian male experienced an SAE of dizziness and anemia from study Day 240 to 251. On study Day 240, after returning home from a regular study visit, the patient experienced severe dizziness and was hospitalized. The patient had been fasting for 8 hours prior to the study visit for scheduled blood collection. A brain CT and brain MRI were unremarkable. An ECG was performed and was determined to be abnormal (details not provided). The hemoglobin level at the time of the ECG was 9.9 g/dL (baseline 14.2 g/dL). On Day 241, the hemoglobin was 8.8 g/dL. On the day of discharge (Day 244), the patient's hemoglobin was 8.7 g/dL. The patient was considered to be in "stable condition." The event of dizziness was considered resolved. On study Day 251, the hemoglobin remained at 8.7 g/dL, but the adverse event of anemia was considered "resolved." There was no change to study treatment. The investigator assessed the SAEs of dizziness and anemia as not related to study drug. The investigator noted that the patient had been fasting and that hypoglycemia and weakness could have contributed to the event of

dizziness. The investigator considered underlying disease as a cause of the patient's low hemoglobin levels.

Table 35. Applicant-Defined Anemia Events, Safety Population, Study PROTECT

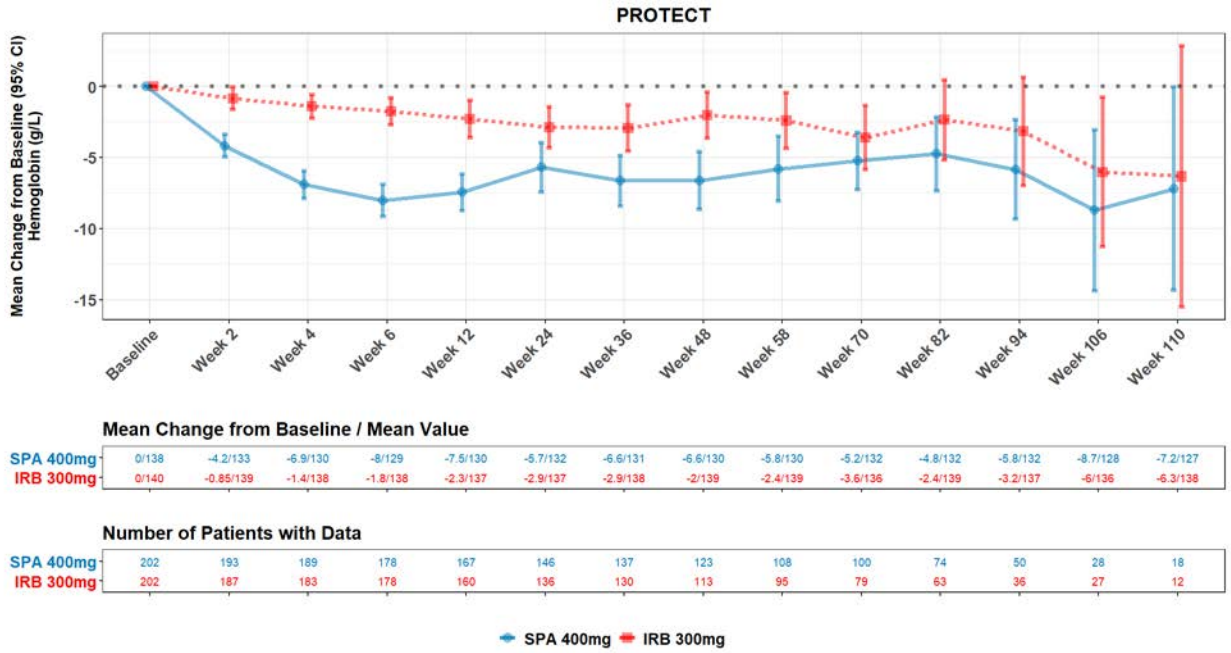
Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE Grouping Related to AESI	13 (6.4%)	6 (3.0%)	3.5 (-0.6, 7.6)
Anemia	8 (4.0%)	5 (2.5%)	1.5 (-2.0, 4.9)
Macrocytosis	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Hemoglobin decreased	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Iron deficiency anemia	2 (1.0%)	1 (0.5%)	0.5 (-1.2, 2.2)
Serious	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Requiring hospitalization	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Resulting in discontinuation	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Relatedness	13 (6.4%)	6 (3.0%)	3.5 (-0.6, 7.6)
Related	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Possibly Related	3 (1.5%)	2 (1.0%)	0.5 (-1.7, 2.7)
Unlikely Related	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Not related	9 (4.5%)	4 (2.0%)	2.5 (-1.0, 5.9)
Maximum severity			
Mild	11 (5.4%)	6 (3.0%)	2.5 (-1.4, 6.4)
Moderate	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Severe	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Outcome	13 (6.4%)	6 (3.0%)	3.5 (-0.6, 7.6)
Recovered	4 (2.0%)	2 (1.0%)	1.0 (-1.4, 3.3)
Recovering	5 (2.5%)	1 (0.5%)	2.0 (-0.4, 4.3)
Not recovered	4 (2.0%)	3 (1.5%)	0.5 (-2.0, 3.0)

Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest

Hemoglobin Decrease

Compared to the irbesartan group, the sparsentan group had a larger mean decrease from baseline in hemoglobin over time (Figure 19). More subjects in the sparsentan group also had hemoglobin decreases exceeding specified levels (Table 36). Given the mechanism of action of sparsentan, the observed changes in hemoglobin are thought to be in part due to hemodilution.

Figure 19. Mean Hemoglobin Change From Baseline Over Time, Safety Population, Study PROTECT



Source: adlb.xpt; Software: R
Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.
Abbreviations: CI, confidence interval; IRB, irbesartan; SPA, sparsentan

Table 36. Subjects With One or More Hemoglobin Values Exceeding Specified Level of Decrease From Baseline, Safety Population, Study PROTECT

Laboratory Parameter	SPA 400 mg N=202 n/N_w (%)	IRB 300 mg N=202 n/N_w (%)	SPA 400 mg vs. IRB 300 mg Risk Difference (%) (95% CI)
Hemoglobin, low (g/dL)			
Level 2 (>1.5 g/dL dec. from baseline)	74/202 (36.6)	35/201 (17.4)	19.2 (10.8, 27.7)
Level 3 (>2 g/dL dec. from baseline)	32/202 (15.8)	18/201 (9.0)	6.9 (0.5, 13.3)

Source: adlb.xpt; Software: R
Threshold levels 1, 2, and 3 as defined by the Standard Safety Tables & Figures Integrated Guide.
Risk difference (with 95% confidence interval) is shown between total treatment and comparator.
Abbreviations: CI, confidence interval; IRB, irbesartan; N, number of patients in treatment arm; n, number of patients meeting criteria; N_w, number of patients with data; SPA, sparsentan

7.6.6.8. Pancreatic-Associated AEs

Adverse Events

In the PROTECT study, there was an unexpected imbalance in the broad FMQ of pancreatitis between sparsentan (7%) and irbesartan (3.5%), driven by the PT of lipase increased (Table 37). The incidence of elevated amylase was only slightly higher for sparsentan compared to irbesartan (Table 37). Compared to the broad FMQ of pancreatitis, the broad SMQ of acute pancreatitis showed a larger imbalance between sparsentan (16%) and irbesartan (9%). The broad SMQ included more nonspecific PTs compared to the broad FMQ, such as nausea, vomiting, abdominal pain, and abdominal distension. The narrow FMQ of pancreatitis included only one PT of "pancreatitis chronic," the incidence of which was similar between the two groups. There were no acute pancreatitis events for either group under the narrow SMQ. Under the recommendation of the data monitoring committee, the Applicant reached out to external experts on pancreatitis who concluded that "not a single factor is solely responsible for AMS/lipase elevations…but a possible synergy of the following factors: low eGFR [and] concomitant medications (commonly used in the [study] population): steroids, loop diuretics, thiazide diuretics, oral contraceptives" may have played a role. Based on the available data, we believe these adverse events are unlikely to represent drug-related adverse reactions and we recommend that the observed imbalance not be included in labeling.

There were no SAEs or severe AEs for the broad FMQ of pancreatitis. One subject in the sparsentan arm discontinued treatment due to an event of mild lipase increased:

- Subject (b) (6): A 46-year-old white male experienced a mild AE of lipase increased that led to drug withdrawal. The patient's lipase at baseline was 30 U/L (normal range: 13 to 60 U/L). On Study Day 19, the patient's lipase increased to 80 U/L. The lipase further increased to 171 U/L after 10 days (Study Day 29). Sparsentan was temporarily discontinued on Day 31 due to the event. On Day 43, the patient's lipase decreased to 43 U/L and the event was considered resolved on Day 47. The study treatment was restarted on Day 55. On Day 75, the patient experienced another episode of increased lipase levels (123 U/L), which was considered mild in severity. The lipase level further increased to 212 U/L on Day 89. On Day 92, study treatment was permanently discontinued. The event of lipase increased (elevated lipase) was considered resolved on Day 173 (lipase level was 44 U/L on that day). The investigator assessed the event of lipase increased (elevated lipase) as related to study treatment.

Table 37. Broad FMQ of Pancreatitis, Safety Population, Study PROTECT

Variable	Sparsentan N=202 n(%)	Irbesartan N=202 n(%)	Absolute Risk Difference (95.0% CI)
AE grouping related to AESI	14 (6.9%)	7 (3.5%)	3.5 (-0.9, 7.8)
Lipase increased	10 (5.0%)	4 (2.0%)	3.0 (-0.6, 6.5)
Amylase increased	5 (2.5%)	4 (2.0%)	0.5 (-2.4, 3.4)
Amylase abnormal	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Lipase abnormal	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Pancreatitis chronic	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Pancreatic enzymes increased	1 (0.5%)	1 (0.5%)	0.0 (-1.4, 1.4)
Hyperamylasemia	0 (0%)	1 (0.5%)	-0.5 (-1.5, 0.5)
Serious	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Resulting in discontinuation	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Relatedness			
Related	1 (0.5%)	0 (0%)	0.5 (-0.5, 1.5)
Possibly related	6 (3.0%)	2 (1.0%)	2.0 (-0.7, 4.7)
Unlikely related	3 (1.5%)	2 (1.0%)	0.5 (-1.7, 2.7)
Not related	7 (3.5%)	3 (1.5%)	2.0 (-1.0, 5.0)
Maximum severity			
Mild	9 (4.5%)	5 (2.5%)	2.0 (-1.6, 5.5)
Moderate	5 (2.5%)	2 (1.0%)	1.5 (-1.1, 4.0)
Severe	0 (0%)	0 (0%)	0.0 (0.0, 0.0)
Outcome			
Unknown	2 (1.0%)	0 (0%)	1.0 (-0.4, 2.4)
Recovered	6 (3.0%)	4 (2.0%)	1.0 (-2.0, 4.0)
Recovering	4 (2.0%)	0 (0%)	2.0 (0.1, 3.9)
Not recovered	2 (1.0%)	3 (1.5%)	-0.5 (-2.7, 1.7)

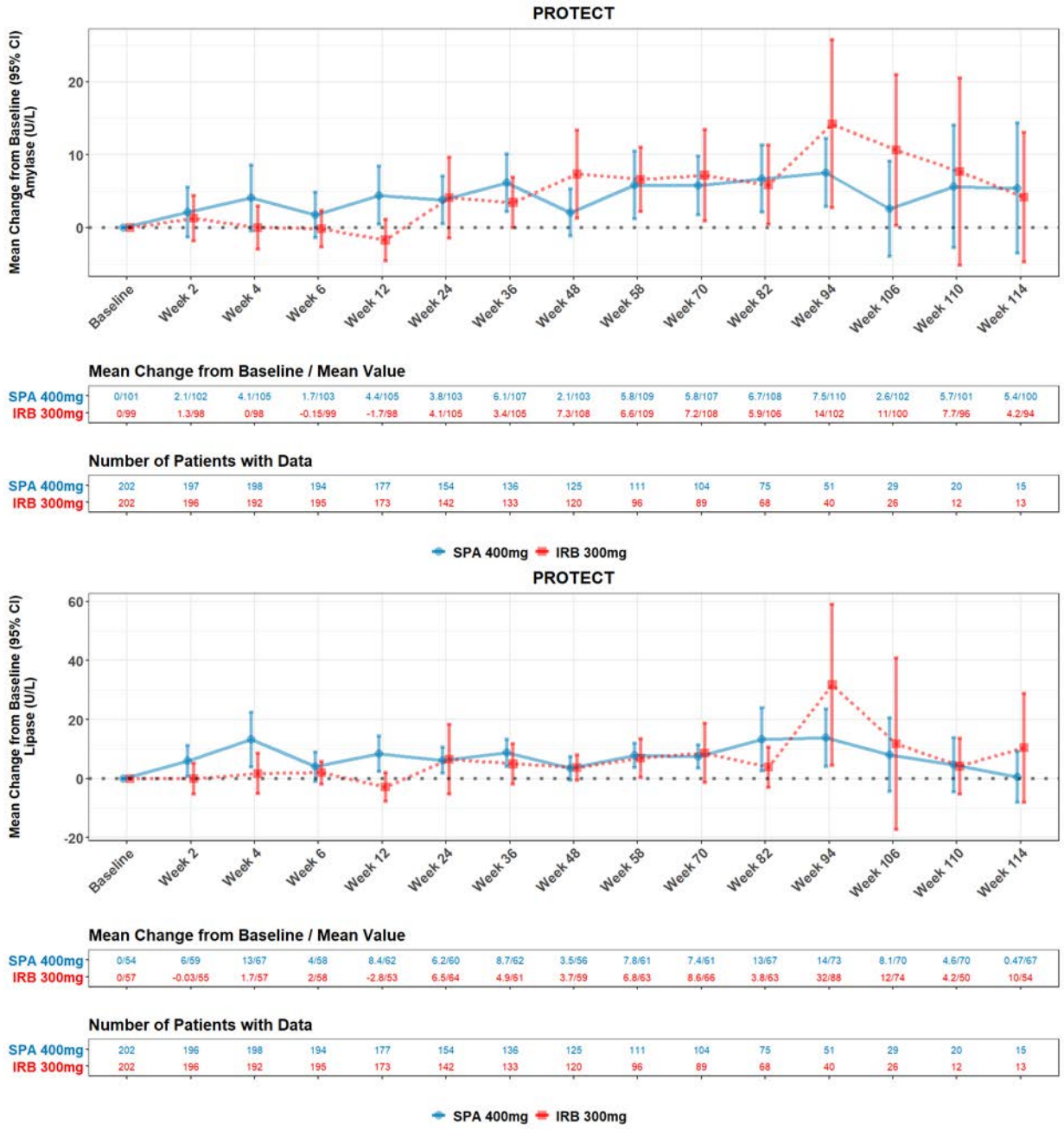
Source: adslir8, adaeir8; Software: R
Abbreviations: AE, adverse event; AESI, adverse event of special interest

Pancreatic Enzymes

There was no significant difference between sparsentan and irbesartan in mean change from baseline of amylase and lipase over time (Figure 20).

More subjects in the sparsentan group had elevated postbaseline amylase and lipase values > ULN than in the irbesartan group. Follow-up analyses revealed that most of these subjects also had baseline amylase and/or lipase values that were elevated (i.e., >ULN). Shifts of lipase level from < ULN at baseline to >3× ULN at any postbaseline visit were observed in 5 of 202 subjects (2.5%) in the sparsentan group and 2 of 202 subjects (1%) in the irbesartan group. No imbalance was observed for a similar shift analysis for amylase for either treatment group.

Figure 20. Mean Changes in Amylase and Lipase From Baseline Over Time, Safety Population, Study PROTECT



Source: adlb.xpt; Software: R
Figures do not include time points with data from fewer than 10% of randomized/enrolled patients in all treatment groups.
Abbreviations: CI, confidence interval; IRB, irbesartan; SPA, sparsentan

7.6.7. Laboratory Findings

Laboratory findings associated with AESIs are described in Section 7.6.6.

Chemistry

Analyses of other biochemistry data did not reveal findings of interest or concern.

Kidney function

There was no difference in mean change from baseline over time for creatinine or eGFR (2009 CKD-EPI) between the sparsentan and irbesartan groups. Analyses evaluating the effect of sparsentan versus irbesartan on loss of renal function over time are described in Section 6.2.

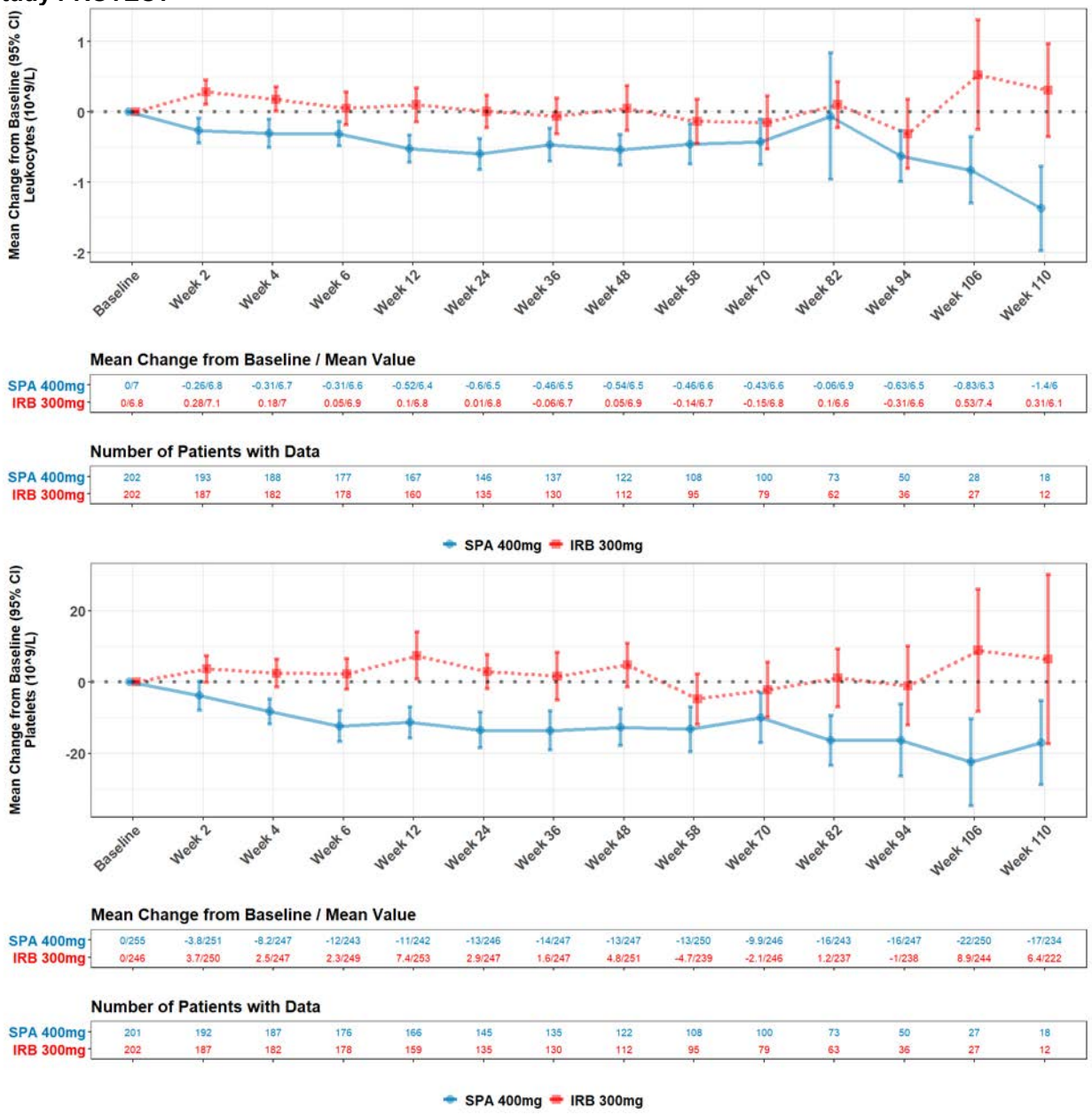
Lipids

There was no difference in change from baseline over time for any lipid lab between the sparsentan and irbesartan groups, including cholesterol, high-density lipoprotein (HDL), low-density lipoprotein, and triglycerides. In female subjects, there was an imbalance between sparsentan and irbesartan in the number of subjects with HDL <50 mg/dL. The analysis showed that 33 out of 63 female subjects (52.4%) in the sparsentan group versus 25 out of 56 female subjects (44.6%) in the irbesartan group had HDL <50 mg/dL. An imbalance was not seen for this subgroup for HDL ≤40 mg/dL. There is no clear mechanistic basis for this finding. The total number of female subjects was low in both treatment groups, and the imbalance may be due to chance.

Hematology

A difference in change from baseline over time for hematology laboratory tests between the sparsentan and irbesartan groups was seen for leukocytes, platelets (Figure 21), and hemoglobin (see Section 7.6.6.7).

Figure 21. Mean Change in Leukocytes and Platelets From Baseline Over Time, Safety Population, Study PROTECT



Source: FDA
Abbreviations: CI, confidence interval; IRB, irbesartan; SPA, sparsentan

7.6.8. Vital Signs

Vital sign findings associated with AESIs are described in Section 7.6.8. There was no significant difference between sparsentan and irbesartan in other mean/median vital signs over time.

7.6.9. Assessment for Potential QT Prolongation

ECGs were only obtained at baseline (screening) in the PROTECT study.

The potential risk for QT prolongation and Torsade de Pointe (TdP) with sparsentan was evaluated in the thorough QT study 021HVOL16002. This study was a randomized, positive-, and placebo-controlled, single-dose, four-arm, four-period crossover study to assess the QTc effects of sparsentan at therapeutic and supratherapeutic exposures in healthy patients. Therapeutic exposures were covered by the 800 mg dose which provided a mean C_{max} of 8.2 µg/mL and is similar to the mean steady state C_{max} values for the 400 mg QD dose (7.1 µg/mL). The highest dose evaluated was a single dose of 1600 mg which provided a mean C_{max} of 11.6 µg/mL and therefore covered 1.2-fold of the high clinical exposure scenario (i.e., CYP3A inhibition). Sparsentan prolonged the QTc interval by 9.0 (90% CI: 6.0 to 11.9) msec at the 800 mg dose (covering clinical exposure) and 8.2 (90% CI: 5.3 to 11.1) msec at the 1600 mg dose (covering high clinical exposure). However, the increase was not dose-dependent and there was a time-delay between peak effects on the QTc interval and maximal sparsentan concentrations. The underlying mechanism behind the observed QTc prolongation is unknown but is unlikely to be mediated via direct inhibition of the hERG channels by sparsentan. Sparsentan did not inhibit the hERG channel (hERG safety margin >2912x) and no QTc prolongation was detected in the in vivo QT study in monkeys at 6× the high clinical exposure.

7.6.10. Pregnancies During the PROTECT Study

By the data cutoff date, there were a total of five pregnancy events in four subjects in the PROTECT study.

In the sparsentan treatment group, one subject (Subject (b) (6)) became pregnant and discontinued study medication. After treatment discontinuation, the subject experienced a spontaneous abortion. The subject did not restart study medication and became pregnant again within 2 months. The pregnancy was ongoing at the time of the interim data lock date, and the estimated delivery date is (b) (6). There was also one pregnancy of a partner (Subject (b) (6)) in the sparsentan treatment group, which resulted in a normal live birth.

One subject (Subject (b) (6)) in the irbesartan group was found to be pregnant 23 days after initiating study medication, which was discontinued the same day. She experienced a spontaneous abortion 6 weeks later. A second subject (Subject (b) (6)) in the irbesartan group became pregnant 10 months after starting study medication, which was stopped following the positive pregnancy test, and the subject had an elective abortion 6 weeks later.

No congenital anomalies have been reported thus far following any pregnancy during the study.

7.7. Key Safety Review Issues

7.7.1. Hepatotoxicity

Issue

FDA-approved ERAs include bosentan, ambrisentan, and macitentan, which are all indicated for the treatment of pulmonary arterial hypertension. ERAs have caused elevations of aminotransferases, hepatotoxicity, and liver failure. FDA-approved labeling includes a boxed warning for hepatotoxicity for bosentan and a Warning and Precaution for hepatotoxicity for bosentan and macitentan. Hepatotoxicity is an AESI for sparsentan. In the PROTECT study, there was a signal for drug-induced liver injury with sparsentan compared to irbesartan. The characterization of the risk of hepatoxicity with sparsentan is limited due to the small existing safety database.

Assessment

In the PROTECT study, compared to irbesartan, there were more patients in the sparsentan group who had a hepatic injury-related AE (broad SMQ) (6.4% sparsentan versus 3.5% irbesartan), discontinued study drug due to a hepatic injury-related AE (2% sparsentan versus 0% irbesartan), or had an ALT or AST elevation >5× ULN during the study (1.5% sparsentan versus 0.5% irbesartan) (see Section 7.6.6.1 for details).

A case level analysis of potential DILI cases from the PROTECT, DUET (phase 2 study in patients with FSGS), and DUPLEX (phase 3 study in patients with FSGS) studies revealed eight cases that were categorized as either probably or possibly related to sparsentan. In general, these cases were characterized by elevations in AST or ALT, a long latency period after initiation of sparsentan, at least a partial resolution after discontinuation of sparsentan, and in most cases, reoccurrence after sparsentan was reinitiated. None of the cases met criteria for Hy's Law (see Section 7.6.6.1 for details).

An assessment of the risk of hepatoxicity with sparsentan based on the potential DILI cases in these studies and the existing sparsentan safety database is limited by the following:

- Small safety database: The total number of subjects with CKD exposed to sparsentan to date is about 500, which is well below the desired threshold to have 95% confidence in observing a Hy's Law case at a rate of 1 in 1000. Around 3000 subjects exposed to sparsentan are needed to reach 95% chance of such detection. Compared to a study setting, transaminase (TA) monitoring and follow-up for elevated TAs are both expected to be less stringent in the postmarketing setting. There is concern for the potential for more severe DILI events than what was observed in the study setting, including events with elevations in bilirubin, in the postmarket setting.
- Long DILI latency: The observed long latency periods (median 7 months) after initiation of sparsentan create challenges for monitoring for DILI in the postmarket setting.

Conclusion

The risk of hepatotoxicity will be mitigated via labeling that mandates monthly transaminase and bilirubin testing every month for the first 12 months (consistent with REMS programs for other ERAs) and every three months thereafter while on treatment. Other dosing considerations related to liver monitoring (e.g., holding and discontinuation criteria) can be found in labeling. Sparsentan will be available through a restricted distribution system (REMS) that requires patients to undergo the mandated liver test monitoring and healthcare providers to be certified. Decreasing the frequency of monitoring to every three months after the first year is considered a reasonable schedule (in comparison to REMS programs for other ERAs) given the nature of the liver events observed in the safety database for sparsentan. See REMS memo for details. A safety study will be required in the postmarketing setting to further characterize liver events.

8. Therapeutic Individualization

8.1. Intrinsic Factors

8.1.1. Hepatic Impairment

A dedicated hepatic impairment study was conducted where participants with normal hepatic function, mild hepatic impairment (Child-Pugh Class A), and moderate hepatic impairment (Child-Pugh Class B) were given a single 400 mg dose of sparsentan (Study 021IHFX16009). The results (Table 38) indicate that mild and moderate hepatic impairment do not appear to significantly affect systemic exposure of sparsentan. The mean C_{max} unbound and AUC_{last} unbound values of the moderate hepatic impairment group were approximately 2 times those of the normal group, however, because of the large variability in the measurement of unbound concentrations, these results should be interpreted with caution (Table 39). The effect of severe hepatic impairment (Child-Pugh Class C) on the PK of sparsentan is unknown.

Table 38. Statistical Analysis of PK Parameters of Sparsentan in Subjects With Hepatic Impairment and Matched Subjects With Normal Hepatic Function

Study Population: Pharmacokinetic

	Hepatic Impairment Group			Normal Function Group					
Parameters (unit)		n[a]	Observed Geometric Means	n[a]	Observed Geometric Means	Comparison	Ratio of Geometric Means[b] (%)	90% CI of the Ratio[b]	p-value[c]
C_{max} (ng/mL)	Mild	8	4440	8	5700	Mild vs Normal	77.9	(57.8, 105)	0.159
	Moderate	8	6790	8	5650	Moderate vs Normal	120	(84.5, 171)	0.355
$C_{max,u}$ (ng/mL)	Mild	8	25.7	8	24.6	Mild vs Normal	105	(62.7, 175)	0.870
	Moderate	8	58.0	8	26.8	Moderate vs Normal	216	(78.5, 596)	0.193
$AUC_{0\text{-}lqc}$ (h*ng/mL)	Mild	8	77300	8	85000	Mild vs Normal	90.9	(60.8, 136)	0.665
	Moderate	8	134000	8	106000	Moderate vs Normal	126	(84.4, 189)	0.309
$AUC_{0\text{-}inf}$ (h*ng/mL)	Mild	8	77400	8	85100	Mild vs Normal	91.0	(60.9, 136)	0.669
	Moderate	8	135000	8	107000	Moderate vs Normal	126	(84.4, 189)	0.309
$AUC_{0\text{-}36,u}$ (h*ng/mL)	Mild	5	241	5	225	Mild vs Normal	107	(50.9, 225)	0.855
	Moderate	6	534	6	273	Moderate vs Normal	195	(64.3, 593)	0.279

Each subject in a hepatic impairment group has 1 matched subject in the normal hepatic function group.
[a] n is the number of subjects used in the analysis.
[b] The ratio and corresponding confidence interval were back-transformed from the mean difference and its confidence interval, which were calculated on the log e scale from the paired t-test.
[c] The p-value was obtained from the paired t-test to assess the difference between either hepatic impairment groups against the normal hepatic function group, for each parameter.

Source: Applicant's 021IHFX16009 report. Table 14.2.2-1 on page 148.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; PK, pharmacokinetic

Table 39. fu Variability Across Hepatic Function Groups

Hepatic Function/Impairment	fu (Min, Max)
Normal	0.000244, 0.0126
Mild	0.000460, 0.0207
Moderate	0.000510, 0.0492

Source: FDA reviewer's summary from Applicant's 021IHFX16009 report.

Based on the results observed with total sparsentan plasma exposures, the impact of mild and moderate hepatic impairment appears to be minimal. The review team agrees that no dose adjustment is required in patients with mild or moderate hepatic impairment.

8.1.2. Renal Impairment

A dedicated renal impairment (RI) study was not conducted. The effect of RI on the PK of sparsentan was assessed as part of the PopPK analyses. In PopPK analyses in subjects with mild and moderate kidney disease, as defined by a creatinine clearance 60 to <90 mL/min and 30 to <60 mL/min, respectively, there was no significant effect of renal impairment on sparsentan PK (Table 40). PK data are available from only one subject with severe renal impairment (creatinine clearance <30 mL/min) so robust conclusions cannot be drawn for this subgroup. The mass balance study (021HVOL16005) shows only 2.2% of the administered dose excreted in urine, with unchanged sparsentan only in trace amounts, suggesting that significant changes in exposure due to impairment of renal function is unlikely in patients with severe renal impairment. Inhibition of hepatic function by uremic toxins could increase exposure in severe renal impairment, however the expected changes in exposure are unlikely to be beyond those seen with moderate hepatic impairment; a setting in which no dose-adjustment of sparsentan is required. No data are available in patients with end stage kidney disease including patients on dialysis.

Table 40. Summary of Median (Min, Max) PK Exposure by Renal Impairment

Subpopulation	N	AUC_{ss} ug·h/mL	$Cmin_{ss}$ ug/mL
Normal (≥90 mL/min)	46	110 (51, 322)	1540 (152, 8509)
Mild (60 to <90 mL/min)	65	111 (61.8, 251)	1344 (249, 5797)
Moderate (30 to <60 mL/min)	62	119.5 (73, 350)	1631 (397, 8784)
Severe (<30 mL/min)	1	162 (162, 162)	2114 (2114, 2114)

Abbreviations: AUC_{ss} = area under the plasma concentration-time curve at steady state; $Cmin_{ss}$ = minimum or trough concentration at steady state; IgAN = immunoglobulin A nephropathy; Max = maximum; Min = minimum; N = number of subjects; QD = once daily; max = maximum; min = minimum; N = number of subjects.
Notes: Exposure values were simulated for 400 mg QD for 21 days and are represented as the median (min, max).
Source: Applicant's RTRX-RE021-304 report. Table A on page 15.

Given these data, the review team agrees that a dose adjustment is not needed for patients with mild, moderate, or severe RI.

8.1.3. Other Intrinsic Factors

The covariate effect analyses in the PopPK model indicated that weight, age, race, and sex did not have a significant effect on the PK of sparsentan. Hence, dose adjustment is not needed for these intrinsic factors.

8.2. Extrinsic Factors

8.2.1. Metabolic Pathway

Sparsentan is predominantly metabolized by CYP3A4, with minor contributions from CYP2C8 and CYP2C9 in vitro. Drugs that are inhibitors or inducers of CYP3A have the potential to affect sparsentan exposures. The Applicant conducted clinical drug-drug interaction (DDI) studies to evaluate the impact of a strong CYP3A inhibitor and a moderate CYP3A inducer on the PK of sparsentan. The effects of strong CYP3A inducers and moderate CYP3A inducers were evaluated by physiologically based pharmacokinetic (PBPK) modeling and simulations.

8.3. Plans for Pediatric Drug Development

Sparsentan received orphan drug designation for the treatment of IgAN (Designation request # DRU-2017-6144, dated January 11, 2021); therefore, sparsentan for the treatment of IgAN is exempt from Pediatric Research Equity Act requirements.

8.4. Pregnancy, Lactation, and Females/Males of Reproductive Potential

Embryo-fetal toxicities in rats and rabbits, male and female fertility in rats as well as potential reproductive organ toxicities are discussed in Section 7.1. Nonclinical data supporting labeling language are shown in the table below by labeling section.

Table 41. Nonclinical Data Supporting Labeling on Fertility, Pregnancy and Lactation

Labeling Section	Nonclinical Data
8.1 Pregnancy	In embryo-fetal developmental (EFD) toxicity studies in pregnant rats and rabbits, teratogenicity and developmental toxicity were observed, which were attributed to the antagonism of ET_AR and AT_1R. In pregnant rats, oral administration of sparsentan throughout organogenesis at doses of 80, 160, and 240 mg/kg/day resulted in dose dependent effects in the form of craniofacial malformations, skeletal abnormalities, embryo-fetal lethality, and reduced fetal weights at all doses tested. The AUC at lowest dose tested (80 mg/kg/day) was approximately 10 times the AUC at the maximum recommended human dose (MRHD) of 400 mg/day. In pregnant rabbits, oral administration of sparsentan throughout organogenesis at doses of 2.5, 10 and 40 mg/kg/day resulted in maternal death and abortions at 10 and 40 mg/kg/day, which provided exposures of approximately 0.1-times and 0.2-times the AUC at the MRHD. An increase in fetal variation (short, supernumerary cervical ribs) occurred at 40 mg/kg/day. In the pre- and postnatal developmental (PPND) study in rats, oral administration of sparsentan during pregnancy and the lactational period at doses of 5, 20, or 80 mg/kg/day resulted in maternal death, body weight loss/reduced body weight gain, and adverse clinical signs at 80 mg/kg/day, and reduced body weight gain at doses ≥20 mg/kg/day. An increase in pup deaths occurred at 80 mg/kg/day, approximately 10 times the AUC at the MRHD, during the neonatal period through weaning, and decreased growth occurred at ≥20 mg/kg/day, approximately 2.6 times the AUC at the MRHD, after weaning. The NOAEL for pre- and postnatal development in rats was 5 mg/kg/day, approximately 0.7 times the AUC at the MRHD.
8.2 Lactation	No animal studies were conducted to assess placental transfer of sparsentan or its secretion in breast milk.
8.3 Females and Males of Reproductive Potential	No reproductive organ toxicity for males and females were observed in the general toxicity studies in rats and monkeys. No fertility impairment was observed in rats (see information below for label section 13.1).
13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility	In a fertility and early embryonic development (FEED) study in male and female rats, oral administration of sparsentan at doses of 20, 80, or 320 mg/kg/day for at least 36 (females) and 49 (males) days did not cause any effects on estrous cycles, mating, fertility, sperm evaluation, or pregnancy incidence at doses up to 320 mg/kg/day, which provided approximately 10 times and 14 times the AUC at the MRHD for males and females, respectively. Male reproductive organ toxicity was not evident in chronic toxicity studies with sparsentan at exposures up to 10 times and 1.3 times the AUC at the MRHD in rats and monkeys, respectively.

8.5. Effects of Other Drugs on Sparsentan

8.5.1. Effects of CYP3A4 Inhibitors on Sparsentan

Itraconazole, a strong CYP3A inhibitor, was dosed (200 mg QD) to steady-state and its effect was evaluated on a single dose of 200 mg sparsentan. Itraconazole increased the AUC and C_{max} of sparsentan by 174% and 25%, respectively. Cyclosporin, a moderate CYP3A inhibitor and an inhibitor of P-gp, was administered once (600 mg) and its effect was evaluated on a single dose of 200 mg sparsentan. Cyclosporin increased the AUC and C_{max} of sparsentan by 70% and 41%, respectively. The effects of two moderate CYP3A inhibitors, erythromycin, and fluconazole, and one weak CYP3A inhibitor, fluvoxamine, were also evaluated using PBPK modeling and simulations (see Section 14.7 for details).

PBPK simulations showed that erythromycin increased the sparsentan AUC and C_{max} by 60% and 25%, respectively, following a single sparsentan dosage of 200 mg. Fluconazole increased the sparsentan AUC and C_{max} by approximately 118% and 24%, respectively, following a single sparsentan dosage of 200 mg. Fluvoxamine, a weak CYP3A inhibitor, increased the sparsentan AUC and C_{max} by approximately 9% and 4%, respectively, following a single sparsentan dosage of 200 mg.

Based on these data, the review team agrees with the Applicant that the use of sparsentan with strong CYP3A inhibitors should be avoided due to the large increase in sparsentan exposure. For use with moderate CYP3A inhibitors, the team does not propose any dosage adjustment, and instead recommends monitoring for the increased risks of adverse reactions that are typical for angiotensin receptor blockers and endothelin receptor antagonists (e.g., hypotension, impaired kidney function, hyperkalemia, fluid retention).

8.5.2. Effects of CYP3A4 Inducers on Sparsentan

The effects of a strong CYP3A inducer, rifampin, and a moderate inducer, efavirenz, were evaluated by PBPK modeling and simulations.

Based on the PBPK simulations, rifampin decreased the sparsentan AUC and C_{max} by approximately 47% and 23%, respectively, following a single sparsentan dosage of 200 mg. Efavirenz, a moderate CYP3A inducer, decreased the sparsentan AUC and C_{max} by approximately 27% and 12%, respectively, following a single sparsentan dosage of 200 mg.

In PROTECT, there were only two patients who remained on a dose of 200 mg. Since the majority of patients were treated with a dose of 400 mg, it is challenging to use these data to assess for an exposure-UP/C reduction relationship. However, analysis of the DUET study (which evaluated sparsentan at 200, 400 and 800 mg doses) demonstrated a significant reduction in UP/C following 200 mg sparsentan in patients with FSGS (Table 6). This suggests that a decrease in sparsentan exposure by 30% at the standard 400 mg dose, due to concomitant use with moderate CYP3A inducers, is unlikely to diminish the efficacy of sparsentan to a clinically significant extent. Furthermore, the in vitro K_i and EC_{80} values for inhibition of ET_AR and AT_1R were lower than 13nM (Table 42). Considering that the average concentration of sparsentan at steady state in patients with FSGS is 3824nM, the unbound sparsentan concentration (approximately 30nM) is at least 2-times higher than the in vitro potency parameters.

Table 42. In Vitro Receptor Inhibition Parameters

Receptor	K_i	EC_{80} (Calcium Mobilization and Antagonism)	EC_{80} (β-Arrestin Translocation and Antagonism)
ET_AR	12.8nM	5.6nM	5nM
AT_1R	0.36nM	1.0nM	3.9nM

Source: Reviewer's summary from Applicant's RE-021-Report016-2015-DMPK and RE-021-Report006-2018-PHARM reports.
Abbreviations: AT_1R, angiotensin II receptor; EC_{80}, drug concentration that causes 80% of maximum effect; ET_AR, endothelin type A receptor

Based on these data, the review team agrees with the Applicant that the use of sparsentan with strong CYP3A inducers should be avoided due to the large decrease in sparsentan exposure. The team also agrees with the Applicant that the use of sparsentan with moderate CYP3A inducers should be allowed without additional instructions or dose-adjustment (See Section 14.7 for details).

8.5.3. Effect of Acid Reducing Agents on Sparsentan

Sparsentan has pH-dependent solubility in the relevant physiological pH range, with solubility that is lower under pH 6.8 (0.055 mg/mL) compared to pH 1.2 (1.48 mg/mL). The solubility of sparsentan within the pH range of 6.0 to 6.8 is less than the proposed dosage (400 mg) divided by 250 mL, and the estimated f2 value (similarity factor) is less than 50 based on the dissolution profiles of sparsentan in 0.1N HCl (pH 1.2) and 50mM phosphate buffer (pH 6.8). Thus, sparsentan is likely to have interactions with acid reducing agents (ARAs).

To address this issue, the Applicant submitted a PopPK analysis (b) (4) However, the Applicant's PopPK analysis is not adequate to characterize the impact of ARAs on sparsentan PK because the Applicant's PopPK analysis treated ARA co-administration status as a binary covariate for each patient and did not account for medication history prior to study entry or the dosing records for concomitant ARAs, which are critical to capture the DDI effect on the absorption phase and therefore relative bioavailability. Therefore, a PMR will be issued for a dedicated DDI study to evaluate the effect of ARAs on sparsentan PK.

8.5.4. Effect of Sparsentan on Other Drugs

In vitro studies indicated that sparsentan is an inhibitor of CYP3A4 and a weak inhibitor of CYP2C8, as well as an inducer of CYP2B6, 2C9, 2C19, and 3A4. Sparsentan is also an inhibitor of transporters P-gp, the breast cancer resistance protein (BCRP), and OATP1B3.

Refer to Section 14.1 for additional information on these in vitro studies with sparsentan.

The effect of sparsentan on substrates of CYP3A was evaluated using midazolam. The induction effect of sparsentan on CYP2B6 as well as its inhibitory effect on OATP1B3 were also tested using bupropion and pitavastatin, respectively.

Induction of CYP2C9 and CYP2C19 by sparsentan was evaluated using PBPK modeling and simulations.

Clinical DDI studies or PBPK modeling and simulations were not conducted for sparsentan to test its inhibition potential on P-gp or BCRP substrates.

8.5.5. Effect of Sparsentan on CYP3A Substrates

Sparsentan is both an inhibitor and an inducer of CYP3A in vitro, a finding that is confirmed by PBPK simulations (See Section 14.7 for details). Steady-state sparsentan did not affect the systemic exposure to midazolam when 2 mg midazolam was coadministered with 800 mg sparsentan (QD administration for 7 days) versus midazolam administered alone. For midazolam, the geometric mean ratios for C_{max}, $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$, and $t_{1/2}$ were near 100% with corresponding 90% CIs entirely contained within the interval of 80% to 125%. The observed result likely indicates that sparsentan's inhibitory effect on CYP3A cancels out its induction effect on CYP3A. The review team agrees with the Applicant's proposal that CYP3A substrates can be taken concomitantly with sparsentan.

8.5.6. Effect of Sparsentan on CYP2B6 Substrates

Steady-state sparsentan decreased the total systemic exposure (AUC) of bupropion by approximately 32%, when 150 mg bupropion was coadministered with 800 mg sparsentan (QD administration for 9 days) versus bupropion administered alone. Hence, the review team recommends monitoring the efficacy of CYP2B6 substrates when used concomitantly with sparsentan.

8.5.7. Effect of Sparsentan on CYP2C9 and CYP2C19 Substrates

Sparsentan is an inducer of CYP2C9 and CYP2C19 in vitro. The Applicant conducted PBPK analyses to evaluate the potential induction effects of sparsentan on substrates of CYP2C9 and CYP2C19. Based on these analyses, sparsentan is predicted to reduce the AUC of CYP2C9 substrate tolbutamide by 34% and CYP2C19 substrate omeprazole by 50% (See Section 14.7 for details). However, it is unclear whether CYP2C induction can be reliably predicted using induction parameters generated from in vitro hepatocyte induction studies. As such, PBPK simulations can only be used for risk assessment. To understand the magnitude of the interaction, a clinical drug interaction study is required. A PMR will be issued to evaluate sparsentan's induction effect on CYP2C9 and CYP2C19 substrates.

8.5.8. Effect of Sparsentan on P-gp and BCRP Substrates

In vitro studies indicate that sparsentan is an inhibitor of P-gp (IC_{50}=36μM) and BCRP (IC_{50}=13μM). Based on the static model, the Igut/IC_{50} for both P-gp and BCRP is greater than 10 (74 and 207, respectively). No clinical DDI studies or PBPK modeling and simulation have been conducted to evaluate the potential interactions between sparsentan and P-gp or BCRP substrates. A dedicated DDI study to evaluate the interaction potential between P-gp and BCRP substrates with sparsentan will be issued as a PMR.

9. Product Quality

The Office of Pharmaceutical Quality review team has assessed NDA 216403 with respect to chemistry, manufacturing, and controls and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports. As such Office of Pharmaceutical Quality recommends approval of this NDA from a quality perspective.

9.1. Device or Combination Product Considerations

Not applicable.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure Review

The Applicant has adequately disclosed financial arrangements with clinical investigators and the PROTECT study appears to have been conducted in compliance with U.S. regulations pertaining to Good Clinical Practice. No clinical sites were inspected because primary efficacy findings were not driven by a single site.

The Agency conducted a clinical inspection of the Applicant, Travere Therapeutics Inc, to evaluate whether blinding procedures and firewalls (according to the Data Access and Dissemination Plan) had been implemented appropriately at the time of the interim analysis to support accelerated approval. Per FDA's Clinical Inspection Summary, no significant concerns regarding the conduct or oversight of the PROTECT study were identified and blinding appeared to be appropriately maintained during the study.

11. Advisory Committee Summary

Because the application did not raise significant or controversial issues that would merit outside expertise or public discussion and due to concerns that the public release of information could impact the integrity of the ongoing study, no Advisory Committee Meeting was held for this application.

III. Additional Analyses and Information

12. Summary of Regulatory History

The original IND for sparsentan for the treatment of IgAN was submitted on October 25, 2018. The opening IND was a phase 3 study, 021IGAN17001, entitled "A Randomized, Multicenter, Double-blind, Parallel-group, Active-control Study of the Efficacy and Safety of Sparsentan for the Treatment of Immunoglobulin A Nephropathy", also known as the PROTECT study.

The Sponsor cross-referenced the existing IND [redacted] (b) (4) (sparsentan indicated for the treatment of FSGS) and an inactivated IND [redacted] (b) (4) for chemistry, manufacturing, and control data, as well as nonclinical and supportive clinical data.

Key Regulatory Interactions

There were several interactions with the Agency over the course of development; a summary of the key regulatory history is provided in Table 43.

Table 43. Summary of Key Regulatory History, PROTECT

Topic	Key Regulatory History
Milestone meetings and key events	• 4/24/2018: End-of-phase 2 meeting • 1/11/2021: Orphan drug status granted (Designation Request No. 2017-6144) • 5/26/2021: The PROTECT study completed enrollment for the double-blind phase of the study • 7/30/2021: Interim data lock (prespecified to occur after approximately 280 patients completed Week 36) • 8/6/2021: Unblinding for the interim analysis (i.e., analysis to evaluate for the primary endpoint) • 8/12/2021: The Applicant provided the topline results of the interim analysis for the PROTECT study • 10/13/2021: In the pre-NDA meeting written responses, the Division of Cardiology and Nephrology (the Division) agreed that based on the information provided, the analyses support filing of an application for accelerated approval under Subpart H. The pre-NDA meeting was later cancelled by the Applicant. • 3/17/2022: The Applicant submitted an NDA under section 505(b)(2) of the Federal Food, Drug, and Cosmetic Act • 5/11/2022: The NDA was granted Priority review designation
Substantial evidence of effectiveness	• The Division agreed that a single, pivotal study could be sufficient to support an NDA for sparsentan for the treatment of IgAN if the "trial were well-conducted and the results were statistically persuasive." • The Division noted that studies from the FSGS program "may be able to provide adequate support for an indication for the treatment of IgA nephropathy when combined with positive results of the PROTECT trial."

Topic	Key Regulatory History
Primary endpoint for accelerated approval	• The Division agreed that a substantial reduction in proteinuria would be reasonably likely to predict a clinical benefit in IgAN and could be used as a basis for accelerated approval. To support accelerated approval, the magnitude of the treatment benefit on proteinuria would need to be sufficient to provide confidence that the anticipated benefit on loss of renal function could be verified with longer term follow-up. • The Division stated that to be granted accelerated approval, the magnitude of the treatment effect on proteinuria must be sufficiently large to provide confidence that the study is adequately powered to confirm the treatment benefit on eGFR (i.e., confirmatory endpoint) accounting for uncertainties in the relationship.
Confirmatory endpoint	• The Division agreed to a confirmatory endpoint based on rate of change in eGFR over a 110-week (approximately 2-year) period • The Division stated that it would be reasonable to assess the treatment effect using the total slope (i.e., slope calculated using all eGFR values from the time of randomization) if the treatment effect over the planned duration of the study is expected to be large enough to overcome the treatment's negative acute pharmacodynamic/hemodynamic effect on eGFR. The Applicant noted that they do not expect there to be a meaningful difference between sparsentan and irbesartan on the acute change in eGFR and that blinded review of accumulating data from PROTECT did not reveal large hemodynamic effects.
Considerations related to accelerated approval	• The Division noted the importance of having sufficiently mature eGFR data in a sufficient number of patients at the time of submission of an application for accelerated approval to provide additional confidence that the postmarketing phase of the study is adequately powered to confirm the treatment benefit. • The Applicant noted that there is uncertainty in the relationship between treatment effects on proteinuria at 9 months and 2-year total eGFR slope, and, to provide further support for approval, they proposed to calculate conditional power for eGFR total slope over 2 years given the available data at the time of the proteinuria analysis. The Applicant indicated that they plan to file for accelerated approval if the analysis of proteinuria at 9 months is statistically significant, the size of the effect predicts a clinically meaningful treatment effect on eGFR slope at 2 years, and the conditional power is ≥70%. The Agency noted that this may be a reasonable approach, but it may not be suitable for every scenario and does not address all sources of uncertainty. The Agency advised the Applicant to submit the necessary data at the time of NDA submission to allow the Agency to reproduce the conditional power calculations. • The Applicant agreed to provide analyses of eGFR data available at the time of submission of an application for accelerated approval to provide additional confidence that the postmarketing phase of the study is adequately powered to confirm the treatment benefit. The Applicant specified that they would evaluate treatment effects on 1-year eGFR slope, which are likely to predict treatment effects on eGFR slope assessed at later timepoints • To provide confidence that the postmarketing phase of the study is completed in a timely manner following accelerated approval, the Division noted that the study should be fully enrolled or nearly so at the time of submission of an application for accelerated approval.

Topic	Key Regulatory History
Statistical considerations	• In an Advice Letter dated 5/27/2021, regarding SAP version 2, the Agency stated, " (b) (4) [redacted] • In an Advice Letter dated 8/27/2021, regarding the submitted topline results of the interim analysis for the PROTECT study, the Agency stated, (b) (4) [redacted]
Data access and blinding plan	• In an Advice Letter dated 5/27/2021, the Division noted that the Applicant should submit a detailed unblinding plan for Agency review and feedback. The plan should address how the Applicant will maintain blinding for those involved in the ongoing study throughout the submission and review of an NDA for accelerated approval. Details regarding unblinding were specified by the Applicant in the Data Access and Dissemination Plan (DADP) dated 7/23/2021.

Abbreviations: IgAN, immunoglobulin A nephropathy; MAR, missing at random; SAP, statistical analysis plan; EOP2, end-of-phase 2; eGFR, estimated glomerular filtration rate; UPCR, urine protein to creatinine ratio

13. Pharmacology Toxicology

13.1. Summary Review of Studies Submitted With the Investigational New Drug Application

13.1.1. Pharmacology

13.1.1.1. Primary Pharmacology

Sparsentan's specificity for human endothelin type A (ET_A) and angiotensin II type 1 (AT_1) receptors was evaluated in in vitro assays including a competitive radioligand binding assay in cells expressing recombinant human receptors, a receptor subtype selectivity assay, and a receptor binding function-potency assay. The data are summarized in the table below.

Table 44. Sparsentan Receptor Specificity, Subtype Selectivity, and Functions

Study	Findings
Receptor binding affinity	Binds human ET_A (K_i =12.8nM) and human AT_2 (K_i =0.36nM) receptors.
Subtype-receptor selectivity	Human subtype-receptor selectivity (>500-fold) over endothelin type B (ET_B) (K_i =6582nM) and angiotensin II type 2 (AT_2) (K_i =190nM) receptors.
Receptor functional assay	Antagonizes ET_A (5.6nM)- and Ang II (1.0nM)-stimulated calcium mobilization (IC_{50}=46.55nM and 13.2nM, respectively). Inhibits ET_A (5nM)- and Ang II (3.9nM)-stimulated β-arrestin translocation (IC_{50}=521nM and 7.86nM, respectively)

Source: Applicant's study reports RE-021-Report016-2015-DMPK, RE-021-0044, and RE-021-Report006-2018-PHARM.
Abbreviations: Ang II, angiotensin II; AT_2, angiotensin II type 2; ET_A, endothelin type A; ET_B, endothelin type B

Dysregulation of endothelin-1 and angiotensin II has been implicated in many facets of the pathophysiology underlying renal disease, which includes vascular effects and structural changes in the glomeruli. Various animal models were used to evaluate potential effects on renal function and structural alterations. Proof-of-concept efficacy of sparsentan was tested in the in vivo animal models discussed below.

In hemodynamic models, sparsentan produced a dose dependent inhibition on big endothelin-1 (1 nmol/kg, intravenous [IV]) or angiotensin II (100 ng/kg, IV) induced mean arterial pressure elevation in rat with an ED_{50} of 7.6 mg/kg or 0.9 mg/kg, respectively. In addition, sparsentan at doses of 6, 18 and 60 mg/kg inhibited the elevation of mean arterial pressure induced by deoxycorticosterone acetate (significant at 18 and 60 mg/kg) in rats; significantly decreased the mean arterial pressure in spontaneously hypertensive rats (significant at ≥6 mg/kg) and inhibited systolic blood pressure dose-dependently in the 5/6 nephrectomy rat model at all doses.

In animal models representing conditions of IgAN, effects of sparsentan are summarized in the table below.

Table 45. Pharmacodynamic Efficacy of Sparsentan in Animal Models Representing Immunoglobulin A Nephropathy Conditions

Study	Findings
Study no.: RE-021-Report054-2018-PHARM Study title: Determination of Sparsentan Efficacy in a gddY Mouse Model of IgAN Species/strain: Mouse/gddY mouse Number/sex/group: 5-10/female/group Dose: 900 or 1800 ppm Route and dosing frequency: Oral, feed/8 weeks	• Elevated urine albumin levels (≥900 ppm) and glomerulosclerosis (GS) (1800 ppm) were dose-dependently attenuated. • Urine albumin levels were dose dependently and significantly decreased.
Study no.: RE-021-Report004-2018-PHARM Study title: Determination of Sparsentan Efficacy in a Passive Mouse Model of IgAN Species/strain: Mouse/NCr nude sp/sp homozygous Number/sex/group: 5/female/group Dose: 60 or 120 mg/kg Route and dosing frequency: Oral gavage/daily for 6 weeks	• Attenuated EICs (Gd-IgA1 and rIgG in proportion of 2:1, six IV injections/every alternate day/total of five doses in 12 days)-induced mesangial cellularity (60 or 120 mg/kg/day, P<0.05). • Dose dependently attenuated EICs-induced K_i-67 positive nuclei. • Ameliorated the increase in plasma creatinine (60 mg/kg, P<0.05) levels. • Prevented increase in glomerular area (120 mg/kg).
Study no.: RE-021-Report003-2018-PHARM Study title: Effect of Sparsentan on Antithymocyte Serum-Induced Mesangioproliferative Glomerulonephritis in the Rat, a Model of the Mesangioproliferative Aspects of IgAN Species/strain: Rat/SD rats Number/sex/group: 8/male/group Dose: 20, 60, or 180 mg/kg/day Route and dosing frequency: Oral gavage/once daily for 7 days	• Dose-dependently attenuated Thy-1 ATS (0.6 mL/100 g, IV, 7 days)-induced proteinuria. • Dose-dependently attenuated glomerular injury (hypercellularity, hypertrophy, and expression of ECM proteins collagen I, IV, and laminin) (≥60 mg/kg, P<0.05), mesangial cell activation (≥60 mg/kg, P<0.05), proliferation (≥20 mg/kg, P<0.05), and macrophage infiltration (≥60 mg/kg, P<0.05), and interstitial myofibroblast activation myofibroblast.

Source: Pharmacology/toxicology reviewer
Abbreviations: IV, intravenous; EIC, engineered immune complexes

Sparsentan was also evaluated in animal models of FSGS as listed below. In TPRC-Transgenic mice and adriamycin-induced nephropathy models, sparsentan was compared with losartan, an angiotensin II receptor blocker, for the measured parameters.

Table 46. Pharmacodynamic Efficacy of Sparsentan in Animal Models Representing Focal Segmental Glomerulosclerosis Conditions

Study	Findings
Study no.: RE-021-0011 Study title: Effects of Sparsentan on TRPC6-Transgenic Mice, a Model of FSGS Species/strain: Mouse/Ren1d-GCaMP5/tdTomato transgenic mice (Healthy-Tg) (healthy model) and Pod-GCaMP5/tdTomato TRPC6 triple transgenic (FSGS-Tg) (disease model). Number/sex/group: 8-10/sex/group Dose: Sparsentan 120 mg/kg and losartan 10 mg/kg. Route and dosing frequency: oral feed/6 weeks (FSGS-Tg model) or 2 weeks (Healthy-Tg model).	Healthy-Tg mice, • Sparsentan dilated both afferent arteriole (AA) (19.61±0.67 versus 15.17±0.43µm in control) and efferent arteriole (EA) (19.33±0.88 versus 9.8±0.47µm in control), which resulted in an increase in capillary blood flow and single nephron glomerular filtration rate (SNGFR) (5.79±0.47 versus 4.0±0.39 nl/min in control). Losartan had no significant effect on glomerular hemodynamic parameters. • Sparsentan attenuated ET-1 (50 ng)/Ang II (400 ng/kg)-induced vasoconstriction of the AA and AA vascular smooth muscle cell (VSMC) calcium. Sparsentan only reduced VSMC calcium but not agonist induced vasoconstriction. • Sparsentan abolished ET-1-induced elevations in AA, VSMC calcium, AA vasoconstriction, and the reduction in glomerular turf area and losartan had no effect on hemodynamic changes. • Sparsentan was more effective in ET-1/Ang-II-induced podocyte injury and in protecting agonist podocyte loss compared to losartan. • FSGS-Tg mice (1.5 years-old) • Significantly improved several chronic disease parameters of glomerular hemodynamics (increased both AA (17.56±1.05 versus 11.44±0.75µm in control) and EA (10.39±0.46 versus 7.53±0.69µm in control) diameters, SNGFR (7.99±0.60 versus 2.94±0.29 nl/min in control), and glomerular capillary blood flow (red blood cell velocity; 2.26±0.14 versus 0.88±0.08µm/s in control)). • Albumin leakage through the GFB (albumin glomerular sieving coefficient; 0.11±0.02 versus 0.21±0.03 in control) and the level of albuminuria (urinary albumin/creatinine ratio, albumin/creatinine ratio normalized to baseline; 0.74±0.04 versus 2.52±0.76 in control) significantly reduced. • Glomerulosclerosis (47.17±2.44 versus 101.70±4.30 in control) and tissue fibrosis (36.66±1.51 versus 86.22±4.40 in control) was reduced. • Podocyte number in the glomeruli is significantly preserved and increasing cyclin dependent kinase inhibitor p57 Kip2 (p57)-positive podocyte number (12.46±0.62 versus 4.39±0.46 in control). • Losartan improved glomerulosclerosis and tissue fibrosis but it was less effective in preserving p57-positive podocytes compared to sparsentan.ET-1 (50 ng)/Ang II (400 ng/kg)-induced podocyte calcium elevations (1.02±0.02-fold of baseline), AA vasoconstriction (88.67±3.85% of baseline diameter), reductions in glomerular diameter (98.10±0.38% of baseline) and glomerular tuft area (97.38±0.83% of baseline) is

Study	Findings
	attenuated. Losartan decrease calcium elevation but had no effect on glomerular hemodynamic parameters.
Study no.: RE-021-Report-28-2016-PHARM Study title: Effect of Sparsentan on Adriamycin-Induced Nephropathy in the Rat, a Model of FSGS-Initial Dose Range Finding Species/strain: Rat/SD Number/sex/group: 5-10/male/group Dose: 6, 18, or 60 mg/kg Route and dosing frequency: Oral gavage/daily for 35 days	• Lower protein-creatinine ratios (22.9 in 60 mg/kg versus 40.1 in control on Day 33, P=0.05), proteinuria (270.2 in 60 mg/kg versus 483.7 mg in ADR control on Day 33). • Glomerulosclerosis (severity (+)/incidence (%) ratio is 1.31 in 18 mg/kg and 1.07 in 60 mg/kg versus 1.92 in ADR control, P<0.05), incidence of interstitial lesions (1.3% in 60 mg/kg versus 3.4% in ADR control, P<0.05), and a trend towards preservation of podocytes.
Study no.: RE-021-0034-Addendum 1 Study title: Effect of Sparsentan on Adriamycin-Induced Nephropathy in the Rat, a Model of FSGS-Repeat Model Execution with Higher Dose of Sparsentan and Addition of Comparator Compounds Species/strain: Rat/SD Number/sex/group: 10/male/group Dose: Sparsentan 20, 60, 180 mg/kg/day, losartan 3, 10 and 30 mg/kg and atrasentan 3 mg/kg. Route and dosing frequency: Oral gavage/daily for 33 days	• Significantly reduced increase in urine protein: creatinine ratio on Day 14 at 180 mg/kg. • Sparsentan (60 and 180 mg/kg) significantly reduced glomerular sclerosis severity scores on Day 33. Both losartan and atrasentan failed to achieve significant glomerular sclerosis reduction. • Increased glomerular basement membrane width and grater glycocalyx staining was attenuated staining at ≥60 mg/kg. Losartan and atrasentan failed to produce any significant improvement in glycocalyx staining. • Podocyte number was significantly increased in the sparsentan 180 mg/kg and losartan 10 mg/kg groups.
Study no.: RE-021-Report017-2015-PHARM Study title: Effects of Sparsentan on the Progression of Renal Failure in Uremic Rats, a 5/6 Nephrectomy Model of FSGS Species/strain: Rat/SD Number/sex/group: 8/male/group Dose: 6, 18, and 60 mg/kg Route and dosing frequency: Oral gavage/8 weeks	• Decreasing hypertension (mean SBP) -18% (p <0.01), -22% (p <0.01), and -31% (p <0.001) lower at 6, 18, and 60 mg/kg/day, respectively. • Slightly but significantly improved GFR (18 mg/kg exhibited a small (18%, p<0.05)), prevent the progressive rise in urine protein (p<0.05; -66% and -84% at Weeks 4 and 8, respectively, at 60 mg/kg) and albumin (-96% [p <0.05], -78% [NS], and -99% [p <0.05] at 6, 18, and 60 mg/kg/day, respectively). • Lowered kidney and heart weights.

Source: Pharmacology/toxicology reviewer
Abbreviations: ADR, adriamycin; GFR, glomerular filtration rate; SBP, systolic blood pressure

In addition, sparsentan demonstrated its desired pharmacodynamic effects in Alport Syndrome model (129Sv Autosomal Transgenic Mouse Model of Collagen 4α3 gene (*COL4a3KO*)) and Membranous Nephropathy model (Fx1A-induced passive Heymann Nephritis in rats).

13.1.1.2. Secondary Pharmacology

In the off-target screening, binding of sparsentan to 105 unique G-protein coupled receptors, and selected ion channels, transporters, and other receptors with abuse potential were evaluated. No off-target binding was noted up to 10μM concentration except for the ET_A, AT_1, and AT_2 receptors.

13.1.1.3. Safety Pharmacology

A complete battery of safety pharmacology (central nervous system, cardiovascular, and respiratory) endpoints was adequately assessed and did not raise significant safety concerns. Decreased blood pressure is considered a desired primary pharmacological action. Results of these studies are summarized in the table below.

Table 47. Safety Pharmacology Studies

Study/Study No.	Key Findings
Study no.: PCO-NC-002 Study title: Effects of BMS-346567 on action potential parameters recorded from isolated rabbit Purkinje fibers Species/tissue: Rabbit/Purkinje fibers Concentration: 3, 10, and 30µM	• No significant effects on resting membrane potential (RMP), overshoot (OS), action potential duration of 50% (APD_{50}) or 90% (APD_{90}).
Study no.: BMS-346567-hERG Study title: Effects of BMS-346567 on hERG/IKr currents Cells/strain: Human embryonic kidney cells (HEK293)/stably expressed recombinant hERG channels (hERG/IKr) Concentration: 10 and 30µM	• Minimal inhibition on cardiac potassium channel ($hERG/IK_r$) current (1.7±1.4% (n=3) at 10µM and 8.1±1.7% (n=3) 30µM).
Study no.: RE-021-Report050-2015-SPHARM Study title: Evaluation of the effect of RE-021 on cloned hERG channels expressed in human embryonic kidney. Cells/strain: Human embryonic kidney cells (HEK293)/stably expressed recombinant hERG channels (hERG/IKr) Concentration: 150 and 500µM	• 7% inhibition of hERG-mediated potassium currents at 500µM and no inhibition at 150µM.
Study no.: PCO-NC-010 (included in PCO-NC-012) Study title: A Cardiovascular Safety Pharmacology Study of PS433540 Administered by Oral Gavage to Telemetered Cynomolgus Monkeys Species/strain: Monkey/cynomolgus Number/sex/group: 4/male/group Dose: 32, 500, and 1000 mg/kg/day Route of administration/dosing frequency: Oral gavage/twice weekly (on Days 1, 4, 9 and 14) NOAEL: 32 mg/kg	• No treatment related moribundity and mortality. • No adverse effect on blood pressure, ECG parameters, or body temperature at 32 mg/kg. • Decrease (up to 27% at ≥500 mg/kg) in diastolic blood pressure, systolic blood pressure and mean blood pressure between 16-18 hours postdose).
Study no.: PCO-NC-011 (Dose range-finding phase) Study title: Acute Oral (Gavage) Central Nervous System (CNS) Safety Pharmacology Study of PS433540 in Rats Species/strain: Rat/Crl:CD(SD) Number/sex/group: 5 (part A) to 10 (part B)/sex/group Dose: mg/kg/day: 31.3, 250 and 1000 mg/kg/day (part A and B) Route of administration/dosing frequency: Oral gavage/single	• No treatment-related effect on clinical signs, body weight or CNS parameters.

Study/Study No.	Key Findings
Study no.: PCO-NC-012 (Definitive phase) Study title: Acute Oral (Gavage) Central Nervous System (CNS) Safety Pharmacology Study of PS433540 in Rats Species/strain: Rat/Crl:CD(SD) Number/sex/group: 6/male/group Dose: mg/kg/day: 31.3, 250 and 1000 mg/kg/day Route of administration and dosing/frequency: Oral gavage/single NOAEL: 250 mg/kg	• No treatment-related effects on CNS parameters. • Dose dependent decreases in body weight gain (31.3 and 250 mg/kg) or body weight losses (1000 mg/kg) in male rats.
Study no.: PCO-NC-013 Study title: A Pharmacological Assessment of the Effect of PS433540 on the Respiratory System of the Albino Rat Species/strain: Rat/Crl:CD(SD) Number/sex/group: 5 (part A) to 10 (part B)/sex/group Dose: mg/kg/day: 31.3, 250 and 1000 mg/kg/day (part A and B) Route of administration and dosing/frequency: Oral gavage/single NOAEL: 1000 mg/kg	• No mortality or treatment-related effects on respiratory parameters; tidal volume, derived minute volume, and respiratory rate.

Source: Pharmacology/toxicology reviewer
Abbreviations: CNS, central nervous system; ECG, electrocardiogram; hERG, human ether-a-go-go related gene; NOAEL; no observed adverse effect level

13.1.2. Pharmacokinetics/ADME/Toxicokinetics

General pharmacokinetics (PK) including absorption, distribution, metabolism, and excretion (ADME), and toxicokinetics of sparsentan were evaluated in rodents (mice and rats), and nonrodents (rabbits, dogs, and monkeys) following IV, intra-arterial, intraperitoneal, and oral (PO) administration. Overall, there was no significant difference in PK profiles between animals and humans that would limit the interpretation of animal toxicology studies.

Absorption

Rapid absorption was reported in mice, rat, dogs, and monkeys with maximum plasma concentration (C_{max}) and time to maximum concentration (T_{max}) values observed within 1 to 2 hours and oral bioavailability ranging from 32% (monkeys) to 56% (rats). Plasma concentration declines in a biphasic manner, rapid distribution phase followed by a slower elimination phase. Plasma half-life ($t_{1/2}$) is ranging from ~3 hours (rat) to 5.6 hours (monkeys).

Distribution

Sparsentan (1μM to 100μM) was highly bound to rat, monkey and human plasma protein (97.2% to >99%) in a concentration-independent manner. Low affinity to red blood cells was noted with a human blood-to-plasma distribution ratio of 0.579 to 0.674 at concentration range from 1μM to 100μM. No studies were conducted to assess placental transfer of sparsentan or its secretion in breast milk. Organ distribution evaluated with quantitative whole-body autoradiography in male rats showed highest amount of radioactivity in bile, blood, and urine, followed by liver, arterial walls, lungs, renal cortex, and intervertebral discs and the lowest amount of radioactivity in noncircumventricular central nervous system tissues, bone, seminal vesicles, abdominal fat and

eyes. The radioactivity that did distribute across the blood: brain barrier was measurable at 1.8 times the lower level of quantitation but was not detectable after 2 hours. No affinity for tissues containing melanin was noted and no quantifiable amount was detected in the eye lens. In all tissues, the elimination of radioactivity was complete by 168 hours postdosing.

Metabolism

Sparsentan is a substrate for CYP3A4 and to a minor degree to CYP2C8 or CYP2C9. The metabolic profile of sparsentan using hepatocytes has generated quantitively similar (16% to 54%) metabolic profile across all the animal species (mice, rat, dogs, monkeys) and humans. All of the metabolites identified in human hepatocytes incubation were detected in one or more animal species (see table below).

Table 48. Interspecies Comparison for Biotransformation of Sparsentan by Hepatocytes From Human, Monkey, Dog, Rat, and Mouse After 4-Hour Incubation

Component ID[a]	% of Total Radioactivity by HPLC					
	m/z[b]	Human (δ)[c]	Monkey (δ)[c]	Dog (δ)[c]	Rat (δ)[e]	Mouse (δ)[e]
M3 Glucuronide conjugate of BMS-346567	769	d	3.1	d	nd	nd
M8 Dihydroxylated BMS-346567	625	0.6	2.1	nd	d	0.8
M9 Dihydroxylated BMS-346567	625	0.3	2.0	nd	nd	0.7
M11 Dihydroxylated BMS-346567	625	d	1.1	nd	d	d
M13 Dihydroxylated BMS-346567	625	d	1.3	nd	d	d
M15 Monohydroxylated Keto-BMS-346567 and M15 Monohydroxylated BMS-346567	623 609	d[f]	1.4[f]	nd d	2.2[f]	0.7[f]
M18 Monohydroxylated BMS-346567	609	0.4	1.4	0.7	1.0	0.6
M19 Monohydroxylated BMS-346567	609	1.0	nd	nd	nd	d
M20 Monohydroxylated BMS-346567 and M20 Des-ethyl BMS-346567	609 565	3.2[f]	10.8[f]	3.4[f]	13.9[f]	8.0[f]
M21 Monohydroxylated BMS-346567	609	2.8	11.3	5.5	5.0	8.2
M22 Monohydroxylated BMS-346567	609	1.4	3.9	1.0	5.1	3.4
M23 Monohydroxylated BMS-346567	609	0.7	1.2	0.2	1.5	1.0
M24 Monohydroxylated BMS-346567 and D1 Ring-opened BMS-346567	609 611	1.5[f]	6.5[f]	1.0[f]	2.6[f]	1.8[f]
M25 Keto-BMS-346567	607	2.1	3.9	1.6	2.5	3.1
P BMS-346567	593	84.0	46.3	83.8	61.1	66.9
Total Characterized		**98.0**	**96.3**	**97.2**	**94.9**	**95.2**
Unknown		2.0	3.7	2.8	5.1	4.8
Total		100	100	100	100	100

[a] Tentative identification based on Q1, PIS, MRM results
[b] *m/z* values listed are for $[M+H]^+$
[c] Cryopreserved hepatocytes
[e] Freshly isolated hepatocytes
[f] More than 1 component present in the peak
d – detected in trace amounts
nd – not detected

Source: Sponsors study report MAP010 Page No 28.
Abbreviations: HPLC, high performance liquid chromatography

There were no unique human metabolites observed. Cross-species comparisons of metabolite exposures (area under the concentration-time curve [AUC] and C_{max}) in FSGS patients (800 mg, Day 57), rats (80 mg/kg/day for 14 days), and monkeys (50 mg/kg/day for 14 days) indicated that all the human metabolites were detected in one or more animal species in vivo. The metabolite profile in humans identified a total of 68 metabolites. Four metabolites detected above 0.5% of total counts were also identified in rat or monkey plasma.

Excretion

The excretion of sparsentan occurred predominantly in feces (93.7%) and little in urine (1.96%) through 336 hours post dose. This is comparable with human studies. Sparsentan was primarily eliminated through CYP-mediated metabolism, followed by biliary excretion of metabolites, and then fecal elimination.

Toxicokinetics

In repeat oral dose studies, systemic exposure was generally increased in a dose-dependent manner and no drug accumulation was noted. In rodents (mice and rats), higher exposure was noted in females at lower doses. The TK parameters in the pivotal toxicology studies are summarized in the table below.

Table 49. Toxicokinetic Data

Study/Study No.	Major Findings
General Toxicology Studies	
Study no: PCO-NC-023 Study title: PS433540: A 13-Week Toxicity Study in Sprague-Dawley Rats with a 4-Week Recovery Sample collection times: Predose, 0.5, 1, 2, 4, and 8 hours Accumulation: No accumulation Dose proportionality: In general, C_{max} and mean AUC_{last} are less than dose proportion with few exceptions. NOAEL: 320 mg/kg/day	**Table 50. Week 13 TK Parameters for the Rat** Group 2, 15 mg/kg/day: Male, Day 1: C_{max} 5710 ng/mL, t_{max} 1 h, t_{last} 24 h, AUC_{last} 35800 ng-h/mL, AUC 36100 ng-h/mL, $t_{1/2}$ 3.6 h; Female, Day 1: 13500, 1, 24, 118000, 139000, 9.0; Male, Day 28: 8630, 1, 24, 35800, 36200, 4.0; Female, Day 28: 18500, 1, 24, 89700, 103000, 8.9; Male, Day 91: 7810, 1, 24, 48100, 51200, 6.2; Female, Day 91: 17200, 1, 24, 84200, 90700, 6.8 Group 3, 80 mg/kg/day: Male, Day 1: 36700, 4, 24, 413000, NE, NE; Female, Day 1: 89700, 8, 24, 1290000, NE, NE; Male, Day 28: 37600, 2, 24, 280000, 291000, 5.0; Female, Day 28: 67700, 2, 24, 692000, 698000, 3.4; Male, Day 91: 28300, 4, 24, 291000, NE, NE; Female, Day 91: 66000, 4, 24, 595000, NE, NE Group 4, 320 mg/kg/day: Male, Day 1: 75700, 4, 24, 1040000, NE, NE; Female, Day 1: 130000, 4, 24, 2070000, NE, NE; Male, Day 28: 75200, 8, 24, 1050000, NE, NE; Female, Day 28: 95600, 2, 24, 1170000, 1200000, 4.2; Male, Day 91: 75100, 4, 24, 754000, NE, NE; Female, Day 91: 96900, 2, 24, 1110000, 1120000, 3.2
Study no: PCO-NC-028 Study title: PS433540: A 26-Week Oral Toxicity Study with Toxicokinetics in Sprague-Dawley Rats with an 8-Week Recovery Sample collection times: predose and 0.5, 1, 2, 4, 8, and 24 hours Accumulation: No accumulation Dose proportionality: In general, C_{max} and mean AUC_{last} are less than dose proportion with few exceptions. NOAEL: 80 mg/kg/day	**Table 51. Week 26 TK Parameters for the Rat** Group 2, 15 mg/kg/day: M, Day 1: C_{max} 9040 ng/mL, t_{max} 1 h, t_{last} 24 h, AUC_{last} 46600 ng-h/mL, AUC 48500 ng-h/mL, $t_{1/2}$ 5.4 h; F, Day 1: 17500, 2, 24, 123000, 135000, 6.6; M, Day 91: 11800, 1, 24, 57600, 59000, 4.6; F, Day 91: 16400, 8, 24, 235000, NE, NE; M, Day 182: 12400, 1, 24, 89000, 89800, 3.5; F, Day 182: 16200, 1, 24, 90600, NE, NE Group 3, 80 mg/kg/day: M, Day 1: 39300, 2, 24, 374000, 416000, 7.0; F, Day 1: 97900, 2, 24, 1440000, 1440000, 2.8; M, Day 91: 31300, 4, 24, 246000, NE, NE; F, Day 91: 60100, 2, 24, 607000, 611000, 3.3; M, Day 182: 46000, 2, 24, 318000, 325000, 4.3; F, Day 182: 26800, 1, 24, 99700, 101000, 4.4 Group 4, 320 mg/kg/day: M, Day 1: 73200, 4, 24, 1170000, NE, NE; F, Day 1: 144000, 4, 24, 2130000, NE, NE; M, Day 91: 66900, 4, 24, 892000, NE, NE; F, Day 91: 119000, 2, 24, 1220000, 1230000, 3.2; M, Day 182: 78200, 4, 24, 795000, NE, NE; F, Day 182: 162000, 2, 24, 1440000, 1450000, 2.9

Study/Study No.	Major Findings
Study no: PCO-NC-024 Study title: PS433540: 13-Week Toxicity Study in Monkeys with a 4-Week Recovery Sample collection times: Predose and 0.5, 1, 2, 4, 6, 8, and 24 hr postdose Accumulation: Potential for accumulation as increased C_{max} and AUC_{last} values from Day 91 were noted. Dose proportionality: Approximately dose proportional NOAEL: 50 mg/kg/day	**Table 52. Week 13 TK Parameters for the Cynomolgus Monkeys**

Group	Dosage (mg/kg/day)	Sex	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 1								
2	10	M	872	0.8	24	4900	4710[b]	9.0[b]
		F	1050	0.8	24	4130	4550	8.0
3	50	M	2640	1.5	24	29300	NE	NE
		F	2300	1	24	14500	19500[c]	13.5[c]
4	250	M	18200	3	24	154000	136000[c]	7.9[c]
		F	10800	2	24	80400	NE	NE
Day 35								
2	10	M	968	0.5	24	5580	6060	7.4
		F	1830	0.5	24	5970	6340	5.3
3	50	M	5080	1	24	27200	31100	10.1
		F	5270	2	24	25700	29600[b]	7.9[b]
4	250	M	17300	3	24	127000	257000[c]	9.8[c]
		F	41000	1.5	24	272000	321000[c]	7.0[c]
Day 91								
2	10	M	1010	1	8	3290	5680[d]	5.3[d]
		F	3090	0.5	24	6510	7480[b]	4.4[b]
3	50	M	4080	1.5	24	25600	28500	7.6
		F	5630	1	24	29700	31500[c]	5.4[c]
4	250	M	40800	4	24	357000	389000[c]	5.4[c]
		F	32900	3	24	253000	487000[c]	4.2[c]

NE: Not estimated, due to insufficient characterization of the terminal phase of the concentration-time curves.
a: Median for t_{max} and t_{last}; n = 4 for Groups 2 and 3; n = 6 for Group 4.
b: n = 3.
c: n = 2.
d: n = 1.

	Table 53. Week 39 TK Parameters for the Cynomolgus Monkeys

Group	Dosage (mg/kg/day)	Sex	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last}[a] (h)	AUC_{last} (µg·h/mL)	AUC_{inf} (µg·h/mL)	$t_{1/2}$ (h)
Day 1								
2[b]	10	M	0.907	0.8	24	7.13	8.25	8.5
		F	1.64	0.8	16	7.04	NE	NE
3	50	M	3.62	5	24	45.5	43.8[c]	4.8[c]
		F	3.92	2	24	22.3	17.9[d]	3.4[d]
4	200	M	27.0	3	24	307	186[c]	8.5[c]
		F	23.6	4	24	263	349[c]	5.3[c]
Day 36								
2[b]	125	M	16.6	1.5	24	101	106[c]	4.3[c]
		F	22.5	2	24	233	388[c]	8.0[c]
Week 13								
3	50	M	7.35	1.3	24	39.0	41.1	5.9
		F	2.07	1.5	24	14.7	17.0[e]	11.8[e]
2[b]	125	M	13.6	1.5	24	148	193[e]	6.1[e]
		F	14.6	2	24	80.4	NE	NE
4	200	M	17.6	1.5	24	118	111[e]	8.4[e]
		F	21.2	4	24	169	179[e]	7.2[e]

Group	Dosage (mg/kg/day)	Sex	C_{max} (µg/mL)	t_{max}[a] (h)	t_{last}[a] (h)	AUC_{last} (µg·h/mL)	AUC_{inf} (µg·h/mL)	$t_{1/2}$ (h)
Week 39								
3	50	M	2.67	1	24	16.7	20.5[e]	8.1[e]
		F	1.46	2.5	24	12.7	11.6[g]	6.3[g]
2[b]	125	M	25.2	3	24	187	156[g]	8.7[g]
		F	9.31	0.8	24	45.7	64.9[g]	15.5[g]
4	200	M	15.2	3	24	98.5	146[g]	11.4[g]
		F	15.3	3	24	134	120[f]	10.9[f]

M = Male; F = Female; NE = Not estimated.
a Median for t_{max} and t_{last} rather than mean; n = 4 for Groups 2 and 3; n = 6 for Group 4.
b 10 mg/kg/day from Day 1 through Day 35; 125 mg/kg/day from Day 36 through Week 39.
c n = 2.
d n = 1.
e n = 3.
f n = 4.

Reproductive Toxicology Studies

Study no: RE-021-Report002-2017-RTOX Study title: An Embryo-Fetal Development Study of RE-021 by Oral Administration (Gavage) in Rats Sample collection times: 0, 0.5, 1, 2, 6 and 24 hours NOAEL: Not established (<80 mg/kg/day)	**Table 54. Rat EFD TK Parameters; GDs 7 and 17**

Day of Gestation	Dose (mg/kg/day)	T_{max} (hours)	C_{max} (ng/mL)	C_{max}/D (ng/mL/(mg/kg))	$AUC_{(0-t)}$ (hr*ng/mL)	$AUC_{(0-t)}/D$ (hr*ng/mL/(mg/kg))	R_{AUC} (Ratio)
7	80	2	78000	975	990000	12400	NA
	160	2	105000	653	1390000	8700	NA
	240	6	92300	385	1270000	5300	NA
17	80	2	91000	1140	805000	10100	0.813
	160	2	104000	653	967000	6040	0.695
	240	2	63400	264	798000	3320	0.627

R_{AUC} = DG 17 $AUC_{(0-t)}$/ DG 7 $AUC_{(0-t)}$; NA = Not applicable

Study/Study No.	Major Findings
Study no: RE-021-Report001-2017-RTOX Study title: An Embryo-Fetal Development Study of RE-021 by Oral Administration (Stomach Tube) in Rabbits Sample collection times: 0, 0.5, 1, 2, 6 and 24 hours Accumulation: NOAEL: Not established (<2.5 mg/kg/day)	**Table 55. Rabbit EFD TK Parameters; GDs 7 and 19** Day of Gestation / Dose (mg/kg/day) / T_{max} (hours) / C_{max} (ng/mL) / C_{max}/D (ng/mL/(mg/kg)) / $AUC_{(0-t)}$ (hr*ng/mL) / $AUC_{(0-t)}$/D (hr*ng/mL/(mg/kg)) / $T_{1/2}$ / R_{AUC} (Ratio) 7 / 2.5 / 1.17 / 15.1 / 6.03 / 26.7 / 10.7 / NC / NC 10 / 0.667 / 28.0 / 2.80 / 98.1 / 9.81 / NC / NC 40[a] / 1 / 234 / 5.85 / 772 / 19.3 / NR / NA 19 / 2.5 / 0.667 / 356 / 142 / 1170 / 468 / 2.20 / 34.6 10 / 0.667 / 1440 / 144 / 9550 / 955 / 4.12 / 80.6 40[a] / 0.5 / 1930 / 48.3 / 13500 / 337 / 5.48 / 17.5 R_{AUC} = DG 19 $AUC_{(0-t)}$/ DG 7 $AUC_{(0-t)}$ NC = Not calculable NR = Result not reported NA = Not applicable [a] Values shown are from rabbit 8690, which was the only pregnant satellite rabbit at 40 mg/kg/day.
Study no: RE-021-0005 Study title: RE-021: A GLP 91-Day Oral Gavage Impurity Toxicity Study in CD® [Crl:CD®(SD)] Rats Sample collection times: Predose, 0.5, 1, 2, 4 and 24 hours post dose NOAEL: 80 mg/kg/day	**Table 56. 91-Day TK Parameters for the Rat** Sex / Day / RE-021 Dose (mg/kg/day) / Treatment / C_{max} (ng/mL) / AUC_{0-24hr} (hr*ng/mL) / AUC_{0-24hr} Treatment Ratio M / 1 / 80 / RE-021 / 24100 / 237000 / NA RE-021 + Impurity / 20700 / 194000 / 0.817 91 / 80 / RE-021 / 27000 / 162000 / NA RE-021 + Impurity / 26300 / 189000 / 1.16 F / 1 / 80 / RE-021 / 49000 / 562000 / NA RE-021 + Impurity / 50400 / 629000 / 1.12 91 / 80 / RE-021 / 51600 / 535000 / NA RE-021 + Impurity / 33800 / 480000 / 0.897 M=male F=female

Source: Pharmacology/toxicology reviewer
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration; EFD, embryo-fetal development; NOAEL; no observed adverse effect level; $t_{1/2}$, terminal half-life; t_{max}, time to maximum plasma concentration; TK, toxicokinetics

13.1.3. Toxicology

13.1.3.1. General Toxicology

Repeat dose toxicology studies were conducted in mice (up to 13 weeks), rats (up to 26 weeks), and monkeys (up to 39 weeks). Key results from these pivotal toxicology studies are summarized in the following subsections.

13.1.3.1.1. Repeat Dose Toxicity Study in Mice

Repeat dose toxicity studies were conducted in CD-1 mice for 4 and 13 weeks with oral dosages from 30 to 750 mg/kg/day. Key findings of the 13-week toxicology study are summarized below.

- Doses: 50 mg/kg/day, 200 mg/kg/day, and 750 mg/kg/day.
- Increase in liver weight and a dose dependent trend of increase in alkaline transferase and alkaline phosphatase were observed at ≥200 mg/kg/day. The microscopic finding in the liver, hypertrophy, was noted at all dose levels and single cell necrosis was noted at 750 mg/kg/day.
- A dose dependent hypertrophy/hyperplasia of the kidney juxtaglomerular apparatus was observed at all doses tested with an increased incidence and severity at 750 mg/kg/day. An increase in blood urea nitrogen (BUN) was observed at ≥200 mg/kg/day.

- The no observed adverse effect level (NOAEL) was determined at 200 mg/kg/day, which is associated with $AUC_{0\text{-last}}$ values of 321,000 h*ng/mL in males and 427000 h*ng/mL in females, approximately 4.2 (male)- and 5.6 (female) times the AUC at the maximum recommended human dose (MRHD) (400 mg).

13.1.3.1.2. Repeat Dose Toxicity Studies in Rats

Repeat dose toxicity studies were conducted in rats for 2 weeks to 26 weeks with oral dose levels from 15 to 400 mg/kg/day. Key findings from the 26-week study are summarized in the table below. The testicular atrophy in rodents reported with other endothelin receptor antagonists was not observed in the rat studies with sparsentan.

26-Week (8-Week Recovery) Toxicology Study

Study Number/Title

A 26-Week Oral Toxicity Study with Toxicokinetics in Sprague-Dawley Rats with an 8-Week Recovery

Key Study Finding

- Based on decrease in the body weight, increased BUN and Cr, and the increased incidence and severity of multifocal renal tubular degeneration and fibrosis with juxtaglomerular apparatus hypertrophy/hyperplasia at 320 mg/kg/day, the NOAEL was determined to be 80 mg/kg/day, which is associated with $AUC_{0\text{-last}}$ values of 318,000 h*ng/mL in males and 99700 h*ng/mL in females, or approximately 4 (male)- and 1.3 (female) times the AUC at MRHD.
- The dose-dependent findings in the kidney were anticipated based on the pharmacological action of sparsentan and most findings at the lower dosages were not considered significant given the low incidence and severity. The findings were reversible except in one high dose male.

Table 57. Study Information

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	0, 15, 80 and 320 mg/kg and once daily
Route of administration	Oral Gavage
Formulation/vehicle	0.5% methylcellulose 4000 cP/0.25% Tween 80
Species/strain	Sprague-Dawley Rat
Number/sex/group	5/sex/group
Age	6-7 weeks
Satellite groups/unique design	4 (control) to 12 (treated)/sex/group
Deviation from study protocol affecting interpretation of results	None

Source: Pharmacology/toxicology reviewer
Abbreviations: GLP, good laboratory practice

Table 58. Observations and Results

Parameters	Major Findings
Mortality	Five early deaths: One female rat at 15 mg/kg/day (accidental death), one female rat at 80 mg/kg (cause of death was not determined), two male rats (one male rat death was likely due to the preexisting adenocarcinoma and one male rat cause of death was not determined), and one female rat (procedure related-gavage error) at 320 mg/kg/day. All these animals exhibited treatment related minimal hypertrophy/hyperplasia of the juxtaglomerular region similar or lesser severity of all the surviving animals within their respective groups. Therefore, it was unlikely that these early deaths could be attributed to the treatment with test article.
Clinical signs	Reversible dose-dependent increased incidence of porphyrin, staining and salivation and wet fur were observed at ≥80 mg/kg/day and not considered adverse.
Body weights	Relative to start date the body weights were significantly reduced (17% in male and 12% in female) at 320 mg/kg and it did not completely reverse in males during the recovery phase.
Ophthalmoscopy	No effect
Hematology	A reversible dose-dependent significant decrease (6-7% at 80 mg/kg/day and 9-12% at 320 mg/kg/day) in mean red blood cell parameters; mean red blood cell count, hemoglobin, and hematocrit values were observed. Additional statistically significant increase in the mean platelet count (13% in male and 17% increase in female), decrease in mean absolute neutrophil count (28% in male and 31% decrease in female) and absolute monocytes count (39% decrease in male), and absolute lymphocyte (50% increase in female) were noted in 320 mg/kg/day group were reversible at the end of the recovery phase (Day 239).
Clinical chemistry	A dose dependent significant increases in BUN (32% and 69% at 80 and 320 mg/kg/day, respectively) and Cr (45% at 320 mg/kg/day) was noted in male animals, which was reversed in the recovery phase except BUN.
Urinalysis	No effects
Gross pathology	Enlarged livers (female, 320 mg/kg/day) were noted on Day 183, which was correlated with the increased organ weights and microscopic examination.
Organ weights	Liver: • Reversible increase (12% to 58%) in absolute (320 mg/kg/day) and relative to body weight (≥15 mg/kg/day in male and ≥80 mg/kg/day in female) and relative to brain weight (320 mg/kg/day) were noted with an associated microscopic liver finding. Kidney: • Absolute and relative kidney weights increased (11% to 23%) significantly at ≥80 mg/kg/day, which was lack of a dose dependent pattern. A slightly increased relative (to body weight) weight was present at the end of recovery in the male 320 mg/kg/day group.

Parameters	Major Findings
Histopathology Adequate battery: Yes	Liver: • Hepatocellular hypertrophy was noted (diffuse hepatocellular hypertrophy: 3/15 male and 2/14 female were minimal at 80 mg/kg, and 2/14 male and 10/14 females were mild at 320 mg/kg/day; centrilobular diffuse hepatocellular hypertrophy: 1/15 male was minimal at 15 mg/kg/day, 3/15 male were minimal at 80 mg/kg/day, and 7/14 male and 3/14 female were mild at 320 mg/kg/day). These findings were not associated with any increase in liver enzymes and fully reversed during the recovery phase. Kidney: • Hypertrophy/hyperplasia of the juxtaglomerular apparatus, were dose dependent (3/15 males and 3/14 females were minimal at 15 mg/kg/day, 9/15 male and 8/14 females were minimal, and 1/15 females were mild at 80 mg/kg/day, and 5/14 males and 6/14 females were minimal, 5/14 males and 6/14 females were mild, and 1/14 males were moderate at 320 mg/kg); At the end of the recovery phase, the severity and occurrence of this finding was reduced (1/4 male was minimal). • Increased incidence/severity of minimal to mild tubular degeneration was noted at 320 mg/kg/day (1/15 male and 1/14 female were minimal at 80 mg/kg/day and 5/14 males, and 1/14 females were minimal, and 3/14 male were mild at 320 mg/kg/day compared to 1/15 male was minimal and 1/15 female was mild in control) and was fully reversible (1/5 male in control and 1/4 male at 320 mg/kg/day were minimal in recovery groups). Interstitial fibrosis was observed at 80 mg/kg/day (1/15 male was mild and 1/14 female was minimal) and 320 mg/kg/day (3/14 males were minimal, 3/14 males and 1/14 females were mild, and 1/14 male was moderate). The finding was present in one 320 mg/kg/day male with minimal severity at the end of the recovery phase. • The kidney findings were attributed to the pharmacodynamic effect of the test article.
[Other evaluations]	n/a

Source: Pharmacology/toxicology reviewer
Abbreviations: BUN, blood urea nitrogen; Cr, creatinine

13-Week (8-Week Recovery) Toxicology Study

- Doses tested: 15 mg/kg/day, 80 mg/kg/day, and 320 mg/kg/day
- Decrease in body weight gain at ≥80 mg/kg/day, red cell mass at 320 mg/kg/day and increase in BUN ≥80 mg/kg/day were observed in the dosing phase and fully resolved at the end of the recovery phase.
- Decreased heart weight at ≥15 mg/kg/day and increase in liver weight at ≥80 mg/kg/day were not associated with histological findings and not considered adverse effects.
- A dose dependent increase in the incidence and degree of severity of hyperplasia of the juxtaglomerular apparatus (8 out of 10 males and 9 of10 females were minimal at 15 mg/kg/day; 10 of 10 males and 9 of 10 females were minimal at 80 mg/kg/day; 1 of 10 females was minimal, and 10 of 10 males and 9 of 10 females were mild at 320 mg/kg/day) was observed and associated with increased kidney weights. This microscopic finding was partially reversed at the end of the recovery period (320 mg/kg/day) with a decreased severity (3 of 5 males and 4 of 5 females were minimal), and the increased kidney weight was fully resolved.

- Increased incidences of interstitial infiltrates of mononuclear cells and tubular degeneration/regeneration was noted mainly at 320 mg/kg/day at the end of dosing phase, which was fully reversed at the end of recovery phase.
- The NOAEL was determined at 320 mg/kg/day, which is associated with $AUC_{0\text{-last}}$ values of 754,000 h*ng/mL in males and 1,110,000 h*ng/mL in females, approximately 10 (male)- and 14 (female) times the AUC at MRHD.

4-Week (4-Week Recovery) Toxicology Study

- Doses tested: 20 mg/kg/day, 80 mg/kg/day and 320 mg/kg/day.
- Reversible mild to moderate decrease in systolic blood pressure was noted at ≥20 mg/kg/day and it was attributed to the pharmacological action of sparsentan.
- A decrease in relative heart weights (up to 15%) was noted at ≥20 mg/kg/day which was not associated with any relevant microscopic findings.
- The liver weight was increased (up to 92%) with a minimal increase in alkaline phosphatase (27%). A slightly increased liver weight (9%) was still present at ≥80 mg/kg/day at the end of the recovery phase.
- Increase in BUN (up to 2-fold) was observed at ≥80 mg/kg/day.
- The NOAEL was determined at 20 mg/kg/day.

13.1.3.1.3. Repeat Dose Toxicity Studies in Monkeys

Repeat dose toxicity studies were conducted in monkeys for 4, 13, and 39 weeks with oral dose levels from 10 to 250 mg/kg/day. Key findings are summarized below with the 39-week study results listed in the table below.

39-Week (8-Week Recovery) Toxicology Study

Study Number/Title

39-Week Toxicity Study in Cynomolgus Monkeys with an 8-Week Recovery

Key Study Findings

- Dose dependent increases in the incidence and severity of kidney juxtaglomerular apparatus hypertrophy/hyperplasia and interstitial fibrosis in the renal cortex were observed at ≥10/125 mg/kg, and a reduced severity of these measures in the high dose group was noted at the end of the recovery phase. These observations were considered related to the pharmacological action of sparsentan.
- The NOAEL was determined at 50 mg/kg/day, which is associated with $AUC_{0\text{-last}}$ values of 16,700 h*ng/mL in males and 12,700 h*ng/mL in females, approximately 0.2 times the AUC at MRHD for both males and females.

Table 59. Study Information

Study Features and Methods	Details
GLP compliance	Yes
Dose and frequency of dosing	0, 50, 10/125 (10 mg/kg for Days 1-35 and 125 mg/kg for Days 36-273) and 200 mg/kg and once daily
Route of administration	Oral gavage
Formulation/vehicle	0.5% methylcellulose 4000 cP/0.25% Tween 80
Species/strain	Cynomolgus monkey
Number/sex/group	4 (main) or 2 (recovery)/sex/group
Age/weight	Not provided/2.0 to 3.5 kg
Satellite groups/unique design	None
Deviation from study protocol affecting interpretation of results	None

Source: Pharmacology/toxicology reviewer
Abbreviations: GLP, good laboratory practice

Table 60. Observations and Results

Parameters	Major Findings
Mortality	One male animal (50 mg/kg/day) was found moribund and euthanized on Day 232. Inflammatory changes in the meninges of the brain and cervical spinal cord were noted with elevated fibrinogen level, which were not observed in other surviving animals. However, the cause for inflammation was not identified. The other microscopic findings in bone marrow (minimal hypocellularity of the erythroid precursors) and kidney (mild hypertrophy/hyperplasia of the juxtaglomerular apparatus) were consistent with the surviving animals and unlikely the cause of the moribundity. Since all other animals including the higher dose groups survived, this single incidence was not considered treatment related.
Clinical signs	Dose-dependent (≥10/125 mg/kg/day) increased incidence of tail lesions; abrasions, scabs, lacerations, ulcerations, discharge and apparent blood on the tail, tail skin peeling or swelling, and tail discoloration, were noted. As these observations were partially reversed in the recovery group, they were not considered adverse. These observations were attributed to the treatment-related decreased blood flow that might exacerbate pre-existing tail chewing behavior in monkeys.
Body weights	Dose dependent decrease in the mean body weight (27.3% in males and 19.7% in females on Day 273 at 200 mg/kg/day), mean body weight gain or loss of body weight at ≥10/125 mg/kg/day (41% versus 22.5% and 14.3% in male and 26.9% versus 1.9% and 5.8% in females for control versus 10/125 and 200 mg/kg/day, respectively) was observed with a statistical difference at 200 mg/kg/day. The effect on body weight persisted until the end of the recovery phase at 200 mg/kg/day.
Feed consumption	Reversible low food consumption at ≥10/125 mg/kg/day.
Ophthalmoscopy	No effect
Hemodynamics and ECG measurements	No effect
Hematology	Statistically significant and dose dependent reduction (16 to 24%) in red cell mass (red blood cell count, hemoglobin concentration, and hematocrit) was noted at 200 mg/kg/day. In addition, absolute reticulocytes were significantly decreased (36 to 56%) at ≥10/125 mg/kg/day. These findings were fully resolved at the end of recovery.
Clinical chemistry	Reversible elevated blood urea nitrogen (significant at 10/125 mg/kg/day in male and at 200 mg/kg/day in male and female) and creatinine levels (200 mg/kg/day in female) were associated with microscopic observations of hypertrophy/hyperplasia noted in the juxtaglomerular apparatus.

Parameters	Major Findings
Urinalysis	No effect
Gross pathology	Treatment related tail skin lesions; crusts and ulcers in all the dose groups with a dose-dependent increase in frequency.
Organ weights	The nonsignificant weight differences noted in mean testes weight were attributed to the variation in the onset of sexual maturity in individual animals.
Histopathology Adequate battery: Yes	Kidney: • Dose-dependent increased incidence and severity in juxtaglomerular apparatus hypertrophy/hyperplasia were observed at all dose levels. • (3/4 in both males and females were minimal and 1/4 females was mild at 50 mg/kg/day, 1/4 in both males and females were minimal, 2/4 both males and females were mild, and 1/4 females were moderate at 10/125 mg/kg/day, 1/4 male was mild, and 3/4 males and 4/4 females were moderate at 200 mg/kg/day). At the end of the recovery phase, the severity was decreased (2/2 in both male and female were minimal). • Minimal to mild interstitial fibrosis was noted in the kidney cortex (1/4 male was minimal and 1/4 female was mild at 10/125 mg/kg/day and 3/4 females were mild at 200 mg/kg/day). At the end of the recovery phase, the severity was decreased (1/2 male was minimal at 200 mg/kg/day). Bone marrow: • Minimal hypocellularity of the erythroid precursors were noted in all treated groups (one female at 50 mg/kg/day, four females at 10/125 mg/kg/day and two male and three females at 200 mg/kg/day) and was correlated with a dose-related decrease in red cell mass. Both were resolved in 200 mg/kg/day at the end of the recovery phase. Tail skin: • Increased incidence of mild to marked ulceration at the tail skin was present in all treated groups on Day 274 and resolved at the end of recovery period.
[Other evaluations]	n/a

Source: Pharmacology/toxicology reviewer
Abbreviations: ECG, electrocardiogram

13-Week (4-Week Recovery) Toxicology Study

- Doses: 10 mg/kg/day, 50 mg/kg/day, and 200 mg/kg/day
- Male body weight was decreased (14-22%) at 250 mg/kg/day and improved but not fully reserved at the end of the recovery period.
- Macroscopic lesions, crusts, and abrasions, of the tail at 250 mg/kg/day at Day 92 correlated with microscopic observations including marked necrosis of the skin, infiltrates of neutrophils, edema, and fibrin accumulation in the dermis.
- Reduction in circulating red cell mass corresponded with associated hypocellularity of the bone marrow (3 of 4 males and 2 of 4 females) at 250 mg/kg/day, which was attributed to diminished erythropoiesis at high dose and fully resolved at the end of recovery period.
- Decreases in absolute and/or relative thymic weights was observed (males at ≥50 mg/kg/day and females at 250 mg/kg/day) without any microscopic correlation.
- Microscopic findings in the kidney included increased incidences and degree of severity of hyperplasia of the vessels at juxtaglomerular region (2 of 4 males and 1of 4 females were minimal at 10 mg/kg/day; 1 of 4 males minimal and 2 of 4 males and 3 of 4 females were

mild, and 1 of 4 male and 1 of 4 females were moderate at 50 mg/kg/day; 1 of 4 both males and females were mild, and 3 of 4 males and 3 of 4 females were moderate at 250 mg/kg/day). These findings were partially reversed at the end of recovery period (Day 120) with decreased severity (2 of 4 males and 1 of 4 females were minimal, and 1 of 4 female was mild at 250 mg/kg/day). An associated elevation of BUN and Cr levels at 250 mg/kg/day were fully resolved at the end of recovery.

- There was no statistically significant difference in male reproductive organ weight (testes, epididymides, and seminal vesicles) between groups. The large variation between individual animals may reflect the difference in the onset of sexual maturity for the individual animals.
- A minimal to moderate, bilateral testicular hypoplasia/interstitial fibrosis was observed in one each of the 50, 250 mg/kg/day and recovery control groups). It was characterized with a decrease in the number of seminiferous tubules with an increased connective tissue, and without an active spermatogenesis. A peer-review of these pathological observations by the expert in the field was conducted, and it concluded that these abnormalities were due to a pre-existing congenital or developmental abnormality but not test article related, as it lacks tubular necrosis, inflammation or repair that would indicate destruction of tubules. This review is further supported by a publication in which minimal to severe testicular fibrous hypoplasia with a unilateral (38.5%) and bilateral (61.5%) occurrence was reported in cynomolgus monkeys (Pereira Bacares et al. 2017). Given that the finding was within the reported incidence/severity range in the untreated cynomolgus monkeys and was also observed in the concurrent control group, it was not considered test article related.
- The NOAEL was determined at 50 mg/kg/day, which is associated with a $AUC_{0\text{-last}}$ values of 25,600 h*ng/mL in males and 29,700 h*ng/mL in females, approximately 0.3 (male)- and 0.4 (female)-times the AUC at MRHD.

13.1.3.2. Genotoxicity Studies

Sparsentan showed no mutagenic or clastogenic activity in a standard battery of genotoxicity studies, which include two bacterial reverse mutation studies, an in vitro chromosomal aberration study in human lymphocytes, and a 3-day in vivo micronucleus study in rats.

Table 61. Genetic Toxicology

Study/Study Number	Key Study Findings
Study no: DS1074 Study title: Ames Reverse-Mutation Study in *Salmonella* and *Escherichia Coli (E.Coli)* Test system: *Salmonella typhimurium* (TA98, TA100, TA1535, and TA1537) and Escherichia Coli (WP2uvrA). Doses tested:25, 80, 250, 800, and 2500 µg/plate for *Salmonella* strains and an additional 5000 µg/plate for *E.coli* stain GLP compliance: Yes Study is valid: Yes	Sparsentan did not produce increases in revertant colonies relative to spontaneous reversion in the solvent control (DMSO); therefore, sparsentan was considered negative for mutagenicity in this bacterial reverse mutation assay. Positive controls demonstrated expected S-9- and strain-dependent increases in revertant colonies. Cytotoxicity (reduction of the bacterial background lawn) was noted at ≥2500 µg/plate in *Salmonella typhimurium* strains and no cytotoxicity observed up to 5000 µg/plate in *E.coli* stain.

Study/Study Number	Key Study Findings
Study no: 961259 Study title: Chromosome Aberration Test Test system: Human lymphocytes Doses tested: 4, 8, 16, 32, 64, 128, 256, 512, 1000, and 2000 µg/mL GLP compliance: Yes Study is valid: Yes	Human peripheral blood lymphocytes were treated with sparsentan up to 2000 µg/mL with and without S-9 metabolic activation. No increase in structural chromosome aberrations in sparsentan treated cells were observed: therefore, sparsentan was considered negative for clastogenicity. The positive controls induced expected increases in aberrations over the solvent control (DMSO).
Study no: DS01022 Study title: Oral Micronucleus Study in Rats Species/strain: Rat/Sprague-Dawley Number/sex/group: 5/sex/group Doses tested: 1500, and 2000 mg/kg Route and duration: Oral, daily for three consecutive days. GLP compliance: Yes	No mortality or drug-related clinical signs were observed. No toxicologically significant decreases in mean polychromatic erythrocyte (PCE) frequency were observed in bone-marrow samples, except in females at 2000 mg/kg (25% reduction). Mean PCE frequencies in male are 46% at both dose levels in males, and 44 and 40% at 1500 and 2000 mg/kg, respectively, in females, while the negative control males and females were at 50 and 53%, respectively. The treated animals mononucleated (MN) PCE frequencies (0.18 and 0.17% in males and 0.21 and 0.13% in females at 1500 and 200 mg/kg, respectively) were not different compared to negative control (0.17 and 0.16% in negative control male and female, respectively). Sparsentan was considered negative for genotoxic potential in this study.

Source: Pharmacology/toxicology reviewer
Abbreviations: DMSO, dimethyl sulfoxide; GLP, good laboratory practice

13.1.3.3. Carcinogenicity Studies

13.1.3.3.1. RE-021: 104-Week Oncogenicity Study in Rats (RE-021-Report056-2016-CARC)

Table 62. Methods of Carcinogenicity Study in Rats

Study Features and Methods	Details
ECAC concurrence	Yes
Dose and frequency of dosing	15, 60, and 240 mg/kg/day, once daily
Route of administration	Oral gavage
Formulation/vehicle	0.5% Methylcellulose/0.25% Tween 80 [weight/volume] in deionized water
Species/strain	Rat/Sprague-Dawley
Number/sex/group	60
Age	6 weeks

Study Features and Methods	Details
Dosing comments	Males given 60 or 240 mg/kg/day showed significant body weight loss in the early stage and were euthanized and discarded without further evaluation during Week 29. Control and low-dose males (given 15 mg/kg/day) were euthanized during Week 93 when the control male survival declined to 20 animals. At Week 89, remaining females in the 240 mg/kg/day group were euthanized when the number of surviving rats declined to 15 animals. All the remaining female groups were euthanized during Week 92 when survival in controls declined to 20 animals.

Source: Pharmacology/Toxicology Reviewer
Abbreviations: ECAC, executive carcinogenicity assessment committee

Table 63. Observations and Results of Carcinogenicity Study in Rats

Parameters	Major Findings
Mortality	For female rats, the number of live females in the 240 mg/kg/day high dose group dropped to the range of 26 to 15 between Week 78 to 89. Early deaths in the high dose females were considered sparsentan related.
Clinical signs	No effect
Body weights	By Week 8, males given 60 or 240 mg/kg/day weighed 17.5% and 16.3% less than the control males, respectively. The differences in body weight continued to increase and by Week 28, males given 60 or 240 mg/kg/day weighed 20.6% and 20.8% less than the control males, respectively.
Necropsy findings	Microscopic non-neoplastic findings in the kidney were observed in sparsentan treated animals, and sparsentan was not carcinogenic in males administered with 15 mg/kg/day for up to 93 weeks, and in females at doses up to 240 mg/kg/day for at least 89 weeks.

Source: Pharmacology/Toxicology Reviewer

The $AUC_{0\text{-last}}$ for males at 15 mg/kg/day and females at 240 mg/kg/day are 53700 and 2030000 ng*hr/mL, respectively, which is associated with $AUC_{0\text{-last}}$ values of 53,700 h*ng/mL in males and 203,0000 h*ng/mL in females, approximately 0.7 (male)- and 26 (female)-times the AUC at MRHD.

Executive Carcinogenicity Assessment Committee Conclusions

Rat

- The Committee concluded that there was no evidence of drug-related neoplasms in males; however, only a single treatment group was assessed in this study.
- The Committee concluded that the carcinogenicity study was adequate and negative in females for the 2-year rat study.

13.1.3.3.2. RE-021: 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice (RE-021-Report004-2016-CARC)

Table 64. Study Information

Study Features and Methods	Details
ECAC concurrence	Yes
Dose and frequency of dosing	0, 60, 200 and 600 mg/kg, once daily
Route of administration	Oral gavage
Formulation/vehicle	0.5% Methylcellulose/0.25% Tween 80 [weight/volume] in deionized water
Species/strain	Mouse/CByB6F1-Tg(HRAS)2Jic
Number/sex/group	25
Age	6 weeks
Dosing comments	The were no early terminations in the 26-week CByB6F1-Tg(HRAS)2Jic mouse study.

Source: Pharmacology/toxicology reviewer
Abbreviations: ECAC, executive carcinogenicity assessment committee

Table 65. Observations and Results of Carcinogenicity Study in Mice

Parameters	Major Findings
Mortality	No effect
Clinical signs	No effect
Body weights	No effect
Necropsy findings	Compared to the vehicle controls, there were no drug-related neoplastic changes at doses up to 600 m/kg/day. Non-neoplastic sparsentan-related microscopic findings were noted in liver and kidney. In kidney, hypertrophy/hyperplasia of the Juxtaglomerular apparatus was observed in both sexes at ≥200 mg/kg/day and the severity of the finding was minimal in males at both dose levels and in females at 200 mg/kg/day, and minimal to mild in females at 600 mg/kg/day. An increased incidence of minimal chronic progressive nephropathy was observed in both sexes at ≥200 mg/kg/day. In liver, panlobular hepatocellular hypertrophy was minimal in males and minimal to mild in females at 600 mg/kg/day. These liver and kidney changes were considered adaptive responses to the pharmacology effect of sparsentan.

Source: Pharmacology/toxicology reviewer

Executive Carcinogenicity Assessment Committee Conclusions

Mouse

- The Committee concluded that the carcinogenicity study was adequate.
- The Committee concluded that there was no evidence of drug-related neoplasms in either males or females for the 6-month Tg mouse study.

13.1.3.4. Reproductive and Developmental Toxicity

13.1.3.4.1. Fertility and Early Embryonic Development

Study Number/Title

PCO-NC-021/Oral (Gavage) Fertility and General Reproduction Toxicity Study of PS433540 in Rats

Key Study Findings

- No sparsentan-related effects on fertility or early embryonic development up to 320 mg/kg/day, the highest dose tested.
- The NOAEL was determined at 320 mg/kg/day which was associated with $AUC_{0\text{-last}}$ values of 75400 ng.hr/mL in male and 1,110,000 ng.hr/mL in female rats based on the exposure of females at dose of 320 mg/kg/day in the 13-week general toxicology study, and approximately 10 to 14-times the AUC at MRHD.
- Good laboratory practice (GLP) compliance: Yes

Table 66. Methods of Fertility and Early Embryonic Development Study in Rats

Parameter	Method Details
Dose and frequency of dosing:	0, 20, 80, and 320 mg/kg/day, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% Methylcellulose/0.25% Tween 80 [weight/volume] in deionized water
Species/strain:	Sprague Dawley rat
Number/sex/group:	25 per sex/group
Satellite groups:	None
Study design:	Animals were treated beginning 28 days prior to cohabitation, through cohabitation (maximum 21 days), and through the day prior to necropsy (Days 50 to 53) for males or 15 days prior to cohabitation through gestation day (GD) 7 for females. TK data were not collected.
Deviation from study protocol affecting interpretation of results:	None

Source: Pharmacology/toxicology reviewer
Abbreviations: TK, toxicokinetic

Table 67. Observations and Results of Fertility and Early Embryonic Development Study in Rats

Parameters	Major Findings
Mortality	No effect
Clinical signs	No effect
Body weights	No effect
Necropsy findings Cesarean section data	No effect

Source: Pharmacology/toxicology reviewer

13.1.3.4.2. Embryo-Fetal Development

Rat Embryofetal Development Toxicity Study

Study Number/Title

RE-021-Report002-2017-RTOX/An Embryo-Fetal Development Study of RE-021 by Oral Administration (Gavage) in Rats

Key Study Findings

- Due to the mortality at ≥160 mg/kg/day and adverse findings noted at all doses, the NOAEL for maternal toxicity was not established (<80 mg/kg/day).
- Based on dose-dependent teratogenic effects in the form of craniofacial malformations, skeletal abnormalities, increased postimplantation loss, and reduced fetal weights observed at ≥80 mg/kg/day, the NOAEL for developmental toxicity could not be established. The lowest dose of 80 mg/kg/day is associated with an $AUC_{0\text{-last}}$ of 805,000 ng*hr/mL, approximately 10-times the AUC at MRHD.
- GLP compliance: Yes

Table 68. Methods of Oral Embryo-Fetal Developmental Study in Rats

Parameter	Method Details
Dose and frequency of dosing:	0, 80, 160, and 240 mg/kg/day, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% Methylcellulose/0.25% Tween 80 [weight/volume] in deionized water
Species/strain:	Sprague Dawley rat
Number/sex/group:	20 females/group
Satellite groups:	6 females/group TK (n=3 for controls)
Study design:	Female animals were dosed from Day 7 to Day 17 of gestation (inclusive). At last, females were euthanized on GD 21.
Deviation from study protocol affecting interpretation of results:	None

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day; TK, toxicokinetic

Table 69. Observations and Results of Embryo-Fetal Development Study in Rats

Parameters	Major Findings
Mortality	Sparsentan-related deaths occurred at 160 and 240 mg/kg/day.
Clinical signs	Dehydration and hunched posture (as early as GD 13 or 15 until 21) and thin body condition (beginning from GD 18 or 19 to 21) were observed at ≥80 mg/kg/day.
Body weights	Sparsentan-related reductions in maternal body weights and body weight gains were observed at ≥80 mg/kg/day.
Necropsy findings Cesarean section data	There were 0, 1, 1, and 3 litters in the 0 (Control), 80, 160, and 240 mg/kg/day dose groups that consisted of all dead or resorbed conceptuses.
Necropsy findings Offspring	Mean fetal body weights (total, male, and female) were significantly decreased at all treated groups, compared to controls. Administration of sparsentan at doses of ≥160 mg/kg/day resulted in a variety of craniofacial malformations that included cleft lower jaw, small upper or lower jaw (maxilla and mandible, respectively), and absent or protruding tongue at external examination. Small tongues were also observed at 160 mg/kg/day and 240 mg/kg/day. Sparsentan-related skeletal abnormalities included misshapen basisphenoids and mandibles at ≥80 mg/kg/day, misshapen hyoid bodies, short mandibles, fused and misshapen pterygoid processes, and absent tympanic annuli at ≥160 mg/kg/day, and fused hyoid bodies and incompletely ossified tympanic annuli at 240 mg/kg/day. Reductions in the mean number of ossified caudal vertebra occurred at ≥80 mg/kg/day.

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day

Rabbit Embryofetal Development Toxicity Study

Study Number/Title

T5082410/Prenatal Developmental Toxicity Study in Rabbits After Administration by Gavage

Key Study Findings

- Due to the death and abortions at ≥10 mg/kg/day, and adverse findings noted at all doses, the NOAEL for maternal toxicity was not established (<2.5 mg/kg/day).
- There were no fetal malformations or change in fetal viability or growth at doses up to 40 mg/kg/day. Increased number of litters with fetuses with short, supernumerary cervical ribs was noted at high dose of 40 mg/kg/day which is associated with an $AUC_{0\text{-last}}$ of 13,500 ng*hr/mL, approximately 0.2-times the AUC at MRHD.
- The NOAEL for developmental toxicity was 10 mg/kg/day, which is associated with an $AUC_{0\text{-last}}$ of 9,550 ng*hr/mL, approximately 0.1-times the AUC at MRHD.
- GLP compliance: Yes

Table 70. Methods of Oral Embryo-Fetal Developmental Study in Rabbits

Parameter	Method Details
Dose and frequency of dosing:	0, 2.5, 10 and 40 mg/kg/day, once daily
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% Methylcellulose/0.25% Tween 80 [weight/volume] in deionized water
Species/strain:	Rabbit/New Zealand White
Number/sex/group:	20 per sex/group
Satellite groups:	3 females/group TK
Study design:	Dams treated once daily from GD 7-19. TK blood sampling was performed on gestation Days 7 and 19.
Deviation from study protocol affecting interpretation of results:	None

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day; TK, toxicokinetic

Table 71. Observations and Results of Embryo-Fetal Developmental Study in Rabbits

Parameters	Major Findings
Mortality	Sparsentan-related death and abortions occurred at ≥10 mg/kg/day. One female rabbit at 40 mg/kg/day was found dead on GD 20. Two rabbits at 10 mg/kg/day and one rabbit at 40 mg/kg/day aborted on GD 24, GD 26, and GD 27, respectively, and were subsequently euthanized. These deaths occurred in does with complete litter losses (two litters each of the dose groups of 10 and 40 mg/kg/day consisting of all dead or resorbed conceptuses)
Clinical signs	Treatment-related adverse clinical signs were noted, including thin body condition at all doses, absent feces at 10 mg/kg/day, and decreased fecal output ≥10 mg/kg/day.
Body weights	At all doses, dose-related reductions in mean maternal body weight during intervals up to GD 16 were noted. At last, mean maternal body weights were generally similar across the four dose groups throughout the entire study and did not significantly differ from controls.
Necropsy findings Cesarean section data	No effect
Necropsy findings Offspring	Fetal evaluations were based on 193, 184, 165, and 183 live, GD 29, Caesarean-delivered fetuses in 19, 17, 15, and 18 litters at 0 (Control), 2.5, 10, and 40 mg/kg/day, respectively. An increase in the number of litters with fetuses with short, supernumerary cervical ribs was observed at 40 mg/kg/day at which severe maternal toxicity was observed.

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day

Rat Pre- and Postnatal Development Study

Study Number/Title

RE-021-Report003-2017-RTOX/A Pre- and Postnatal Developmental Toxicity Study of RE-021 by Oral Administration (Gavage) in Rats, Including a Postnatal Behavioral/Functional Evaluation

Key Study Findings

- Maternal mortality and adverse findings were observed at ≥20 mg/kg/day. The maternal NOAEL was 5 mg/kg/day.
- Decreases in pup body weights occurred at ≥20 mg/kg/day; increased pup mortality during the preweaning period (reduced maternal nursing and nesting behaviors) occurred at 80 mg/kg/day.
- The NOAEL for pre- and postnatal development was 5 mg/kg/day, which is associated with an $AUC_{0\text{-last}}$ of 50,313 ng*hr/mL, approximately 0.7-times the AUC at MRHD.
- GLP compliance: Yes

Table 72. Methods of Oral PPND Study in Rats

Parameter	Method Details
Dose and frequency of dosing:	0, 5, 20 and 80 mg/kg/day
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% Methylcellulose 4000 cps/0.25% TWEEN 80 (w/v)
Species/strain:	Sprague Dawley rat
Number/sex/group:	22 females/group 22 F1 offspring/group
Satellite groups:	None
Study design:	Dams treated once/day from GD 7-LD 20
Deviation from study protocol affecting interpretation of results:	None

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day; PPND, pre- and postnatal development

Table 73. Observations and Results of PPND Study in Rats

Parameters	Major Findings
Mortality	Sparsentan-related death was found in one F0 female rat exposed to 80 mg/kg/day.
Clinical signs	At 80 mg/kg/day, there was a slight increase in the number of F0 female rats observed with hunched posture, thin body condition, dehydration (mild or moderate), pale extremities, and/or piloerection in comparison with the control group.
Body weights/food consumption	During the gestation period, there were sparsentan-related body weight gains decreases (21% and 40% lower compared to controls) observed at 20 and 80 mg/kg/day. Reduced body weight was also observed in these two groups, beginning on GD 10, and continuing through the gestation and until DL 19 of the lactation period. There were decreases in food consumption values at all intervals during the gestation period (12% to 42%) at ≥20 mg/kg/day and at all intervals during the lactation period (16% to 27%) at 80 mg/kg/day.
Necropsy findings Cesarean section data	Pregnancy occurred in all of the mated female rats in the RE-021 treated groups. At 80 mg/kg/day, there was a statistically significant increase in the number of pups found dead or presumed cannibalized on PNDs 2 through 7 and PNDs 11 through 21, which contributed to a statistically significant decrease in the viability index (PND 0-4), the lactation index (PNDs 4 to 21), and the number of surviving pups/litter on PNDs 7, 10, 14, and 21.

Parameters	Major Findings
Clinical Observations Offspring	At 80 mg/kg/day, there was a sparsentan-related increase in the total frequency and the number of F1 generation litters observed not nesting or nursing with no milk band present, mild or moderate dehydration, and/or thin body condition.
Body weights/Food consumption Offspring	Average body weights of F1 male rats at ≥20 mg/kg/day were decreased beginning on PND 78 and continuing through the remainder of the postweaning period (PND 113). However, there were no changes in body weight gains in F1 males or in body weights or body weight gains in F1 females, and food consumption was not affected postweaning.
Necropsy findings Offspring	At the terminal necropsy, no RE-021-related findings were observed in F1 generation.

Source: Pharmacology/toxicology reviewer
Abbreviations: GD, gestation day; PND, postnatal day

13.1.4. Other Toxicology/Specialized Studies

Phototoxicity: As sparsentan does not absorb light (between 290 nm and 700 nm) and no sparsentan-derived radioactivity was noted in eye or in melanin-containing tissues in pigmented rats, phototoxic potential is considered negligible and an in vivo phototoxicity study with sparsentan was not required.

13.1.5. Impurities/Degradants

(b) (4) is a (b) (4) impurity present in all batches of sparsentan. The Applicant proposed a specification limit of not more than (b) (4)% for (b) (4). Based on in silico analysis that was accepted by FDA/Division of Applied Regulatory Science, (b) (4) was not identified as a structural alert for bacterial mutagenicity.

A 91-day general toxicity study (Study No. RE-021-0005/RE-021) was conducted to compare two batches of RE-021 (sparsentan) with standard level of impurity (b) (4) and a higher level of impurity ((b) (4)%) at a sparsentan dose level of 80 mg/kg/day. The qualification threshold for the impurity per Q3A is 0.15% or 1 mg (whichever is lower for the MRHD ≤2 g/day). The results showed similar decreased body weight gain and microscopic changes in the kidney (tubular degeneration, tubular dilation, and chronic inflammation) in both groups, which were generally consistent with observations in the previous toxicity studies with sparsentan. As no differences in toxicity profile were observed in rats between sparsentan (80 mg/kg/day) containing (b) (4)% (b) (4) and (b) (4)% (b) (4), the proposed specification limit of not more than (b) (4)% was considered acceptable.

13.2. Individual Reviews of Studies Submitted With the New Drug Application

Genotoxic potential for multiple [redacted] and potential impurities were evaluated using in silico (Q)SAR analysis to identify structural alerts for bacterial mutagenicity and/or in bacterial reverse mutation (Ames) assays.

(Q)SAR evaluation: The submitted (Q)SAR analysis reports were acceptable upon consultation with Division of Applied Regulatory Science. The results are listed below.

- [redacted] were not identified with any structural alerts for bacterial mutagenicity.
- [redacted] were identified with structural alerts for bacterial mutagenicity.

Bacterial reverse mutation (Ames) assay: The following compounds were tested in Ames assay and the results are listed below.

- [redacted] were tested negative. See the studies reviewed in the table below.
- [redacted] was reported equivocal (previously reviewed in the IND). No confirmatory Ames assay has been conducted.

The compounds that lacked structural alerts in (Q)SAR analysis or tested negative in an Ames assay are treated as Class 5 impurities consistent with International Council for Harmonisation M7(R^2). The compound with structural alerts but not evaluated in an Ames assay, [redacted], and the compound with Ames equivocal response, [redacted], are proposed to be treated as Class 2 impurities.

Table 74. Genetic Toxicology Studies

Study No./Study Title	Key Study Findings
Study no. RE-021-0038/GLP Bacterial Reverse Mutation Assay and Dose Formulation Analysis for [redacted] Concentration tested and tester strains: Dose up to [redacted] µg/plate was tested for all the strains (TA98, TA100, TA1535, TA1537 and WP2uvrA). No precipitation was observed in the mutagenicity study. GLP compliance: Yes Study is valid: Yes	Cytotoxicity: No background lawn toxicity was observed, and reduction in revertant count was observed at [redacted] µg/plate with tester stain TA1535 in the absence of S9 mixture. Method: Plate incorporation method was used, and commercial liver homogenate (S9) mixture (MolTox) was used for metabolic activation. Genotoxic effects: The study is considered valid and under the conditions tested impurity, [redacted], did not cause a positive mutagenic response with any of the tester strains with or without the presence of S9 mixture. Therefore, the result of the study is considered negative.

Study No./Study Title	Key Study Findings
Study no. RE-021-Report046-2015-G TOX/(b) (4): A GLP Bacterial Reverse Mutation Assay Concentration tested and tester strains: Dose up to (b) (4) µg/plate was tested for all the strains (TA98, TA100, TA1535, TA1537 and WP2uvrA). GLP compliance: Yes Study is valid: Yes	No precipitate was observed in the mutagenicity study. Cytotoxicity: No background lawn toxicity was observed, and toxicity was observed was observed (b) (4) or (b) (4) µg per plate concentration with tester strains, TA100 and TA1535 in the presence or absence of S9 mixture. Method: Preincubation method was used, and commercial liver homogenate (S9) mixture (MolTox) was used for metabolic activation. Genotoxic effects: The study is considered valid and under the conditions tested impurity, (b) (4), did not cause a positive mutagenic response with any of the tester strains with or without the presence of S9 mixture and with or without GSH (5mM). Therefore, the result of the study is considered negative.
Study no. RE-021-Report008-2016-GTOX/(b) (4): A GLP Bacterial Reverse Mutation Assay Concentration tested and tester strains: Dose up to (b) (4) µg/plate was tested for all the strains (TA98, TA100, TA1535, TA1537 and WP2uvrA). GLP compliance: Yes Study is valid: Yes	Precipitate was observed at (b) (4) µg/plate in the mutagenicity study. Cytotoxicity: No background lawn toxicity was observed and reduction in revertant count was observed at (b) (4) µg per plate concentration with WP2uvrA strain without S9 action and GSH. Method: Plate incorporation method was used, and commercial liver homogenate (S9) mixture (MolTox) was used for metabolic activation. Genotoxic effects: The study is considered valid and under the conditions tested impurity, (b) (4), did not cause a positive mutagenic response with any of the tester strains with or without the presence of S9 mixture and with or without GSH (5mM). Therefore, the result of the study is considered negative.
Study no. RE-021-Report006-2016-GTOX/(b) (4): A GLP Bacterial Reverse Mutation Assay Concentration tested and tester strains: Dose up to (b) (4) µg/plate was tested for all the strains (TA98, TA100, TA1535, TA1537 and WP2uvrA). GLP compliance: Yes Study is valid: Yes	Precipitate was observed beginning at (b) (4) or (b) (4) µg/plate in the presence of absence of S9 mixture in the mutagenicity assay. Cytotoxicity: No background lawn toxicity was observed, and no toxicity was observed. Method: Preincubation method was used, and liver homogenate (S9) mixture (MolTox) was used for metabolic activation. Genotoxic effects: The study is considered valid and under the conditions tested impurity, (b) (4), did not cause a positive mutagenic response with any of the tester strains with or without the presence of S9 mixture and with or without GSH (5mM). Therefore, the result of the study is considered negative.

Source: Pharmacology/toxicology reviewer
Abbreviations: GLP, good laboratory practice; GSH, glutathione

14. Clinical Pharmacology

14.1. In Vitro Studies

14.1.1. Plasma Protein Binding

The plasma protein binding of sparsentan, determined ex vivo over the therapeutic plasma concentration range, averaged 99.1% (±0.5%). In vitro, sparsentan was similarly highly protein bound at ≥97% across a concentration range of 1 to 100μM (0.59 to 59 μg/mL), with concentration-independent preferential binding to albumin (>90%). Binding to α1-acid glycoprotein is concentration dependent, with approximately 50% at 10 and 40μM, and approximately 80% at 1μM with larger coefficient of variation.

14.1.2. Distribution in Red Blood Cells

Sparsentan was not preferentially partitioned into red blood cells (RBCs), with geometric mean blood-to-plasma ratio of 0.647 from ex vivo samples of a clinical study. In vitro, the blood-to-plasma ratio ranged from 0.579 to 0.674 across a concentration range of 0.1 to 100μM (0.059 to 59 μg/mL).

14.1.3. Metabolism Studies

Sparsentan as Substrate

The metabolism of sparsentan was studied in human liver microsomes (HLM) with and without CYP enzyme–specific chemical inhibitors and human hepatocytes.

The biotransformation of sparsentan was investigated in vitro using cryopreserved hepatocytes from human. Oxidation at various sites of the molecule (via the addition of 1, 2, 3, or 4 oxygen atoms) was the most common biotransformation pathway.

Incubation of sparsentan with recombinant CYP enzymes suggested that sparsentan is metabolized primarily by CYP3A4 and, to a lesser extent, CYP2C8 and CYP2C9.

Potential for CYP Enzyme Inhibition

Sparsentan was evaluated as a direct, time-dependent, and metabolism-dependent inhibitor of CYP enzymes over the concentration range of 0.1 to 200μM in HLM. Under the experimental conditions, sparsentan was a direct inhibitor of CYP2C8 and CYP3A4/5 and a metabolism-dependent inhibitor of CYP3A4.

The extent of metabolism-dependent inhibitory potential was determined by half maximal inhibitory concentration (IC_{50}) shift from the 10-fold dilution of the preincubation mixture with the appropriate marker substrate mixture. Assays were also conducted to determine the maximal rate of inactivation (k_{inact}) and concentration that supports half of the maximal rate of inactivation (K_I) for sparsentan on CYP3A4/5 marker substrates.

Sparsentan exhibited direct inhibition of CYP2C8 (IC_{50} 17.4μM), CYP3A4/5 (IC_{50} 5.1μM; midazolam), and metabolism-dependent inhibition of CYP3A4/5. The IC_{50}, k_{inact}, and K_I values were normalized with protein binding of sparsentan to HLM. Estimated R1, $R1_{gut}$, and R^2 values suggest the potential for sparsentan to cause drug-drug interactions (DDIs) resulting from inhibition of CYP3A4/5, but not other CYP enzymes.

Potential for CYP Enzyme Induction

Sparsentan was evaluated as an inducer of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 activity and/or expression in human hepatocytes. Treatment of cultured human hepatocytes with up to 250μM sparsentan (100μM in the study with CYP1A2) caused concentration-dependent increases in CYP2B6, CYP2C19 and CYP3A4 enzyme activity and mRNA expression of CYP2B6, CYP2C9, CYP2C19 and CYP3A4. Calculated maximum drug-induced effect and concentration of drug that achieved half-maximal effect values for induction of CYP enzyme activity and mRNA expression are summarized in Table 75. Based on these in vitro data, sparsentan is an inducer of CYP2B6, CYP2C9, CYP2C19, and CYP3A4.

Table 75. Induction Parameters: E_{max}, EC_{50}, and Associated R3 Values

Enzyme	EC_{50} (μM)	E_{max} (Fold Change)	R3 Values
CYP2B6			
Activity	2.81 – 7.23	5.46 – 10.6	0.159 – 0.389
mRNA	2.42 – 3.17	6.48 – 8.76	0.333 – 0.372
CYP2C9			
mRNA	2.65	1.54	0.665
CYP2C19			
Activity	1.75	1.91	0.552
CYP3A4			
Activity	8.32 – 19.2	2.03 – 2.28	0.0566 – 0.120
mRNA	8.08 – 19.1	2.60 – 3.05	0.0593 – 0.127

Source: Applicant's RE-021-report016-2016-dmpk report. Table on page 14. Applicant's RE-021-0027 report. Table on page 11
Abbreviations: CYP, cytochrome P450 isoenzyme; EC_{50}, concentration of drug that achieved half-maximal effect; E_{max}, maximum drug-induced effect

14.1.4. Transporter Characterization

Sparsentan as Substrate

To determine if sparsentan is a substrate of P-gp, the bidirectional permeability of sparsentan at three concentrations (1, 10, and 100μM) across Caco-2 cells was measured in the presence and absence of the P-gp inhibitor, verapamil (PCO-NC-018).

The apparent permeability of sparsentan (1, 10, and 100μM) in the B-to-A direction (457±18.2, 359±23.6, and 205±13.2 nm/sec, respectively) was greater than in the A-to-B direction (66.8±9.96, 72.4±8.38, and 113±3.65 nm/sec, respectively). The resulting B-to-A/A-to-B efflux ratios were >2 (except for the 100μM set, may likely be due to saturation of the transporters), indicating that sparsentan is actively transported across Caco-2 cells. The transport of sparsentan was also evaluated in the presence of verapamil (100μM). The ratios of the two transport rates, B→A/A→B, were calculated to be 1.08, 1.17, and 1.24 at 1, 10, and 100μM. The decrease in the ratios from >2.0 to ~1.0 in the presence of verapamil indicates that sparsentan is a substrate for efflux transporters and specifically a substrate for the P-gp efflux transporter.

To determine if sparsentan is a substrate of the breast cancer resistance protein (BCRP), the bidirectional permeability of sparsentan at two concentrations (1 and 10μM) across Madin-Darby canine kidney cells was measured in the presence and absence of the BCRP inhibitors, Ko143 and lopinavir (RE-021-Report-038-2018-DMPK). The efflux ratios measured for 1 and 10μM sparsentan were 8.25 and 6.88, respectively. The efflux ratio for the 10μM concentration was decreased at least 50% to 3.39 and 1.17 in the presence of the BCRP inhibitors Ko143 and lopinavir, respectively. The results show that sparsentan is a substrate of BCRP. In BCRP-overexpressing Sf9 insect or Madin-Darby canine kidney cells sparsentan efflux ratios were 8.28, 6.88, and approximately 1 at 1, 10, and 400μM, respectively, indicating that sparsentan is a substrate that could saturate or inhibit BCRP at higher concentrations.

The potential for sparsentan as a substrate of solute carrier transporters was assessed in HEK-293 cells stably expressing human OATP1B1 and OATP1B3 (RE-021-Report039-2015-DMPK). Sparsentan uptake was similar in the absence and presence of rifampin (transporter inhibitor, uptake ratio <2), indicating that sparsentan is not an OATP1B1 or OATP1B3 substrate.

Potential Transporter Inhibition

The ability of sparsentan to inhibit human ABC efflux transporters P-gp (ABCB1/MDR1), MRP2 (ABCC2), MRP3 (ABCC3), BCRP (ABCG2/MXR), and BSEP (ABCB11/sP-gp) was evaluated by assessing indirect inhibition of ATPase activity, inhibition of vesicular transport, or inhibition of calcein-AM efflux (PCO-NC-029). The ability of sparsentan (0.14μM to 300μM) to indirectly inhibit stimulation of ATPase activity by reference activators verapamil (40μM, P-gp), sulfasalazine (100μM, MRP2; 10μM, BCRP), and benzbromarone (50μM, MRP3) was assessed using inside-out membrane vesicles prepared from Sf9 insect cells overexpressing MDR1, MRP2, MRP3, or BCRP. The ability of sparsentan (0.14μM to 300μM) to inhibit the transport of reference substrates was assessed using inside-out membrane vesicles prepared from Sf9 insect cells overexpressing MRP2 (estradiol-17-beta-glucuronide, E217βG), MRP3 (E217βG), BCRP (E3S), or BSEP (taurocholate) transporters. The ability of sparsentan (0.07μM to 150μM) to inhibit P-gp activity (calcein-AM efflux) was assessed in K562 MDR cells overexpressing P-gp. The results indicate that sparsentan could inhibit P-gp, BCRP, MRP2, and MRP3 with IC_{50} values of 36μM, 13μM, 191μM, and 50μM, respectively.

The ability of sparsentan to inhibit human uptake transporters NTCP, OATP1B1 (OATP2, OATP-C), OATP1B3 (OATP8), and OATP2B1 (OATP-B) was evaluated indirectly by assessing inhibition of substrate uptake in intact cells overexpressing individual uptake transporters (PCO-NC-029). The ability of sparsentan to inhibit the uptake of reference substrates was assessed in CHO cells overexpressing human OATP1B1 (E3S), OATP1B3 (Fluo-3), OATP2B1 (E3S), or NTCP (taurocholate). The results indicate that sparsentan inhibits OATP1B1, OATP1B3, OATP2B1, and NTCP with IC_{50} values of 87μM, 2μM, 31μM, and 47μM, respectively.

Sparsentan (0.1 to 100μM) was evaluated as an inhibitor of the solute carrier transporters OAT_1, OAT3, OCT2, MATE1, and MATE2-K by measuring the accumulation of probe substrates after incubation with HEK293 cells (RE-021-Report-038-2018-DMPK). The results suggest that sparsentan is an inhibitor of OAT_1, OAT3, and OCT2 with IC_{50} values of 2.78μM, 1.36μM, and 13.7μM, respectively, and may be an inhibitor of MATE1 with a maximum of 37.4% inhibition (at 100μM) and an IC_{50} value >100μM; sparsentan does not inhibit MATE2-K.

Based on the comparison of calculated Igut/IC_{50} values, R values with the current FDA recommended thresholds, there is a potential for DDIs with P-gp, BCRP, and OATP1B3 substrates.

The clinically relevant effect of sparsentan on the systemic exposures of selected transporter substrate drugs for OATP1B3 was investigated via a dedicated DDI study (021HVOL16007).

14.2. In Vivo Studies

14.2.1. Study 021HVOL16001: A Prospective, Randomized, Open-Label, Nonreplicate Crossover Study To Compare the Bioavailability of a Tablet Formulation of Sparsentan (RE-021) to a Capsule Formulation of Sparsentan in Healthy Volunteer Subjects

Study Design

Two groups of 16 subjects each were randomized to receive one of two sequences (i.e., Sequence 1: one 400-mg tablet of sparsentan/four 100-mg capsules of sparsentan; Sequence 2: four 100-mg capsules of sparsentan/one 400-mg tablet of sparsentan). The single doses administered in Periods 1 and 2 were separated by an interval of at least 7 days. All doses were administered orally after a 10-hour fast, and subjects continued to fast until 4 hours postdose. Blood samples for analysis of sparsentan in plasma were collected predose and up to 72 hours postdose.

Results

Following a single 400 mg dose of sparsentan as one 400 mg tablet or four 100 mg capsules, the mean plasma concentration versus time profiles were characterized by a rapid absorption phase, with approximately 7.5% lower geometric mean C_{max} observed for the tablet compared to capsules. After reaching C_{max}, the disposition of sparsentan appeared to be multiphasic, with a similar arithmetic mean $t_{1/2}$ for each formulation, 13.0 and 10.7 hours for the tablet and capsule formulations, respectively. Geometric mean apparent clearance (CL/F)(124.2 and 4.3 L/hour), Vd/F (71.5 and 63.4 L/hour) and mean resistance time (18.5 and 15.1 hour) values were also similar between the two formulations.

In this relative bioavailability study comparing tablet and capsule formulations, the exposures of the two formulations were similar (Table 76). Within-subject variability was considered low with a within-subject coefficient of variation values less than 18.1%.

Table 76. Secondary Statistical Analysis of Relative Bioavailability of Sparsentan Administered as a Tablet Versus Capsule

Parameters (Units)	n[a]	LS Mean[b] 1 x 400-mg sparsentan tablet(Test)	n[a]	LS Mean[b] 4 x 400-mg sparsentan capsule(Ref)	Geometric Mean Ratio[c] (90% CI of Ratio)[d](%)	CV_W(%)[e]
$AUC_{0\text{-}lqc}$ (h*ng/mL)	32	91700	31	92900	98.7 (92.5, 105.3)	15.2
$AUC_{0\text{-}inf}$ (h*ng/mL)	32	93600	31	93000	100.7 (94.0, 107.8)	15.7
C_{max} (ng/mL)	32	5820	32	6290	92.5 (85.6, 100.0)	18.1

Source: Applicant's analysis 021HVOL16001 report. Table 11-3 on page 32.
Abbreviations: $AUC_{0\text{-}inf}$, area under the concentration-time curve extrapolated to infinity, $AUC_{0\text{-}lqc}$, area under the concentration-time curve from hour 0 to the last quantifiable concentration, C_{max}, maximum observed concentration, CI, confidence interval, CV, coefficient of variance; LS, least squares

Reviewer's Comment

Bioequivalence was established between the capsule formulation of sparsentan used in early development phases, and the tablet formulation of sparsentan used in other clinical studies including the pivotal phase 3 studies. The geometric least squares mean ratios and 90% confidence intervals (CIs) of $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$ and C_{max} were within the 80 to 125% bioequivalence range.

14.2.2. Study RTRX-RE021-101: Open-Label, Randomized, Two-Period, Two-Way Crossover Study To Evaluate the Single-Dose Bioequivalence of Sparsentan 400-mg Tablets Compared to Sparsentan 200-mg Tablets in Healthy Adult Subjects

Study Design

Two groups of 18 subjects each were randomized to receive one of two sequences (i.e., Sequence 1: one 400-mg tablet of sparsentan/two 200-mg tablets of sparsentan; Sequence 2: two 200-mg tablets of sparsentan/one 400-mg tablet of sparsentan). The single doses administered in Periods 1 and 2 were separated by an interval of at least 7 days. All doses were administered orally after a 10-hour fast, and subjects continued to fast until 4 hours postdose. Blood samples for analysis of sparsentan in plasma were collected predose and up to 120 hours postdose.

Results

Following a single 400-mg dose of sparsentan as one 400-mg tablet and two 200-mg tablets, sparsentan exposure, as measured by geometric mean $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max}, were similar after oral administration of 1× 400-mg tablet (Test) in comparison to 2× 200-mg tablets (Reference). Median sparsentan T_{max} occurred slightly earlier, following Test compared to Reference, at 3.8 hours and 4.5 hours, respectively. Mean plasma sparsentan $t_{1/2}$ (11.9 and 12.0 hours), CL/F (4.2 and 4.4 L/hour), and Vz/F (67.5 and 66.9 L) were similar for both treatment groups.

In this relative bioavailability study, the exposures of sparsentan from 1× 400-mg tablet and 2× 200-mg tablets were similar (Table 77). Within-subject variability was considered low, with a within-subject coefficient of variation values less than 29.8%.

Table 77. Statistical Analysis of Relative Bioavailability of Sparsentan Administered as One 400 mg Tablet Versus Two 200 mg Tablets

	Treatment B (Test)	Treatment A (Reference)				
Parameter	Geometric LSM	Geometric LSM	n	GMR (%)	90% Confidence Interval	Intra-subject CV%
AUC_{0-t} (ng*hr/mL)	95930	90380	36	106.14	101.06 - 111.48	12.36
AUC_{0-inf} (ng*hr/mL)	96440	91000	36	105.98	100.91 - 111.31	12.35
C_{max} (ng/mL)	6073	5658	36	107.35	95.58 - 120.57	29.77

Source: Applicant's analysis RTRX-RE021-101 report. Table 14.2.1.6.1 on page 86.
Abbreviations: AUC_{0-inf}, area under the concentration-time curve extrapolated to infinity, AUC_{0-t}, area under the concentration-time curve to time t; CV, coefficient of variance; GMR, geometric mean ratio; LSM, least squares mean

Reviewer's Comment

Bioequivalence was established between the 200-mg tablet and the 400-mg tablet of sparsentan.

14.2.3. Study RTRX-RE021-103: Open-Label, Parallel Group, Fixed Dose Study To Assess the Pharmacokinetic Profile and Safety of Sparsentan Following Single-Dose Administration Under Fed and Fasted Conditions, and Following Multiple Doses Administered Once Daily for 14 Days Under Fasted Conditions in Healthy Adult Subjects

Study Design

Six cohorts of six subjects each were randomized to receive a single dose of sparsentan (50, 100, 200, 400, 800, or 1600 mg) under fasted condition on Day 1, a single dose of sparsentan under fed condition on Day 5, and once daily (QD) of sparsentan under fasted condition from Days 9 to 22. Single and multiple dose sparsentan under fasted condition was administered after a 10-hour fast, and subjects continued to fast until 4 hours postdose, blood samples for analysis of sparsentan in plasma were collected predose and up to 96 hours postdose. Single dose sparsentan under fed condition was administered 30 minutes after a standardized FDA high-fat meal. Blood samples for analysis of sparsentan in plasma were collected predose and up to 96 hours postdose. All doses were administered using tablet sparsentan compounded as an oral suspension.

Results

A total of 36 subjects was randomized in the study, and all subjects were included in the PK and cardiodynamic analyses.

PK Analysis

SAD Under Fasting Conditions

Mean plasma sparsentan concentrations increased when the dose was increased from 50 to 1600 mg. At the 50 and 100 mg dose levels, all subjects had measurable sparsentan concentrations up to 48 hours postdose; at the 200, 400, 800, and 1600 mg dose levels, all subjects had measurable sparsentan concentrations up to 96 hours postdose. Mean plasma sparsentan concentrations increased and peaked at 2.5 to 5 hours postdose and then declined in a biphasic fashion across all doses. The mean t½ values ranged from approximately 8.4 to 19.6 hours across dose levels (Table 78). Based on the dose proportionality analysis of sparsentan plasma PK parameters, overall exposure (based on AUC_{0-24}, AUC_{0-last}, AUC_{0-inf} and C_{max}) was approximately dose-proportional in the range of 50 to 200 mg, and increased in a less than dose-proportional manner with sparsentan doses from 50 to 1600 mg. The slopes for AUC_{0-24}, AUC_{0-last}, AUC_{0-inf} and C_{max} were approximately 0.72, 0.84, 0.87 and 0.60, respectively.

Table 78. Summary of Plasma Sparsentan Pharmacokinetics Following Single Ascending Doses of Sparsentan Under Fasting Conditions

Pharmacokinetic Parameters	SD 50 mg sparsentan	SD 100 mg sparsentan	SD 200 mg sparsentan	SD 400 mg sparsentan	SD 800 mg sparsentan	SD 1600 mg sparsentan
AUC_{0-24} (ng*hr/mL)	11280 (13.9) [n=6]	23100 (46.9) [n=6]	38290 (37.7) [n=6]	61840 (45.0) [n=6]	105400 (25.0) [n=6]	133600 (41.6) [n=6]
AUC_{0-last} (ng*hr/mL)	11980 (14.9) [n=6]	25010 (52.7) [n=6]	44100 (44.4) [n=6]	82740 (45.8) [n=6]	159600 (26.2) [n=6]	200900 (28.5) [n=6]
AUC_{0-inf} (ng*hr/mL)	12050 (15.0) [n=6]	25090 (52.7) [n=6]	45010 (42.9) [n=6]	83000 (45.9) [n=6]	161100 (26.7) [n=6]	206300 (7.5) [n=3]
C_{max} (ng/mL)	1391 (27.1) [n=6]	2846 (41.6) [n=6]	4634 (31.8) [n=6]	6966 (34.0) [n=6]	8623 (20.5) [n=6]	12260 (46.2) [n=6]
C_{last} (ng/mL)	5.184 (57.4) [n=6]	5.319 (64.0) [n=6]	7.164 (141.2) [n=6]	15.58 (91.7) [n=6]	49.71 (166.6) [n=6]	180.8 (106.8) [n=6]
T_{max} (hr)	3.281 (2.04, 4.00) [n=6]	3.253 (2.00, 5.00) [n=6]	2.507 (1.50, 4.00) [n=6]	3.499 (2.02, 5.00) [n=6]	4.248 (2.50, 6.14) [n=6]	4.999 (2.49, 8.00) [n=6]
$t_{½}$ (hr)	8.444 ± 3.3890 [n=6]	9.733 ± 2.3947 [n=6]	19.574 ± 22.3860 [n=6]	10.169 ± 1.5834 [n=6]	12.178 ± 2.4898 [n=6]	17.931 ± 7.0399 [n=3]
CL/F (L/hr)	4.151 (15.0) [n=6]	3.986 (52.7) [n=6]	4.444 (42.9) [n=6]	4.819 (45.9) [n=6]	4.965 (26.7) [n=6]	7.756 (7.5) [n=3]
V_z/F (L)	46.95 (35.2) [n=6]	54.50 (41.2) [n=6]	89.98 (119.5) [n=6]	70.03 (39.7) [n=6]	85.85 (28.5) [n=6]	188.5 (55.3) [n=3]

Source: Applicant's analysis RTRX-RE021-103 report. Table 11-2 on page 67.
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration; CL/F, apparent clearance; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life; Vz/F, apparent volume of distr bution

SAD Under Fed Conditions

Mean plasma sparsentan concentrations increased when the dose was increased from 50 to 1600 mg. At the 50 and 100 mg dose levels, all subjects had measurable sparsentan concentrations up to 48 hours; at the 200 and 400 mg dose levels, all subjects had measurable concentrations up to 72 hours; at the 800 and 1600 mg dose levels, all subjects had measurable sparsentan concentrations up to 96 hours postdose. Median plasma sparsentan T_{max} values ranged from approximately 4 hours at the 50 to 200 mg doses to 6 hours at the 400 to 1600 mg dose levels. The mean $t_{1/2}$ values ranged from approximately 7.7 to 15.9 hours across dose levels (Table 79). A greater magnitude of effect of a high-fat meal on the PK of sparsentan (based on overall and peak exposure) was seen at sparsentan doses of 800 and 1600 mg, whereas a limited food effect was observed at the 50, 100, 200, and 400 mg dose levels.

Table 79. Summary of Plasma Sparsentan Pharmacokinetics Following Single Ascending Doses of Sparsentan Under Fed Conditions

Pharmacokinetic Parameters	SD 50 mg sparsentan	SD 100 mg sparsentan	SD 200 mg sparsentan	SD 400 mg sparsentan	SD 800 mg sparsentan	SD 1600 mg sparsentan
AUC_{0-24} (ng*hr/mL)	9843 (19.3) [n=6]	18220 (57.0) [n=6]	36310 (40.0) [n=6]	66660 (41.5) [n=6]	162700 (35.1) [n=6]	317500 (28.9) [n=6]
AUC_{0-last} (ng*hr/mL)	10410 (20.6) [n=6]	19860 (64.2) [n=6]	39300 (42.9) [n=6]	71470 (46.3) [n=6]	176500 (33.8) [n=6]	339300 (31.5) [n=6]
AUC_{0-inf} (ng*hr/mL)	10490 (20.7) [n=6]	19960 (63.9) [n=6]	39510 (42.4) [n=6]	71590 (46.3) [n=6]	176600 (33.8) [n=6]	310700 (24.2) [n=5]
C_{max} (ng/mL)	1351 (24.5) [n=6]	2314 (37.1) [n=6]	4951 (28.4) [n=6]	8083 (30.2) [n=6]	17300 (29.1) [n=6]	30820 (26.6) [n=6]
C_{last} (ng/mL)	6.154 (67.2) [n=6]	6.007 (62.0) [n=6]	4.405 (71.1) [n=6]	7.286 (67.1) [n=6]	6.262 (50.2) [n=6]	29.31 (121.1) [n=6]
T_{max} (hr)	4.244 (3.00, 6.00) [n=6]	4.262 (3.00, 6.00) [n=6]	4.257 (3.49, 6.16) [n=6]	5.608 (4.00, 6.21) [n=6]	6.200 (6.17, 6.29) [n=6]	6.002 (5.99, 8.01) [n=6]
$t_{½}$ (hr)	7.704 ± 2.1694 [n=6]	9.465 ± 1.9454 [n=6]	13.625 ± 11.4409 [n=6]	9.830 ± 2.5825 [n=6]	11.051 ± 1.9505 [n=6]	15.855 ± 5.6418 [n=5]
CL/F (L/hr)	4.768 (20.7) [n=6]	5.010 (63.9) [n=6]	5.062 (42.4) [n=6]	5.588 (46.3) [n=6]	4.529 (33.8) [n=6]	5.150 (24.2) [n=5]
V_z/F (L)	51.26 (15.5) [n=6]	67.24 (54.9) [n=6]	81.64 (95.2) [n=6]	76.76 (30.8) [n=6]	71.40 (33.7) [n=6]	111.1 (62.6) [n=5]

Source: Applicant's analysis RTRX-RE021-103 report. Table 11-4 on page 73.
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration; CL/F, apparent clearance; SD, single dose; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life; Vz/F, apparent volume of distribution

MAD Under Fasting Conditions

A total of 36 subjects was randomized in the study and completed all scheduled study dosing through Days 9 to 22. Mean plasma sparsentan concentrations increased in a less than dose-dependent manner from 50 to 800 mg following administration of single and multiple oral doses of 50 to 800 mg sparsentan QD (Days 9 to 22). Mean plasma sparsentan concentrations observed after multiple oral doses of 1600 mg (Days 9 to 22) were similar to that of 800 mg. Median T_{max} ranged from 2.5 to 5 hours after a single dose, and from 2.0 to 4.5 hours after multiple doses. After repeated daily dosing, sparsentan exposures were similar on Days 9 and 22 at the lower dose levels (50 mg to 800 mg QD) but were lower on Day 22 compared with Day 9 at the highest dose level (1600 mg QD) (Table 80). Mean RAUC ranged from 0.80 for 1600 mg QD to 1.24 for 200 mg QD. Mean RC_{max} ranged from 0.75 for 1600 mg QD to 1.21 for 1600 mg QD. Based on plasma C_{trough} values, sparsentan reached steady state within 7 days of QD dosing. Geometric mean CL/F generally increased after multiple daily dosing, i.e., from 4.2 and 3.8 L/hour after 50 mg QD and 100 mg QD, respectively, to 15.6 L/hour after 1600 mg QD. Based on the dose proportionality analysis of sparsentan plasma PK parameters, the quadratic effect was statistically significant suggesting a potential departure from linearity. The statistical accumulation analysis across all sparsentan doses of plasma sparsentan AUC_{0-24} and C_{max} following single (Day 1) and multiple (Days 9 to 22) sparsentan QD administration demonstrated that there was no apparent accumulation after multiple QD administration of sparsentan.

Table 80. Summary of Plasma Sparsentan Pharmacokinetics Following Multiple Ascending Doses of Sparsentan Under Fasting Conditions (Day 22)

Pharmacokinetic Parameters	MD 50 mg sparsentan	MD 100 mg sparsentan	MD 200 mg sparsentan	MD 400 mg sparsentan	MD 800 mg sparsentan	MD 1600 mg sparsentan
$AUC_{0\text{-}tau}$ (ng*hr/mL)	11900 (21.0) [n=6]	26600 (33.0) [n=5]	46710 (57.7) [n=5]	63560 (30.3) [n=6]	96920 (18.8) [n=6]	102400 (32.3) [n=5]
$AUC_{0\text{-}last}$ (ng*hr/mL)	12550 (22.5) [n=6]	29160 (35.7) [n=5]	53640 (63.9) [n=5]	77690 (23.6) [n=6]	129600 (27.5) [n=6]	158100 (48.1) [n=5]
$AUC_{0\text{-}inf}$ (ng*hr/mL)	12630 (22.2) [n=6]	29290 (35.4) [n=5]	54370 (63.8) [n=5]	78040 (23.4) [n=6]	131700 (28.7) [n=6]	171400 (57.8) [n=4]
C_{max} (ng/mL)	1511 (27.7) [n=6]	3011 (23.9) [n=5]	5534 (31.8) [n=5]	6471 (35.2) [n=6]	9808 (19.0) [n=6]	8927 (38.1) [n=5]
T_{max} (hr)	2.015 (2.00, 3.51) [n=6]	4.500 (1.50, 5.99) [n=5]	2.500 (1.50, 3.50) [n=5]	2.000 (1.00, 3.00) [n=6]	3.249 (2.00, 5.00) [n=6]	3.500 (1.00, 4.50) [n=5]
$t_{½}$ (hr)	8.672 ± 3.9498 [n=6]	12.587 ± 7.6976 [n=5]	14.657 ± 10.7148 [n=5]	10.750 ± 3.7585 [n=6]	13.895 ± 8.3524 [n=6]	15.524 ± 5.3221 [n=4]
CL/F (L/hr)	4.203 (21.0) [n=6]	3.760 (33.0) [n=5]	4.281 (57.7) [n=5]	6.293 (30.3) [n=6]	8.254 (18.8) [n=6]	15.62 (32.3) [n=5]
Vz/F (L)	49.08 (37.9) [n=6]	60.08 (74.3) [n=5]	77.48 (95.7) [n=5]	93.01 (47.6) [n=6]	146.8 (56.1) [n=6]	331.5 (38.3) [n=4]
RA, AUC	1.058 ± 0.084613 [n=6]	0.9845 ± 0.12560 [n=5]	1.239 ± 0.43209 [n=5]	1.049 ± 0.22058 [n=6]	0.9277 ± 0.13991 [n=6]	0.8046 ± 0.13116 [n=5]
RA, C_{max}	1.094 ± 0.15017 [n=6]	0.9567 ± 0.27782 [n=5]	1.210 ± 0.24291 [n=5]	0.9891 ± 0.39171 [n=6]	1.192 ± 0.41549 [n=6]	0.7546 ± 0.17881 [n=5]

Source: Applicant's analysis RTRX-RE021-103 report. Table 11-6 on page 85.
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration; CL/F, apparent clearance; MD, multiple dose; RA, accumulation ratio; T_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life; Vz/F, apparent volume of distribution

Reviewer Comment

Sparsentan exposure (C_{max} and AUC) increased less than proportionally with dose, from 50 mg to 1600 mg, following both single and multiple dosing. The less than dose-proportional increase in exposure at the 800 and 1600 mg dose levels could be due to solubility limited absorption or induction of an enzyme responsible for metabolism of sparsentan (CYP3A4) or both. Exposure-response modeling demonstrated a mild effect of sparsentan on the QTcF interval under both fasting and fed conditions, the predicted values for dQTcF indicated the highest upper limits of the 90% CI were lower than 10 msec under both fasting and fed conditions, which does not reach the regulatory threshold of concern.

14.2.4. Study 021HVOL109: A Phase 1, Open-Label, Randomized, Single-Dose, Four-Period, Crossover Study To Investigate the Effect of Food on the Pharmacokinetics of Sparsentan in Healthy Subjects

Study Design

A total of 16 subjects were randomized to receive one of four treatment sequences (ABDC, BCAD, CDBA, or DACB). Treatment A: one 200-mg tablet of sparsentan administered under fasted condition; Treatment B: one 200-mg tablet of sparsentan administered under fed condition; Treatment C: two 400-mg tablet of sparsentan administered under fasted condition; Treatment D: two 400-mg tablet of sparsentan administered under fed condition. All doses designed to be taken under fasted condition were administered after a 10-hour fast, and subjects continued to fast until 4 hours postdose, blood samples for analysis of sparsentan in plasma were collected predose and up to 72 hours postdose. All doses designed to be taken under fed condition were administered 30 minutes after a standardized FDA high-fat meal, blood samples for analysis of sparsentan in plasma were collected predose and up to 72 hours postdose.

Results

A total of 16 subjects were enrolled in the study, and 15 subjects completed the study. The ratio of geometric least-squares means of AUC_{0-72} under fed versus fasted conditions for the 200 mg and the 800 mg groups were 0.86 and 1.22, respectively (Table 81). The ratio of geometric least-squares means of C_{max} under fed versus fasted conditions for the 200 mg and the 800 mg groups were 1.22 and 2.08, respectively (Table 81). T_{max} ranged from 3 to 5 hours postdose across all the dosing arms.

Table 81. Summary of the Statistical Analysis of Sparsentan Pharmacokinetic Parameter Data – Food Effect on 200 mg and 800 mg Sparsentan Single Oral Dose

Parameter	Treatment	n	Geometric least squares means	Ratio of geometric least squares means (Test : Reference)	90% CI for the ratio (Test : Reference) Lower	Upper	Within-subject CV%
AUC_{0-72} (h*ng/mL)	Treatment A(Reference)	15	58800	86.1	73.8	100.5	25.4
	Treatment B(Test)	15	50620				
C_{max} (ng/mL)	Treatment A(Reference)	15	5230	121.7	107.0	138.5	21.2
	Treatment B(Test)	15	6370				
t_{max} (h)#	Treatment A(Reference)	15	4.00	0.00	-1.00	1.00	
	Treatment B(Test)	15	4.50				
AUC_{0-72} (h*ng/mL)	Treatment C(Reference)	15	138240	122.1	104.7	142.5	25.4
	Treatment D(Test)	15	168810				
C_{max} (ng/mL)	Treatment C(Reference)	15	7770	208.1	183.0	236.6	21.2
	Treatment D(Test)	16	16180				
t_{max} (h)#	Treatment C(Reference)	15	4.50	1.00	0.00	2.25	
	Treatment D(Test)	16	3.00				

Source: Applicant's 021HVOL109 report. Table 10 on page 41 and Table 11 on page 42.
Treatment A: 200 mg sparsentan (fasted); Treatment B: 200 mg sparsentan (fed); Treatment C: 800 mg sparsentan (fasted); Treatment D: 800 mg sparsentan (fed)
Hodges Lehmann location shifts along with HL 90% CIs of the location shifts are presented.
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration; CL/F, apparent clearance; RA, accumulation ratio; t_{max}, time to maximum concentration

Reviewer's Comment

The design of this food effect study is acceptable with a standard high-fat meal and sufficiently long washout period. The tablet formulation used in this food effect study is the same as used in the pivotal phase 3 study. The food effect was minimal at the 200 mg dose, but larger at the 800 mg dose. The effect of food on exposure of sparsentan following the clinically recommended dose of 400 mg was not tested, however, the expected effects are likely to be bracketed by the results observed at 200 and 800 mg.

14.2.5. Study 021HVOL16005: A Phase 1 Study To Investigate the Absorption, Metabolism, and Excretion of [^{14}C]-Sparsentan Following a Single Oral Dose in Healthy Male Subjects

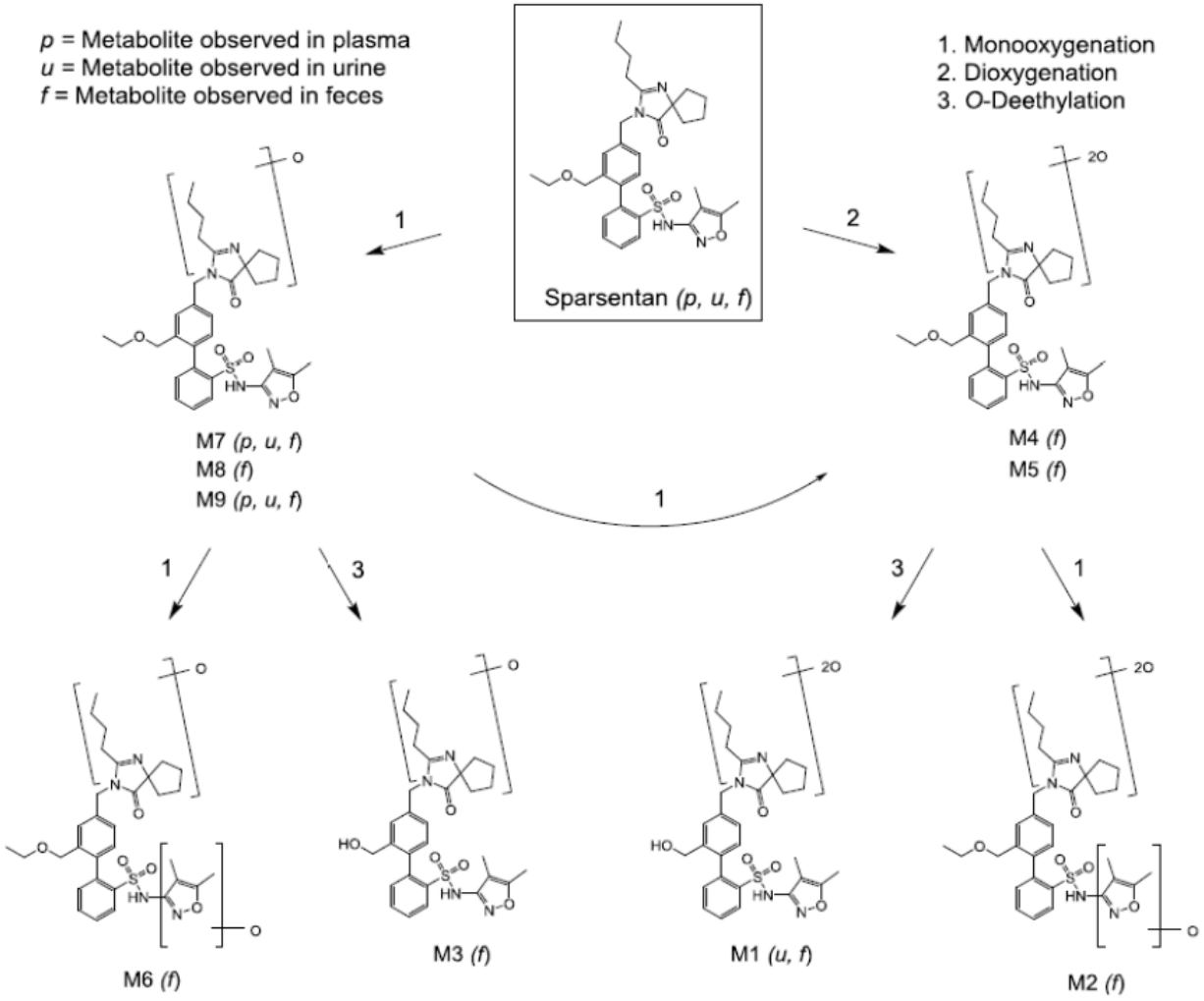
Study Design

Study 021HVOL16005 was an open-label study to evaluate the ADME of oral sparsentan. Healthy male subjects received a single 400 mg oral dose of [^{14}C]sparsentan (containing approximately 1 μCi). [^{14}C]sparsentan was administered after an 8-hour fast, and subjects remained fasted for a minimum of 4 hours postdose. Blood samples for radioanalysis and metabolite profiling were collected from predose to 240 hours postdose, urine and feces for sparsentan concentrations, total radioactivity, and metabolite profiling and identification were also collected from predose to 24 hours postdose, and at 24-hour intervals thereafter until discharge.

Results

Eight subjects were enrolled and completed the study and were included in the pharmacokinetics analysis set. After oral dosing, total radioactivity appeared rapidly in plasma and whole blood with a median T_{max} of 2.3 and 2.0 hours, respectively (ranging from 1.5 to 3.5 hours). The geometric mean AUC ratio of total radioactivity in whole blood to plasma was 0.647, indicating minimal association of radioactivity with RBCs. The overall mean recovery of administered radioactivity was 82.3% over the 240-hour collection period. Approximately 80.2% of the administered radioactivity was recovered in feces and 2.2% was recovered in urine. Unchanged sparsentan recovered in feces was 9%, suggesting that most fecal radioactivity was related to sparsentan metabolites. Only trace amounts (<1%) of unchanged sparsentan was recovered in urine. A total of nine metabolites was characterized/identified in plasma, urine, and feces (Figure 22). The metabolites M5, M1, and M6 were the most abundant metabolites in urine and feces. Based on the metabolite structures, the primary metabolic pathways for [^{14}C]sparsentan in human subjects involved oxidation (monooxygenation, sequential oxidation, and O-deethylation) followed by biliary excretion. Each metabolite represented <10% of the total radioactivity in the pooled plasma samples.

Figure 22. Proposed Biotransformation Pathways of Sparsentan in Human



Source: Applicant's 021HVOL16005 report. Figure 5 on page 36.

Reviewer's Comment

The mass balance data indicate that sparsentan is well absorbed and undergoes extensive hepatic metabolism. Renal excretion is not the major elimination pathway for unchanged sparsentan.

14.2.6. Study 021IHFX16009: A Phase 1, Open-Label, Single-Dose Study to Evaluate the Pharmacokinetics and Safety of Sparsentan (RE-021) in Male Subjects With Mild or Moderate Hepatic Impairment Compared to Healthy Subjects

Study Design

The primary objective of this study was to evaluate the PK profile of sparsentan, and the secondary objective was to assess the safety and tolerability following a single oral 400 mg dose in subjects with mild and moderate hepatic impairment relative to control subjects with normal hepatic function. The study population consisted of male subjects who were ≥18 to ≤65 years, with mild hepatic impairment (Group 2; eight subjects, Child-Pugh Class A, score of 5 to 6), moderate hepatic impairment (Group 3; eight subjects; Child-Pugh Class B, score of 7 to 9) and normal hepatic function (Group 1; 12 subjects). Subjects with normal hepatic function were to be matched by age (±5 years), sex and body mass index (BMI; ±20%) to subjects with hepatic impairment. Sparsentan tablet of 400 mg under fasted condition was administered after an 8-hour fast, and subjects continued to fast until 4 hours postdose. Blood samples for analysis of sparsentan in plasma were collected predose and up to 120 hours postdose. Blood samples to determine unbound concentration and fraction unbound of sparsentan were collected at 2, 4, and 36 hours postdose.

Results

Following treatment with sparsentan, point estimates of the geometric LSM ratios of the primary parameters AUC and C_{max} plasma total are presented in Table 82. The point estimates of the test/reference (i.e., mild or moderate hepatic impairment/normal hepatic function) mean ratios (90% CI) of the C_{max} for sparsentan were 77.9% (57.8%, 105%) and 120% (84.5%, 171%), ratios of the $AUC_{0\text{-}lqc}$ were 90.9% (60.8%, 136%) and 126% (84.4%, 189%), and the ratio of $AUC_{0\text{-}inf}$ were 91.0% (60.9%, 136%) and 126% (84.4%, 189%), respectively. Following a single oral dose of 400 mg sparsentan, C_{max}, $AUC_{0\text{-}lqc}$, and $AUC_{0\text{-}inf}$ were slightly lower in the mild hepatic impairment group compared to the normal hepatic function group and slightly higher in the moderate hepatic impairment group compared to the normal hepatic function group.

The PK parameters for unbound sparsentan are summarized in Table 83. The test/reference ratios (90% CI) of geometric means for $C_{max,u}$ were 105% (62.7%, 175%) and 216% (78.5%, 596%) for mild and moderate hepatic impairment groups, respectively. The test/reference ratios (90% CI) of geometric means for $AUC_{0\text{-}36,u}$ were 107% (50.9%, 225%) and 195% (64.3%, 593%) for mild and moderate hepatic impairment groups, respectively (Table 83). Following a single oral dose of 400 mg sparsentan, the PK parameters $C_{max,u}$ and $AUC_{0\text{-}36,u}$ were higher in both the mild and moderate hepatic impairment groups compared to the matched normal hepatic function groups, but wide 90% CIs were observed for each comparison.

Table 82. Statistical Comparison of the Pharmacokinetic Parameters of Sparsentan

Parameters (unit)		N[a], Observed Geometric Mean: Hepatic Impairment Groups	N[a], Observed Geometric Mean: Hepatic Normal Function Group	Ratio (90% CI) of Geometric Means[b]	p-value[c]
C_{max} (ng/mL)	Mild	8, 4440	8, 5700	77.9 (57.8, 105)	0.159
	Moderate	8, 6790	8, 5650	120 (84.5, 171)	0.355
$C_{max,u}$ (ng/mL)	Mild	8, 25.7	8, 24.6	105 (62.7, 175)	0.870
	Moderate	8, 58.0	8, 26.8	216 (78.5, 596)	0.193
$AUC_{0\text{-}lqc}$ (h*ng/mL)	Mild	8, 77300	8, 85000	90.9 (60.8, 136)	0.665
	Moderate	8, 134000	8, 106000	126 (84.4, 189)	0.309
$AUC_{0\text{-}inf}$ (h*ng/mL)	Mild	8, 77400	8, 85100	91.0 (60.9, 136)	0.669
	Moderate	8, 135000	8, 107000	126 (84.4, 189)	0.309
$AUC_{0\text{-}36,u}$ (h*ng/mL)	Mild	5, 241	5, 225	107 (50.9, 225)	0.855
	Moderate	6, 534	6, 273	195 (64.3, 593)	0.279

Source: Applicant's 021IHFX16009 report. Table 9 on page 33.
Abbreviations: AUC, area under the concentration-time curve; C, plasma concentration

Table 83. Summary of the Plasma Pharmacokinetic Parameters of Unbound Sparsentan

Parameter	Hepatic Function Group: Normal (N = 12)	Mild (N = 8)	Normal (matched with Mild) (N = 8)	Moderate (N = 8)	Normal (matched with Moderate) (N = 8)
$AUC_{0\text{-}36h,u}$ (h*ng/mL)	214 (66.2) [11]	231 (85.4) [6]	207 (34.2) [7]	594 (125.0) [7]	236 (87.5) [7]
$C_{max,u}$ (ng/mL)	26.0 (63.1) [12]	25.7 (87.5) [8]	24.6 (38.2) [8]	58.0 (134.1) [8]	26.8 (78.1) [8]
$t_{max,u}$[a] (h)	4.25 (2.00-5.00) [12]	4.00 (2.00-14.0) [8]	4.75 (2.00-5.00) [8]	5.00 (2.00-7.00) [8]	4.50 (4.00-5.00) [8]

Source: Applicant's 021IHFX16009 report. Table 10 on page 36.
Abbreviations: AUC, area under the concentration-time curve, C_{max}, maximum plasma concentration; t_{max}, time to maximum concentration

Reviewer's comment

High variability was observed in the measurement of sparsentan unbound concentrations (Table 83), thus the results with unbound concentrations should be interpreted with caution. Based on the total plasma sparsentan exposures, there seems to be no significant impact of mild or moderate hepatic impairment on sparsentan PK.

14.2.7. Study 021HVOL16006: A Study To Evaluate the Individual Effects of Cyclosporine and Itraconazole on the Pharmacokinetics, Safety, and Tolerability of Sparsentan in Healthy Male Subjects

Study Design

This was a phase 1, open-label study to evaluate the individual effects of single-dose cyclosporine (moderate CYP3A4 and P-gp inhibitor) and multiple-dose itraconazole (strong CYP3A4 and P-gp inhibitor) on the single-dose PK, safety, and tolerability of sparsentan in healthy males.

The primary objectives of this study were to assess the effect of a single oral dose of cyclosporine 600 mg on the plasma PK profile of a single oral dose of sparsentan 200 mg and to assess the effect of repeated oral doses of itraconazole 200 mg once daily (1st day twice daily, 9 days QD) on the plasma PK profile of a single dose of oral sparsentan 200 mg. Two 100 mg

sparsentan capsules under fasted condition were administered after an 8-hour fast, and subjects continued to fast until 2 hours postdose. Cyclosporine was administered orally under fasted condition after a 6.5-hour fast, 1.5 hours prior to dosing with sparsentan, and continued to fast by at least 2 hours following administration of sparsentan. Itraconazole 200 mg (administered as 20 mL of a 10-mg/mL oral solution) was administered twice on Day 1 of Period 3 (Study Day 29) and QD on Days 2 through 10 of Period 3 (Study Days 30 through 38). On Day 6 of Period 3 (Study Day 34), itraconazole was administered after at least an 8-hour fast and at the same time as that day's dose of sparsentan, on all other days when itraconazole was administered, it was given with or just after a meal. Blood samples for analysis of sparsentan in plasma were collected predose and up to 120 hours postdose.

Results

Systemic exposure to sparsentan was higher when co-administered with cyclosporine compared with administration of sparsentan alone. Statistical analysis of sparsentan PK parameters after administration of a single dose of sparsentan 200 mg given alone and with a single oral dose of cyclosporine 600 mg are shown in Table 84. In comparison with sparsentan administered alone, coadministration with cyclosporine had a statistically significant effect on the dose-dependent parameters C_{max}, $AUC_{0\text{-}lqc}$, and $AUC_{0\text{-}inf}$. The least squares means ratios of the test (sparsentan coadministered with cyclosporine) versus reference (sparsentan alone) were 141.4%, 170.3%, and 169.6% for C_{max}, $AUC_{0\text{-}lqc}$, and $AUC_{0\text{-}inf}$, respectively.

Table 84. Statistical Analysis for Sparsentan PK Parameters With and Without Coadministration of Cyclosporine or Itraconazole in Healthy Subjects

	Treatment		
Parameter	Sparsentan (N=32)	Sparsentan + Cyclosporine (N=29)	Sparsentan + Itraconazole (N=30)
$AUC_{0\text{-}lqc}$ (h*ng/mL)	42900 (38.8)[a]	72200 (89.9)	115000 (59.2)[b]
$AUC_{0\text{-}\infty}$ (h*ng/mL)	43100 (38.2)	72300 (89.4)	117000 (65.4)
C_{max} (ng/mL)	3560 (19.3)	5040 (73.7)	4460 (34.9)
t_{max}[c] (h)	3.00 (1.50-5.00)	4.00 (2.00-7.03)	5.00 (1.50-23.9)
$t_{1/2}$[d] (h)	9.92 (2.68)	9.13 (1.94)	20.9 (12.7)
CL/F (L/h)	4.64 (38.2)	2.77 (89.4)	1.70 (65.4)
V_Z/F (L)	64.0 (35.0)	35.6 (77.4)	43.4 (24.0)
MRT (h)	14.0 (26.4)	14.5 (24.5)	26.3 (54.3)

Source: Applicant's 021HVOL16006 report. Table 11-1 on page 40.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CL/F, apparent clearance; MRT, mean residence time; t_{max}, time to maximum concentration; $t_{1/2}$, terminal half-life; Vz/F, apparent volume of distr bution

Following multiple doses of itraconazole there was an approximately 2.7-fold increase in total exposures of sparsentan while C_{max} increased by 25%. Statistical analysis of sparsentan PK parameters after administration of a single dose of sparsentan 200 mg alone and after multiple doses of itraconazole are shown in Table 84.

Reviewer's Comment

Clinically meaningful pharmacokinetic drug interaction is observed for sparsentan with cyclosporine (moderate CYP3A inhibitor) and itraconazole (strong CYP3A inhibitor). The product insert should carry appropriate instructions for use with moderate and strong CYP3A inhibitors.

14.2.8. Study 021HVOL16007: A Phase 1, Open-Label, Randomized, Two-Period, Two-Sequence, Crossover, Drug-Drug Interaction Study To Evaluate the Effect of Sparsentan (RE-021) on the Pharmacokinetics of Single-Dose Pitavastatin, a Sensitive OATP1B1 and OATP1B3 Substrate, in Healthy Female and Male Subjects

Study Design

This was a phase 1, open-label study to assess the effect of sparsentan on the PK of pitavastatin in healthy female and male subjects, as well as to evaluate the safety, and tolerability of sparsentan in healthy males.

Two groups of 14 subjects each were randomized to receive one of two sequences (i.e., Sequence 1: one single 4-mg dose of pitavastatin on Days 1 and 8 and 800 mg (2 × 400-mg tablets) of sparsentan QD on Days 7 through 10; Sequence 2: one single 4-mg dose of pitavastatin on Days 2 and 8 and 800 mg (2 × 400-mg tablets) of sparsentan QD on Days 1 through 4). Each subject was to receive two single oral doses of pitavastatin 4 mg and 4 days of QD dosing with sparsentan 800 mg with a washout of 4 to 6 days between periods. Sparsentan tablets and pitavastatin tablets were administered after an 8-hour fast, and subjects continued to fast until 4 hours postdose. Blood samples for analysis of pitavastatin and pitavastatin lactone in plasma were collected predose and up to 72 hours postdose.

Results

Systemic exposure to pitavastatin was decreased when co-administered with sparsentan compared with administration of pitavastatin alone. Statistical analysis of pitavastatin PK parameters after administration of a single dose of pitavastatin 4 mg given alone and with administration of sparsentan 800 mg are shown in Table 85. In comparison with pitavastatin administered alone, coadministration with sparsentan decreased C_{max}, $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$, and $t_{1/2}$ of pitavastatin. The least squares means ratios of the test (sparsentan coadministered with pitavastatin) versus reference (pitavastatin alone) were 70.1%, 69.7%, 81.3%, and 74.7% for $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$, C_{max}, and $t_{1/2}$, respectively.

Table 85. Statistical Analysis for Pitavastatin PK Parameters With and Without Coadministration of Sparsentan in Healthy Subjects

	Pitavastatin + Sparsentan (Test)		Pitavastatin Alone (Reference)		Ratio of Test/Reference[c]	90% CI of Test/Reference[d]
PK Parameters (Units)	n[a]	LS Mean[b]	n[a]	LS Mean[b]	(%)	(%)
$AUC_{0\text{-}lqc}$ (h*ng/mL)	27	103	27	146	70.1	(65.7, 74.8)
$AUC_{0\text{-}inf}$ (h*ng/mL)	24	119	26	171	69.7	(65.6, 74.0)
C_{max} (ng/mL)	27	56.4	27	69.4	81.3	(70.0, 94.3)
$t_{1/2}$ (h)	25	8.58	26	11.5	74.7	(63.4, 88.0)

Source: Applicant's 021HVOL16007 report. Table 8 on page 39.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; LS, least squares; $t_{1/2}$, terminal half life

Systemic exposure to pitavastatin lactone decreased when pitavastatin was coadministered with sparsentan versus alone. The pitavastatin lactone geometric mean ratios of pitavastatin coadministered with sparsentan compared to pitavastatin alone were 57.8%, 61.7%, 67.1%, and 99.7% for $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$, C_{max}, and $t_{1/2}$, respectively (Table 86).

Table 86. Statistical Analysis for Pitavastatin Lactone PK Parameters With and Without Coadministration of Sparsentan in Healthy Subjects

	Pitavastatin + Sparsentan (Test)		Pitavastatin Alone (Reference)		Ratio of Test/Reference[c]	90% CI of Test/Reference[d]
PK Parameters (Units)	n[a]	LS Mean[b]	n[a]	LS Mean[b]	(%)	(%)
$AUC_{0\text{-}lqc}$ (h*ng/mL)	25	315	27	545	57.8	(53.7, 62.3)
$AUC_{0\text{-}inf}$ (h*ng/mL)	25	350	27	568	61.7	(58.3, 65.2)
C_{max} (ng/mL)	27	31.1	27	46.2	67.1	(62.5, 72.2)
$t_{1/2}$ (h)	25	14.3	27	14.4	99.7	(93.3, 106.6)

Source: Applicant's 021HVOL16007 report. Table 11 on page 43.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; LS, least squares; PK, pharmacokinetic; $t_{1/2}$, terminal half life

Reviewer's Comment

When sparsentan was administered in combination with pitavastatin (a substrate of UGT, P-gp, BCRP, OATP1B1, OATP1B3, CYP2C9), the exposure of pitavastatin and pitavastatin decreased about 30%. This study was designed to evaluate the potential inhibitory effect of sparsentan towards OATP1B3, however, the expected increase in pitavastatin exposure was not observed. Induction effect of sparsentan towards enzymes responsible for the metabolism of pitavastatin is less likely since sparsentan was only administered 1 day before coadministration of pitavastatin. The decrease in pitavastatin exposure could be related to OATP2B1, an uptake transporter present in the intestine. Sparsentan is known to inhibit OATP2B1 in vitro (IC_{50}=31µM) and pitavastatin is known to be a substrate of OATP2B1 (Shirasaka et al. 2011). This plausible mechanism of interaction, mediated by OATP2B1 inhibition, probably also explains the reduced

exposure to pitavastatin lactone. Inhibition of OATP2B1 may also explain why substate exposure was reduced in several clinical DDI studies for unclear reasons.

14.2.9. Study 021HVOL16008: A Phase 1, Single-Sequence, Open-Label, Two-Period, Crossover Study To Evaluate the Effect of Steady-State Sparsentan (RE-021) on the Single-Dose Pharmacokinetic Profile of Midazolam, a Sensitive CYP3A4 Substrate, and Bupropion, a Sensitive CYP2B6 Substrate, in Healthy Male Subjects

Study Design

This is a phase 1, open-label, three-part study to evaluate DDIs of sparsentan with midazolam (a CYP3A4 substrate), and bupropion (a CYP2B6 substrate) in healthy male subjects.

The primary objective was to assess the effect of repeated oral doses of sparsentan (800 mg QD) at steady state on the PK of a single oral dose of midazolam (2 mg) and a single oral dose of bupropion (150 mg). Two 400 mg sparsentan tablets were administered QD orally on Days 7 through 19, one 1-mL dose of 2 mg/mL midazolam syrup was administered orally on Days 1 and 14, one 150-mg dose of bupropion was administered orally on Days 3 and 16. All doses were administered after at least 8-hour fast, followed by a fast for at least 4 hours postdose. Blood samples for midazolam and its metabolite were obtained through 24 hours postdose after each midazolam dose. Blood samples for bupropion and the metabolites were obtained through 96 hours postdose after each bupropion dose. Trough samples for sparsentan were obtained predose on Days 12, 13, 14, and 16.

Results

Systemic exposure to midazolam was not altered when co-administered with sparsentan. Statistical analysis of midazolam C_{max} and AUC values after administration of a single dose midazolam 4 mg given alone and with multiple doses of sparsentan 800 mg are shown in Table 87. The geometric mean ratios of midazolam coadministered with sparsentan compared to midazolam administered alone were 105.3%, 98.4%, 99.1%, and 101.3% for C_{max}, $AUC_{0\text{-}lqc}$, $AUC_{0\text{-}inf}$, and $t_{1/2}$, respectively.

Table 87. Summary of the Statistical Analysis of the Effect of Sparsentan on Pharmacokinetic Parameters of Midazolam

Parameters (Units)	n[a]	LS Mean[b] M+S(Test)	n[a]	LS Mean[b] M(Ref)	Geometric Mean Ratio[c] (Test/Reference)(90% CI of Ratio)[d](%)	p-value[e]
$AUC_{0\text{-}lqc}$ (h*ng/mL)	28	18.5	28	18.8	98.4 (89.8, 107.8)	
$AUC_{0\text{-}inf}$ (h*ng/mL)	28	20.2	28	20.3	99.1 (90.7, 108.3)	
C_{max} (ng/mL)	28	7.44	28	7.07	105.3 (97.7, 113.6)	
$t_{1/2}$ (h)	28	3.79	28	3.74	101.3 (90.5, 113.3)	
t_{max} (hr)	28	0.50	28	1.00	-0.25 (-0.25, 0.00)	0.0768

Source: Applicant's 021HVOL16008 report. Table 7 on page 32.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; LS, least squares; $t_{1/2}$, terminal half life

Systemic exposure to bupropion decreased when bupropion was coadministered with sparsentan versus bupropion administered alone. Statistical analysis of bupropion PK parameters after administration of a single dose bupropion 150 mg given alone and with multiple doses of sparsentan 800 mg are shown in Table 88. The geometric mean ratios of bupropion coadministered with sparsentan compared to bupropion administered alone were 68.2%, 65.7%, and 66.8% for C_{max}, $AUC_{0\text{-}lqc}$, and $AUC_{0\text{-}inf}$, respectively.

Table 88. Summary of the Statistical Analysis of the Effect of Sparsentan on Pharmacokinetic Parameters of Bupropion

Parameters (Units)	n[a]	LS Mean[b] B+S(Test)	n[a]	LS Mean[b] B(Ref)	Geometric Mean Ratio[c] (Test/Reference)(90% CI of Ratio)[d](%)	p-value[e]
$AUC_{0\text{-}lqc}$ (h*ng/mL)	28	516	28	786	65.7 (62.0, 69.5)	
$AUC_{0\text{-}inf}$ (h*ng/mL)	28	539	28	808	66.8 (63.1, 70.7)	
C_{max} (ng/mL)	28	99.0	28	145	68.2 (62.6, 74.2)	
$t_{1/2}$ (h)	28	19.2	28	19.1	100.8 (92.9, 109.5)	
t_{max} (hr)	28	1.28	28	1.50	-0.10 (-0.25, 0.00)	0.2016

Source: Applicant's 021HVOL16008 report. Table 10 on page 39.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; LS, least squares; $t_{1/2}$, terminal half life; t_{max}, time to maximum plasma concentration

Reviewer's Comment

Steady-state sparsentan did not affect the single-dose PK of midazolam., This is possibly due to the canceling of inhibition and induction effects of sparsentan towards CYP3A4. Steady-state sparsentan decreased exposure of bupropion by approximately 35%. The review team recommends noting in the product insert that sensitive substrates of CYP2B6 may require monitoring of efficacy and dose modification, if needed.

14.3. Bioanalytical Method Validation and Performance

Two bioanalytical methods were used to quantify sparsentan in human plasma during drug development. Table 89 highlights the key features of these methods. The primary method used in most clinical studies was RE-021-Report037-2014-MVA, with the exception of DDI studies 021HVOL16006, 021HVOL16007, and 021HVOL16008, and hepatic impairment study 021HFX16009004 which used the assay M8349671. Table 90 and Table 91 lists the validation and performance results of methods RE-021-Report037-2014-MVAL and M8349671, respectively.

Table 89. Bioanalytical Methods Overview

	Method Validation RE-021-Report037-2014-MVAL	Method Validation RE-021-Report037-2014-MVAL Addendum 1	Method Validation RE-021-Report037-2014-MVAL Addendum 2
Analyte	Sparsentan	Sparsentan	Sparsentan
Validation type	Full	Addendum	Addendum
eCTD reference number	Not available		
Method ID	RE-021-Report037-2014-MVAL	RE-021-Report037-2014-MVAL	RE-021-Report037-2014-MVAL
Duration of time method is in use	18 Dec 2013 to 16 Jan 2014	02 Jul 2014 to 10 Oct 2016	14 Feb 2019 to 15 Feb 2019
Bioanalytical site	(b) (4)		
Matrix	Plasma	Plasma	Plasma
Platform	Protein precipitation with LC-MS/MS		
Format	NA		
Stock reference, lot number, expiration date	Sparsentan, LTPI1A1001 Expiry 18 Sep 2014 Sparsentan-d_5, 1539-068A3 Expiry Nov 2016	Sparsentan, LTPI1A1001 Expiry 24 Sep 2016[a] Sparsentan-d_5, 1539-068A3 Expiry Nov 2016	Sparsentan, C14052048-RF16001 Expiry Jul 2019 Sparsentan-d_5, 1539-068A3 Expiry 15 Aug 2019
Calibration range from LLOQ to ULOQ	2.00 to 4000 ng/mL	2.00 to 4000 ng/mL	2.00 to 4000 ng/mL
Matrix study population	Healthy subjects	Healthy subjects	Healthy subjects
Link to reports and applicable amendments	RE-021-Report037-2014-MVAL	RE-021-Report037-2014-MVAL Addendum 1	RE-021-Report037-2014-MVAL Addendum 2
Synopsis of amendment history	NA	Addition of lipemic effect and extension of stability	Addition of whole blood stability

	Method Validation 8349671	Method Amendment 8349671 Amendment 1	Method Addendum 8349671 Addendum 1
Analyte	Sparsentan	Sparsentan	Sparsentan
Validation type	Full	Amendment	Addendum
eCTD reference number	Not available		
Method ID	M8349671	M8349671	M8349671
Duration of time method is in use	21 Sep 2016 to 08 Feb 2017	21 Sep 2016 to 08 Feb 2017	02 May 2017 to 19 Jul 2021
Bioanalytical site	(b) (4)		
Matrix	Plasma	Plasma	Plasma
Platform	Protein precipitation with LC-MS/MS		
Format	NA		
Stock reference, lot number, expiration date	Sparsentan, L0503567, Expiry 31 Mar 2018 Sparsentan-d_5, 1539-068A3, Expiry 15 Aug 2019	Sparsentan, L0503567, Expiry 31 Mar 2018 Sparsentan-d_5, 1539-068A3, Expiry 15 Aug 2019 Cyclosporine A, 12-SSR-178-1, Expiry 25 Jan 2020 Cyclosporine A-d_4, 13-MAR-65-4, Expiry 18 Apr 2019	Sparsentan, L0503567, Expiry 31 Mar 2018 Sparsentan, C14052048-RF16001, Expiry 10 May 2022 Sparsentan-d_5, 1539-068A3, Expiry 15 Aug 2019 Sparsentan-d_5, RE-0004862-005-001LD, Expiry 30 Jun 2021
Calibration range from LLOQ to ULOQ	2.00 to 4000 ng/mL	2.00 to 4000 ng/mL	2.00 to 4000 ng/mL
Matrix study population	Healthy subjects	Healthy subjects	Healthy subjects
Link to reports and applicable amendments	8349671	8349671 Amendment 1	8349671 Addendum 1
Synopsis of amendment history	NA	Addition of cyclosporine method transfer	Addition of interference testing and extension of stability

Source: Applicant's summary of biopharmaceutic studies and associated analytical methods report. Table 9 on page 23. Table 11 on page 32.
Abbreviations: eCTD, electronic common technical document; LLOQ, lower limit of quantification; NA, not applicable; LC-MS/MS, liquid chromatography with tandem mass spectrometry; ULOQ, upper limit of quantification

Table 90. Summary of Method Validation and Performance in Clinical Studies for Sparsentan in Human Plasma: Method RE-021-Report037-2014-MVAL

Bioanalytical method validation report name, amendments, and hyperlinks	Method validation for the quantitation of RE-021 in human plasma by turbo ion spray LC-MS/MS Method report RE-021-Report037-2014-MVAL, Addendum 1, and Addendum 2.		
Method description	Human plasma samples were prepared by addition of internal standard (sparsentan-d_5) and protein precipitation with acetonitrile. After centrifugation, supernatant was diluted and analyzed by LC-MS/MS		
Materials used for standard calibration curve and concentration	Sparsentan, LTPI1A1001 and C14052048-RF16001 2, 4, 10, 50, 250, 1000, 2000, 3750, and 4000 ng/mL		
Validated assay range	2.00 to 4000 ng/mL		
Material used for QCs and concentration	Sparsentan, LTPI1A1001 and C14052048-RF16001 2, 6, 100, 1500, 3000, and 25 000 ng/mL		
Minimum required dilutions	NA		
Source and lot of reagents	Human plasma: (b) (4) Acetonitrile and methanol: (b) (4) Ammonium acetate: (b) (4) Sodium hypochlorite solution: (b) (4) Water: (b) (4)		
Regression model and weighting	Linear, weighted $1/x^2$		
Validation parameters	**Method validation summary**		**Source location**
Standard calibration curve performance during accuracy and precision runs	Number of standard calibrators from LLOQ to ULOQ	9	RE-021-Report037-2014-MVAL, Table 3
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-5.3% to 4.0%	RE-021-Report037-2014-MVAL, Table 3
	Cumulative precision (%CV) from LLOQ to ULOQ	≤5.9%	RE-021-Report037-2014-MVAL, Table 3

Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 4 QCs**	-4.0% to 6.0%	RE-021-Report037-2014-MVAL, Table 4
	Inter-batch %CV	≤5.8%	RE-021-Report037-2014-MVAL, Table 4
	Total error	NR	NA
Selectivity and matrix effect	The mean bias using 6 different lots of spiked blank plasma was 1.0% and the CV was 4.4% for the LLOQ, and the mean bias was -9.8% and the CV was 0.9% for the ULOQ The sparsentan matrix effect CV was ≤3.3% The sparsentan-d_5 matrix effect CV was ≤1.9% There was no sparsentan or sparsentan-d_5 response in any of 6 blank matrix lots		RE-021-Report037-2014-MVAL, Table 9, Table 10, Table 11, and Table 14
Interference and specificity	No interference or contribution was observed from sparsentan to the sparsentan-d_5 channel.		RE-021-Report037-2014-MVAL, Figure 10
Hemolysis effect	Hemolyzed QC samples met acceptance criteria at each QC level (bias = -1.5% and CV = 6.8% for the low QC; bias = -2.0% and CV = 1.7% for the high QC)		RE-021-Report037-2014-MVAL, Table 12
Lipemic effect	Lipemic QC samples met acceptance criteria at each QC level (bias = 2.5% and CV = 6.6% for the low QC; bias = -2.0% and CV = 1.5% for the high QC)		RE-021-Report037-2014-MVAL, Table 13
Dilution linearity and hook effect	10-fold dilution of 25 000 ng/mL		RE-021-Report037-2014-MVAL, Table 5
Bench-top/process stability	In plasma for 48 hours at room temperature Reinjection reproducibility for 404 hours at room temperature Processed samples for 144 hours at room temperature		RE-021-Report037-2014-MVAL, Table A-7, Table 26, and Table 27
Freeze-thaw stability	5 cycles		RE-021-Report037-2014-MVAL, Table 21 and Table 22
Long-term stability	343 days at -20°C 440 days at -70°C		RE-021-Report037-2014-MVAL, Table A-8 and Table A-9
Parallelism	NA		NA
Carry over	There was no significant carryover in a blank sample following a ULOQ sample		RE-021-Report037-2014-MVAL, Figure 11

Method performance in Study 021HVOL16001 **Bioanalytical report:** RE-021-Report090-2016-BIOA		
Assay passing rate	90%	RE-021-Report090-2016-BIOA, Table 1
Standard curve performance	Cumulative bias range: -10.5% to 9.2% Cumulative precision: ≤5.1% CV	RE-021-Report090-2016-BIOA, Table 3
QC performance	Cumulative bias range: -5.7% to 8.0% Cumulative precision: ≤10.5% CV	RE-021-Report090-2016-BIOA, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 10.5% of study samples (130 of 1235) and 90.0% of these were within ±20% of the original result	RE-021-Report090-2016-BIOA, Section 3 and Table 7
Study sample analysis/stability	Samples were analyzed as received, within the established freezer stability limit of 440 days at −70°C	RE-021-Report090-2016-BIOA, Section 3
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 18 analytical batches (total 36 per concentration [grand total 324]) – 11 excluded (3.4%)	
Method performance in Study 021HVOL16002 **Bioanalytical report:** RE-021-Report035-2017-BIOA		
Assay passing rate	92.9%	RE-021-Report035-2017-BIOA, Table 1
Standard curve performance	Cumulative bias range: -4.5% to 5.6% Cumulative precision: ≤5.2% CV	RE-021-Report035-2017-BIOA, Table 3
QC performance	Cumulative bias range: -3.2% to 6.0% Cumulative precision: ≤8.8% CV	RE-021-Report035-2017-BIOA, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 10.5% of study samples (200 of 1901) and 98.5% of these were within ±20% of the original result	RE-021-Report035-2017-BIOA, Section 3 and Table 7
Study sample analysis/stability	Samples were analyzed as received, within the established freezer stability limit of 440 days at −70°C	RE-021-Report035-2017-BIOA, Section 3
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 26 analytical batches (total 52 per concentration [grand total 468]) – 6 excluded (1.3%)	

Method performance in Study 021HVOL109 Bioanalytical report: RE-021-Report051-2018-BIOA		
Assay passing rate	100%	RE-021-Report051-2018-BIOA, Table 1
Standard curve performance	Cumulative bias range: -8.0% to 7.0% Cumulative precision: ≤5.7% CV	RE-021-Report051-2018-BIOA, Table 3
QC performance	Cumulative bias range: 0.7% to 8.0% Cumulative precision: ≤4.6% CV	RE-021-Report051-2018-BIOA, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 10.1% of study samples (111 of 1095) and 96.3% of these were within ±20% of the original result	RE-021-Report051-2018-BIOA, Table 7
Study sample analysis/stability	Samples were analyzed as received, within the established freezer stability limit of 440 days at −70 °C	RE-021-Report051-2018-BIOA, Section 4
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 12 analytical batches (total 24 per concentration [grand total 216]) – 1 excluded (0.5%)	
Method performance in Study RTRX-RE021-101 Bioanalytical report: RE-021-0048		
Assay passing rate	83.3%	RE-021-0048, Table 1
Standard curve performance	Cumulative bias range: -4.8% to 5.0% Cumulative precision: ≤7.2% CV	RE-021-0048, Table 3
QC performance	Cumulative bias range: -2.7% to 4.3% Cumulative precision: ≤5.9% CV	RE-021-0048, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 8.7% of study samples (125 of 1437) and 89.6% of these were within ±20% of the original result	RE-021-0048, Table 7 and Section 6.4
Study sample analysis/stability	Samples were analyzed within the established freezer stability limit of 440 days at −70°C	RE-021-0048, Section 4
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 15 analytical batches (total 30 per concentration [grand total 270]) – 9 excluded (3.3%)	

Method performance in Study RTRX-RE021-103 Bioanalytical report: RE-021-0050		
Assay passing rate	93.3%	RE-021-0050, Table 1
Standard curve performance	Cumulative bias range: -5.0% to 5.0% Cumulative precision: ≤7.0% CV	RE-021-0050, Table 3
QC performance	Cumulative bias range: -1.0% to 6.0% Cumulative precision: ≤12.9% CV	RE-021-0050, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 7.0% of study samples (196 of 2810) and 93.4% of these were within ±20% of the original result	RE-021-0050, Table 7 and Section 6.4
Study sample analysis/stability	Samples were analyzed within the established freezer stability limit of 440 days at −70°C	RE-021-0050, Section 4
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 28 analytical batches (total 56 per concentration [grand total 504]) – 19 excluded (3.8%)	
Method performance in Study RET-D-001 (DUET) **Bioanalytical report:** RE-021-Report084-2016-BIOA		
Assay passing rate	88.0%	RE-021-Report084-2016-BIOA, Table 1
Standard curve performance	Cumulative bias range: -3.5% to 2.4% Cumulative precision: ≤6.0% CV	RE-021-Report084-2016-BIOA, Table 3
QC performance	Cumulative bias range: -2.0% to 3.0% Cumulative precision: ≤7.4% CV	RE-021-Report084-2016-BIOA, Table 2
Method reproducibility	Incurred sample reanalysis was performed in approximately 10.6% of study samples (142 of 1344) and 97.2% of these were within ±20% of the original result	RE-021-Report084-2016-BIOA, Table 7 and Section 3
Study sample analysis/stability	All samples were analyzed within 398 days after collection (within established stability of 440 days)	RE-021-Report084-2016-BIOA, Section 3
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 22 analytical batches (total 44 per concentration [grand total 396]) – 3 excluded (0.8%)	

Method performance in Study 021FSGS16010 (DUPLEX) **Bioanalytical report:** RE-021-Report037-2017-BIOA-1		
Assay passing rate	91.7%	RE-021-Report037-2017-BIOA-1, Table 1
Standard curve performance	Cumulative bias range: -5.9% to 3.0% Cumulative precision: ≤8.4% CV	RE-021-Report037-2017-BIOA-1, Table 3
QC performance	Cumulative bias range: 0.0% to 7.0% Cumulative precision: ≤10.7% CV	RE-021-Report037-2017-BIOA-1, Table 2
Method reproducibility	Incurred sample reanalysis was performed in 11.6% of study samples (60 of 519) and 91.7% were within ±20% of the original result	RE-021-Report037-2017-BIOA-1, Section 6.4 and Table 7
Study sample analysis/stability	All samples were analyzed within 379 days after collection (within established stability of 440 days)	RE-021-Report037-2017-BIOA-1, Section 4
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 11 analytical batches (total 22 per concentration [grand total 198]) – 5 excluded (2.5%)	
Method performance in Study 021IGAN17001 (PROTECT) **Bioanalytical report:** RE-021-Report050-2018-BIOA-1		
Assay passing rate	92.3%	RE-021-Report050-2018-BIOA-1, Table 1
Standard curve performance	Cumulative bias range: -5.1% to 4.0% Cumulative precision: ≤4.9% CV	RE-021-Report050-2018-BIOA-1, Table 3
QC performance	Cumulative bias range: 1.3% to 5.3% Cumulative precision: ≤15.5% CV	RE-021-Report050-2018-BIOA-1, Table 2
Method reproducibility	Incurred sample reanalysis was performed in 11.5% of study samples (85 of 739) and 95.3% were within ±20% of the original result	RE-021-Report050-2018-BIOA-1, Section 6.4 and Table 7
Study sample analysis/stability	All reported samples were analyzed within the established stability of 440 days.	RE-021-Report050-2018-BIOA-1, Section 4
Standard calibration curve performance during accuracy and precision runs	2 × 9 concentrations of calibration standard in 12 analytical batches (total 24 per concentration [grand total 216]) – 1 excluded (0.46%)	

Source: Applicant's summary of biopharmaceutic studies and associated analytical methods report. Table 10 on page 26.
Abbreviations: CV, coefficient of variation; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLOQ, lower limit of quantification; NA, not applicable; NR, not reported; QC, quality control; ULOQ, upper limit of quantification.

Table 91. Summary of Method Validation and Performance in Clinical Studies for Sparsentan in Human Plasma: Method M8349671

Bioanalytical method validation report name, amendments, and hyperlinks	Validation of a method for the determination of sparsentan in human plasma by liquid chromatography/tandem mass spectrometry Method report 8349671, Amendment 1, and Addendum 1		
Method description	Human plasma samples were prepared by addition of internal standard (sparsentan-d_5) and protein precipitation with acetonitrile. After centrifugation, supernatant was evaporated to dryness, reconstituted, and analyzed by LC-MS/MS. API 6500 and API 4000 mass spectrometers were used and accuracy and precision are reported separately below.		
Materials used for standard calibration curve and concentration	Sparsentan, lot number: L0503567 2, 4, 20, 200, 800, 2400, 3600, and 4000 ng/mL		
Validated assay range	2.00 to 4000 ng/mL		
Material used for QCs and concentration	Sparsentan, lot number: L0503567 2, 6, 100, 1600, 3200, and 16 000 ng/mL		
Minimum required dilutions	NA		
Source and lot of reagents	Human plasma: (b) (4) Acetonitrile and methanol, HPLC grade: (b) (4) Ammonium acetate, ACS grade: (b) (4) N,N-dimethylformamide, HPLC grade: (b) (4) Water, deionized, HPLC grade: NR		
Regression model and weighting	API 6500: Quadratic, weighted 1/x API 4000: Linear, weighted $1/x^2$		
Validation parameters	**Method validation summary**		**Source Location**
Standard calibration curve performance during accuracy and precision runs API 6500	Number of standard calibrators from LLOQ to ULOQ	8	8349671, Table 6.2
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-7.5% to 3.5%	8349671, Table 6.2
	Cumulative precision (%CV) from LLOQ to ULOQ	≤9.5%	8349671, Table 6.2
Performance of QCs during accuracy and precision runs API 6500	Cumulative accuracy (%bias) in 4 QCs	-11.0% to 8.0%	8349671, Table 6.6
	Inter-batch %CV	≤9.1%	8349671, Table 6.6
	Total error	NR	NA

Standard calibration curve performance during accuracy and precision runs API 4000	Number of standard calibrators from LLOQ to ULOQ	8	8349671, Table 7.2
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-7.2% to 8.5%	8349671, Table 7.2
	Cumulative precision (%CV) from LLOQ to ULOQ	≤5.5%	8349671, Table 7.2
Performance of QCs during accuracy and precision runs API 4000	**Cumulative accuracy (%bias) in 4 QCs**	-2.2% to 7.2%	8349671, Table 7.6
	Inter-batch %CV	≤6.4%	8349671, Table 7.6
	Total error	NR	NA
Selectivity and matrix effect	No significant interference observed in blank plasma from 6 lots using either API 6500 or API 4000 The matrix effect using the API 6500 was -9.0% (CV=6.5%) The matrix effect using the API 4000 was -3.5% (CV=5.3%)		8349671, Figure 8.8 through Figure 8.13, Figure 9.21 through Figure 9.26, Table 6.10, and Table 7.14
Interference and specificity	Bupropion, cyclosporine A, itraconazole, midazolam, hydroxymidazolam, pitavastatin, and pitavastatin lactone (all at 8000 ng/mL) did not affect the analysis of sparsentan at 6 ng/mL Sparsentan did not affect the analysis of midazolam, hydroxymidazolam, itraconazole, or cyclosporine A		8349671, Table 7.10, Table 7.11, and Table 7.12, 8349671, Appendix, and 8349671 Addendum 1, Table 8.5
Hemolysis effect	Hemolyzed QC samples met acceptance criteria at each QC level (bias = 5.3% and CV = 6.0% for the low QC; bias = 5.6% and CV = 2.9% for the high QC)		8349671, Table 6.14
Lipemic effect	Lipemic QC samples met acceptance criteria at each QC level (bias = 3.7% and CV = 5.4% for the low QC; bias = 7.5% and CV = 3.2% for the high QC)		8349671, Table 6.15
Dilution linearity and hook effect	10-fold dilution of 16000 ng/mL		8349671, Table 6.9 and Table 7.9
Bench-top/process stability	In plasma for 8 hours at room temperature Processed samples for 169 hours at room temperature		8349671, Table 6.20 and Table 6.23

Freeze-thaw stability	4 cycles at -20°C 5 cycles at -70°C	8349671, Table 6.21 and Table 7.16
Long-term stability	62 days at -20°C 233 days between -60°C and -80°C	8349671, Table 7.17 and Table 7.18 8349671 Addendum 1, Table 8.6
Parallelism	NA	NA
Carry over	There was no significant carryover (ie <20% of LLOQ) in a blank sample following a ULOQ sample	8349671, Figure 8.7 and Figure 9.20
Method performance in Study 021HVOL16006 **Bioanalytical report:** 8349679		
Assay passing rate	90.9%	8349679, Table 1
Standard curve performance	Cumulative bias range: -6.3% to 5.0% Cumulative precision: ≤7.9% CV	8349679, Table 5
QC performance	Cumulative bias range: -10.6% to 7.2% Cumulative precision: ≤12.6% CV	8349679, Table 9
Method reproducibility	Incurred sample reanalysis was performed in 12.2% of study samples (236 of 1928) and 84.7% were within ±20% of the original result	8349679, Section 4.4 and Table 22
Study sample analysis/stability	All samples were analyzed within the established stability of 233 days	8349679, Section 4.1 and Table 1
Standard calibration curve performance during accuracy and precision runs	2 × 8 concentrations of calibration standard in 20 analytical batches (total 40 per concentration [grand total 320]) – 23 excluded (7.2%)	

Method performance in Study 021HVOL16007 Bioanalytical report: 8349674		
Assay passing rate	66.7%	8349674, Table 1
Standard curve performance	Cumulative bias range: -5.0% to 5.4% Cumulative precision: ≤7.8% CV	8349674, Table 3
QC performance	Cumulative bias range: -8.1% to 4.8% Cumulative precision: ≤7.7% CV	8349674, Table 5
Method reproducibility	Incurred sample reanalysis was performed in 18.0% of study samples (24 of 133) and 82.9% were within ±20% of the original result	8349674, Section 4.4 and Table 7
Study sample analysis/stability	All samples were analyzed within the established stability of 233 days	8349674, Section 4.1 and Table 1
Standard calibration curve performance during accuracy and precision runs	2 × 8 concentrations of calibration standard in 4 analytical batches (total 8 per concentration [grand total 64]) – 2 excluded (3.1%)	
Method performance in Study 021HVOL16008 Bioanalytical report: 8349765		
Assay passing rate	75%	8349765, Table 1
Standard curve performance	Cumulative bias range: -4.7% to 5.5% Cumulative precision: ≤8.5% CV	8349765, Table 4
QC performance	Cumulative bias range: -6.3% to 3.5% Cumulative precision: ≤6.7% CV	8349765, Table 8
Method reproducibility	Incurred sample reanalysis was performed in 19.6% of study samples (22 of 112) and 100% were within ±20% of the original result	8349765, Table 25
Study sample analysis/stability	All samples were analyzed within the established stability of 233 days	8349765, Section 4.1 and Table 1
Standard calibration curve performance during accuracy and precision runs	2 × 8 concentrations of calibration standard in 3 analytical batches (total 6 per concentration [grand total 48]) – 2 excluded (5.6%)	

Method performance in Study 021HFX16009 Bioanalytical report: 8349856		
Assay passing rate	100%	8349856, Table 1
Standard curve performance	Cumulative bias range: -9.8% to 7.0% Cumulative precision: ≤6.6% CV	8349856, Table 3
QC performance	Cumulative bias range: -3.8% to 16.7% Cumulative precision: ≤22.9% CV	8349856, Table 5
Method reproducibility	Incurred sample reanalysis was performed in approximately 10% of study samples and all but 1 were within ±20% of the original result	8349856, Table 7
Study sample analysis/stability	All samples were analyzed within the established stability of 233 days	8349856, Section 4.1
Standard calibration curve performance during accuracy and precision runs	2 × 8 concentrations of calibration standard in 7 analytical batches (total 14 per concentration [grand total 112]) – none excluded	

Source: Applicant's summary of biopharmaceutic studies and associated analytical methods report. Table 12 on page 34.
Abbreviations: ACS, American Chemical Society; CV, coefficient of variation; HPLC, high performance liquid chromatography; LC-MS/MS, liquid chromatography with tandem mass spectrometry; LLOQ, lower limit of quantification; NA, not applicable; NR, not reported; QC, quality control; ULOQ, upper limit of quantification.

All bioanalytical methods satisfied the method validation criteria in accordance with the FDA guidance (May 2018). The performance of the assays are considered acceptable for sample analysis.

14.4. Immunogenicity Assessment—Impact of PK/PD, Efficacy, and Safety

Not applicable.

14.5. Pharmacometrics Assessment

14.5.1. Summary of Applicant's Population PK Analysis

A PopPK analysis for sparsentan was conducted with PK data from healthy volunteers (HV) and subjects with FSGS. The PopPK model developed using the results from HV and subjects with FSGS was then evaluated externally using the PK data from IgAN patients in PROTECT. This section of the review summarizes the following two population PopPK reports: (1) Population Pharmacokinetic Analysis of Sparsentan in Healthy Volunteers and Subjects with Focal Segmental Glomerulosclerosis (referred to as "PopPK report" in this review), and (2) Population Pharmacokinetic and Efficacy and Safety Exposure Response Analyses of Sparsentan in Patients with Immunoglobulin A Nephropathy (IgAN) (referred to as "PROTECT PopPK/E-R report").

Data

The PopPK analysis was conducted using PK data collected from nine clinical studies: seven phase 1 studies in HV, and one phase 2 study (DUET) and one phase 3 study (DUPLEX) in subjects with primary and genetic FSGS. For an overview of clinical studies that are included in the analysis and the details about the PK sampling, refer to the PopPK report. Noted exclusions of PK data include 753 BLQ samples and 1139 samples were also excluded as these samples were taken after oral sparsentan dose with standardized high-fat meal. The PK absorption profile at this fed condition differed from the profile in fasted subjects, and subjects with FSGS were instructed to take sparsentan before the morning meal (or at least 8 hours fasting) during the site visit. A summary of the baseline covariates is provided for continuous covariates (Table 92) and categorical covariates (Table 93).

Table 92. Summary of Baseline Continuous Covariates

Covariate Statistics	Healthy Subjects (N=236)	FSGS (N=194)	Hepatic Impairment (N=16)	Overall (N=446)
Age (years)				
Mean (SD)	38.5 (9.66)	41.0 (16.9)	56.8 (5.65)	40.3 (13.6)
Median (CV%)	38.0 (25.1)	43.0 (41.2)	58.0 (10.0)	40.0 (33.9)
[Min, Max]	[18.0, 65.0]	[8.00, 74.0]	[49.0, 65.0]	[8.00, 74.0]
Body Weight (kg)				
Mean (SD)	79.8 (12.4)	80.6 (21.8)	82.2 (14.1)	80.2 (17.2)
Median (CV%)	78.0 (15.6)	79.3 (27.1)	84.7 (17.2)	78.6 (21.4)
[Min, Max]	[54.6, 122]	[21.1, 154]	[53.0, 103]	[21.1, 154]
BSA (m^2)				
Mean (SD)	1.92 (0.195)	1.90 (0.289)	1.95 (0.178)	1.91 (0.240)
Median (CV%)	1.92 (10.1)	1.89 (15.2)	1.98 (9.1)	1.91 (12.5)
[Min, Max]	[1.42, 2.51]	[0.830, 2.61]	[1.59, 2.17]	[0.830, 2.61]
Missing	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
BMI (kg/m^2)				
Mean (SD)	26.9 (2.79)	28.1 (6.18)	27.9 (4.11)	27.5 (4.65)
Median (CV%)	27.0 (10.4)	27.7 (22.0)	27.8 (14.8)	27.4 (16.9)
[Min, Max]	[19.1, 34.0]	[15.1, 47.0]	[18.8, 34.4]	[15.1, 47.0]
Missing	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Lean Body Weight (kg)				
Mean (SD)	58.4 (9.29)	55.3 (12.0)	60.5 (6.96)	57.1 (10.6)
Median (CV%)	59.0 (15.9)	54.0 (21.7)	62.2 (11.5)	57.7 (18.5)
[Min, Max]	[35.8, 83.1]	[18.5, 84.8]	[45.6, 68.8]	[18.5, 84.8]
Missing	0 (0%)	1 (0.5%)	0 (0%)	1 (0.2%)
Albumin (g/dL)				
Mean (SD)	4.40 (0.359)	3.50 (0.727)	4.12 (0.574)	4.00 (0.711)
Median (CV%)	4.40 (8.1)	3.70 (20.8)	4.25 (13.9)	4.10 (17.8)
[Min, Max]	[3.40, 5.50]	[1.40, 4.80]	[3.00, 5.00]	[1.40, 5.50]
Total Protein (g/dL)				
Mean (SD)	7.23 (0.501)	5.82 (1.03)	7.38 (0.726)	6.62 (1.05)
Median (CV%)	7.25 (6.9)	5.90 (17.7)	7.60 (9.8)	6.90 (15.9)
[Min, Max]	[5.60, 8.80]	[3.20, 7.90]	[5.90, 8.70]	[3.20, 8.80]

Covariate Statistics	Healthy Subjects (N=236)	FSGS (N=194)	Hepatic Impairment (N=16)	Overall (N=446)
CrCL (mL/min)				
Mean (SD)	127 (26.9)	90.2 (43.7)	128 (29.7)	111 (39.7)
Median (CV%)	123 (21.3)	78.4 (48.5)	126 (23.3)	112 (35.8)
[Min, Max]	[73.8, 253]	[26.0, 361]	[87.4, 189]	[26.0, 361]
Creatinine (µmol/L)				
Mean (SD)	79.1 (16.6)	115 (46.8)	67.8 (14.2)	94.5 (38.0)
Median (CV%)	79.6 (21.0)	111 (40.5)	66.3 (21.0)	86.6 (40.2)
[Min, Max]	[41.5, 122]	[35.4, 240]	[44.2, 97.2]	[35.4, 240]
SGOT/AST (U/L)				
Mean (SD)	21.5 (6.01)	23.0 (11.5)	63.8 (50.0)	23.7 (14.9)
Median (CV%)	21.0 (27.9)	20.0 (49.9)	37.0 (78.5)	21.0 (62.8)
[Min, Max]	[11.0, 43.0]	[8.00, 95.0]	[17.0, 167]	[8.00, 167]
SGPT/ALT (U/L)				
Mean (SD)	22.0 (10.3)	21.3 (12.0)	60.6 (52.4)	23.1 (16.3)
Median (CV%)	19.0 (46.9)	18.0 (56.3)	38.5 (86.6)	19.0 (70.4)
[Min, Max]	[5.00, 59.0]	[5.00, 82.0]	[14.0, 163]	[5.00, 163]
Missing	1 (0.4%)	0 (0%)	0 (0%)	1 (0.2%)
Total Bilirubin (µmol/L)				
Mean (SD)	9.64 (5.63)	6.41 (3.88)	21.9 (14.6)	8.67 (6.29)
Median (CV%)	8.60 (58.4)	5.10 (60.5)	18.8 (66.7)	6.80 (72.5)
[Min, Max]	[1.70, 46.2]	[1.70, 27.0]	[5.10, 51.3]	[1.70, 51.3]
Missing	3 (1.3%)	0 (0%)	0 (0%)	3 (0.7%)
ALKP (U/L)				
Mean (SD)	67.2 (19.8)	79.6 (36.2)	95.1 (35.0)	73.6 (29.5)
Median (CV%)	65.0 (29.5)	73.0 (45.4)	91.5 (36.8)	68.0 (40.1)
[Min, Max]	[30.0, 174]	[25.0, 269]	[47.0, 165]	[25.0, 269]

Source: Applicant's PopPK Report. Table 4. Page 27.
Abbreviations: ALKP, alkaline phosphatase; ALT, alanine transferase; AST aspartate transferase; BMI, body mass index; BSA, body surface area; CrCl, creatinine clearance; CV, coefficient of variance; FSGS, focal segmental glomerulosclerosis; SGPT, serum glutamic pyruvic transaminase; SGOT, serum glutamic oxaloacetic transaminase

Table 93. Summary of Baseline Categorical Covariates

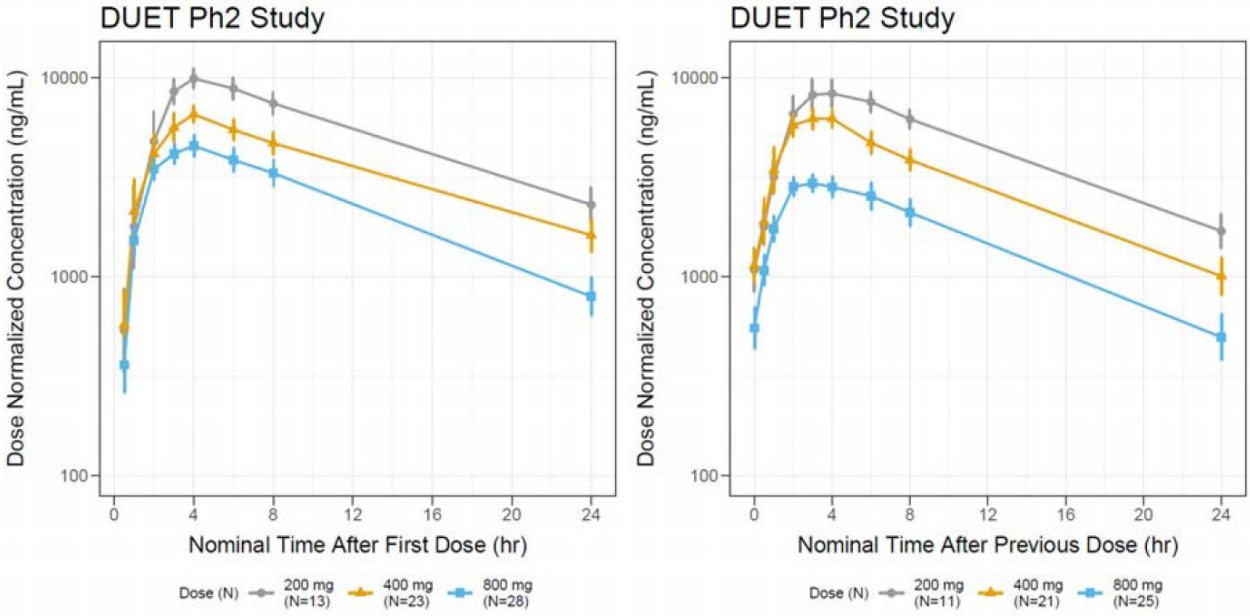
Covariate	Healthy Subjects (N=236)	FSGS (N=194)	Hepatic Impairment (N=16)	Overall (N=446)
Sex				
Female	62 (26.3%)	86 (44.3%)	0 (0%)	148 (33.2%)
Male	174 (73.7%)	108 (55.7%)	16 (100%)	298 (66.8%)
Race				
White	141 (59.7%)	146 (75.3%)	14 (87.5%)	301 (67.5%)
Black or African American	89 (37.7%)	15 (7.7%)	1 (6.2%)	105 (23.5%)
Asian	4 (1.7%)	22 (11.3%)	1 (6.2%)	27 (6.1%)
Multiple	2 (0.8%)	1 (0.5%)	0 (0%)	3 (0.7%)
Other	0 (0%)	10 (5.2%)	0 (0%)	10 (2.2%)
Renal Function				
Normal	231 (97.9%)	85 (43.8%)	15 (93.8%)	331 (74.2%)
Mild	5 (2.1%)	54 (27.8%)	1 (6.2%)	60 (13.5%)
Moderate	0 (0%)	53 (27.3%)	0 (0%)	53 (11.9%)
Severe	0 (0%)	2 (1.0%)	0 (0%)	2 (0.4%)
Hepatic Function				
Normal	12 (5.1%)	0 (0%)	0 (0%)	12 (2.7%)
Mild	0 (0%)	0 (0%)	8 (50.0%)	8 (1.8%)
Moderate	0 (0%)	0 (0%)	8 (50.0%)	8 (1.8%)
Severe	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Missing	224 (94.9%)	194 (100%)	0 (0%)	418 (93.7%)
Food				
Fasting	236 (100%)	0 (0%)	16 (100%)	252 (56.5%)
Fed	51 (21.6%)	0 (0%)	0 (0%)	51 (11.4%)
Unknown	0 (0%)	194 (100%)	0 (0%)	194 (43.5%)
Formulation				
Capsule	137 (58.1%)	71 (36.6%)	0 (0%)	208 (46.6%)
Tablet	95 (40.3%)	123 (63.4%)	16 (100%)	234 (52.5%)
Crushed Tablet	36 (15.3%)	0 (0%)	0 (0%)	36 (8.1%)
P-gp Inhibitor				
No Co-administration	220 (93.2%)	174 (89.7%)	16 (100%)	410 (91.9%)
Co-administration	16 (6.8%)	20 (10.3%)	0 (0%)	36 (8.1%)
CYP3A4 Inhibitor				
None	236 (100%)	118 (60.8%)	16 (100%)	370 (83.0%)
Unknown	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Weak	0 (0%)	57 (29.4%)	0 (0%)	57 (12.8%)
Moderate	30 (12.7%)	17 (8.8%)	0 (0%)	47 (10.5%)
Strong	30 (12.7%)	2 (1.0%)	0 (0%)	32 (7.2%)
CYP3A4 Inducer				
None	236 (100%)	140 (72.2%)	16 (100%)	392 (87.9%)
Unknown	0 (0%)	54 (27.8%)	0 (0%)	54 (12.1%)

Covariate	Healthy Subjects (N=236)	FSGS (N=194)	Hepatic Impairment (N=16)	Overall (N=446)
Weak	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Strong	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Acid Reducing Agent				
No Co-administration	236 (100%)	135 (69.6%)	16 (100%)	387 (86.8%)
Co-administration	0 (0%)	59 (30.4%)	0 (0%)	59 (13.2%)

Source: Applicant's PopPK Report. Table 5. Page 29.
Abbreviations: CYP, cytochrome P450 isoenzyme; FSGS, focal segmental glomerulosclerosis; P-gp, p-glycoprotein

The DUET study had samples in FSGS patients in the clinical dose range (200 to 800 mg) both after the first dose and at steady state (Day 57). Subjects with FSGS have a gradual increase to C_{max} at approximately 4 hours, with a monophasic decline out to 24 hours and plasma concentration profiles are less than dose proportional though the curves are parallel. The steady-state concentrations are lower than concentrations after the first dose.

Figure 23. PK Profiles After Single Dose (Left) and at Steady State (Right), Study DUET



Source: Applicant's PopPK Report. Figure 1. Page 32.

Base Model

The base model was a 2-compartment model with first-order absorption and an absorption lag time (T_{lag}), with proportional plus additive residual error. Between-subject variability terms were included on CL/F, Vc/F and the absorption rate constant (KA). To account for the dose-dependent bioavailability, a saturable relationship on relative bioavailability (Frel) was modeled. To account for decreasing PK concentrations going from single dose to steady state, an induction term on CL/F was modeled as a rapid increase to steady state occurring after the first dose during multiple-dose regimens. Covariate relationships for strong and moderate CYP3A4 inhibition were included to describe the known influence of cyclosporine and itraconazole. At steady state,

CL/F is 5.25 L/h, which is increased from the single-dose clearance (CL) by 1.16 L. The central volume of Vc is 56.2 L. The parameters are estimated with precision (RSE <20%). Between-subject variability were 43.6% for CL/F, 50.6% for Vc/F and 69.7% for Ka. Shrinkages from 4% for CL/F to 23% for the KA.

Covariate Analysis

The covariates evaluated in the PopPK analysis are listed in Table 94. A univariate forward and a stepwise backward covariate analysis resulted in six covariate-parameter relationships retained in the model: ALKP, creatine clearance (CrCL), and sex on CL; race on Vc; formulation on T_{lag}; and formulation on KA.

Table 94. Covariates Evaluated in the PopPK Analysis

Category	Covariate	PK Parameter	Reason for Investigation
Demographics	Body size at baseline (WT, BMI, LBW, BSA)	CL, Vc	CL and Vc assumed to be increase with body size
	Age	CL, Vc	Standard covariate
	Sex (Male/Female)	CL, Vc	Standard covariate
	Race	CL, Vc	Standard covariate
Hepatic function	Serum albumin	CL	General liver function marker
	SGOT/AST	CL	Liver enzyme
	SGPT/ALT	CL	Liver enzyme
	Total bilirubin	CL	Liver enzyme
	Alkaline phosphate	CL	Liver enzyme
Renal function	Creatinine clearance	CL	Renal function indicator
Food	Food (fast/fed/nonspecified)	Frel, KA, Tlag	Absorption parameter
Formulation	Dose	Frel, KA, Tlag	Absorption parameter
	Formulation (capsule/tablet/crushed tablet)	Frel, KA, Tlag	Absorption parameter
Population	FSGS vs non-FSGS	CL, Vc	General effect of disease
Comedications	Acid-reducing agent	Frel, KA, Tlag	Absorption parameter
	CYP3A4 inducer	CL	Drug-drug interaction
	CYP3A4 inhibitors	CL	Drug-drug interaction
	P-gp inhibitor	CL	Drug-drug interaction

Abbreviations: ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; BSA = body surface area; CL = clearance; CYP = cytochrome P450; Frel = relative bioavailability; KA = absorption rate constant; LBW = lean body weight; P-gp = P-glycoprotein transporter; PK = pharmacokinetic; SGPT/ALT = alanine transaminase; SGOT/AST = aspartate transaminase; Tlag = absorption lag time; Vc = central volume of distribution; WT = body weight.

Source: Applicant's PopPK report. Table 2 on page 22.

Final Model

The final model parameters are summarized in Table 95. The typical value of CL/F is 3.88 L/h after a single 400-mg dose, increasing by 1.23 L/h at steady state. At the 800-mg dose, the typical value of CL/F is 5.47 L/h, increasing to 7.21 L/h at steady state. Terminal half-life is also 9.6 hour at steady state. The parameter estimates for dose nonlinearity (Frel) suggests less than dose-proportional exposures. Between-subject variability in CL, Vc, and the KA were moderate and estimated as 40%, 48%, and 69%, respectively.

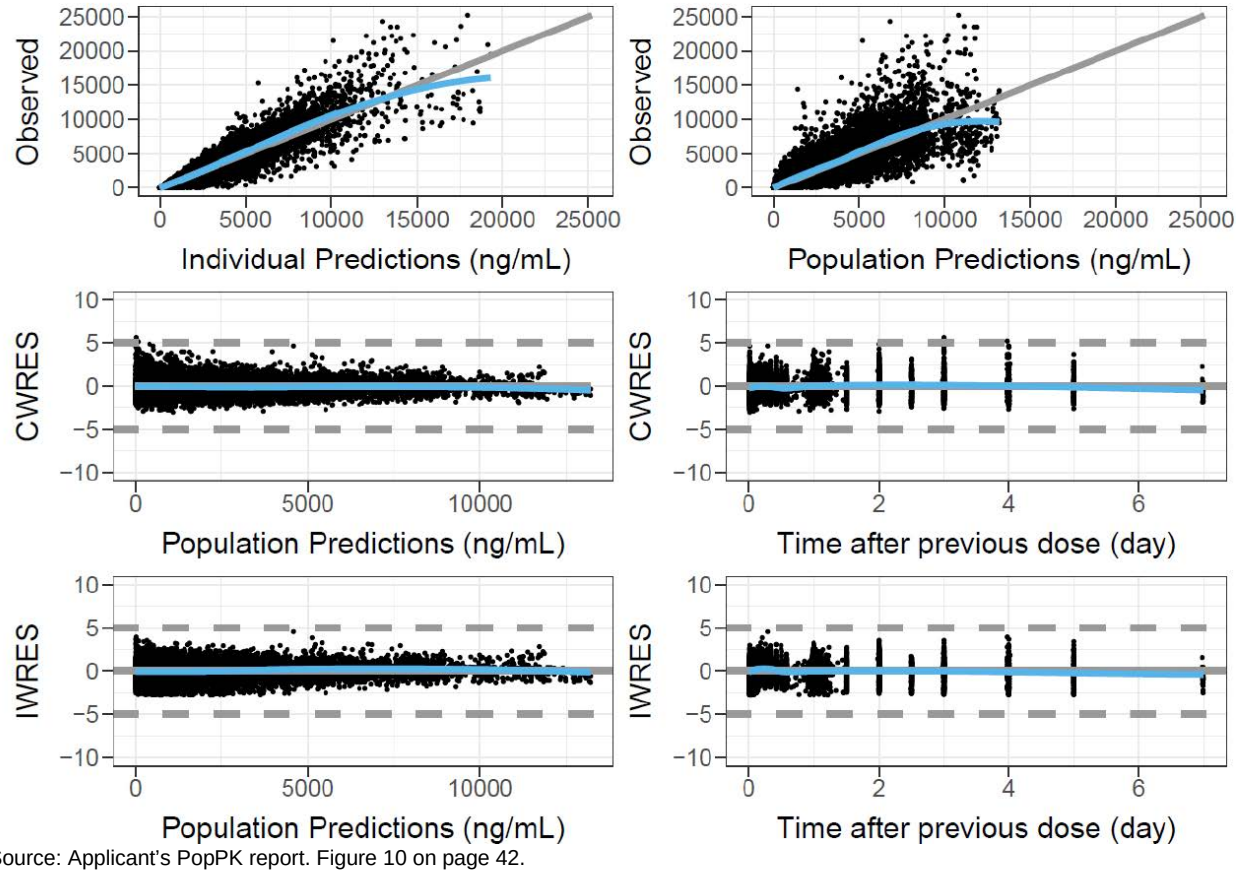
Table 95. Parameter Estimates for Final PopPK Model

Parameter	Estimate	RSE (%)	IIV (%)	Shrinkage (%)
Apparent Clearance (CL/F, L/h)	3.88	4.6	39.5%	4.7%
Apparent Central Volume (Vc/F, L)	49.3	4.3	48.4%	17.2%
Apparent Distribution Clearance (Q/F, L/h)	2.03	12.0	--	--
Apparent Peripheral Volume (Vp/F, L)	12.1	10.5	--	--
Absorption Rate (KA, 1/h)	0.740	6.9	68.9%	22.2%
Absorption Lag Time (Tlag, h)	0.32	4.0		
Induction Change in Clearance (L/h)	1.23	13.6		
Induction Half-Life ($T_{1/2}$, day)	0.001	FIXED		
Dose on Relative Bioavailability	-0.495	5.1		
Moderate CYP3A4 on CL	-0.273	18.8		
Strong CYP3A4 on CL	-1.069	10.0		
Alkaline Phosphatase on CL	-0.208	27.5		
Creatinine Clearance on CL	0.222	26.5		
Male on CL	0.139	32.8		
Black or African American on Vc	0.309	18.4		
Asian on Vc	0.265	48.4		
Tablet on KA	-0.306	34.8		
Crushed Tablet on KA	0.080	159.1		
Tablet on Tlag	-0.269	29.1		
Crushed Tablet on Tlag	-1.175	28.7		
Variance CL	0.156	8.7		
Variance Vc	0.234	11.3		
Variance KA	0.474	10.2		
SD of Additive Error (ng/mL)	2	FIXED		
SD of Proportional Error	0.365	1.9		
Single Dose Clearance at 800 mg (L/h)	5.47	--		
Single Dose Clearance in Males at 800 mg (L/h)	6.29	--		
Steady-State Clearance at 400 mg (L/h)	5.12	--		
Steady-State Clearance at 800 mg (L/h)	7.21	--		
Steady-State Clearance in Males at 800 mg (L/h)	8.29	--		
Steady-State Central Volume at 800 mg (L)	69.5	--		
Steady-State Peripheral Volume at 800 mg (L)	17.0	--		

Parameter	Estimate	RSE (%)	IIV (%)	Shrinkage (%)
Absorption Rate Tablet (1/h)	0.545	--		
Absorption Lag Time Tablet (h)	0.24	--		
Steady-State Terminal Half-Life at 800 mg	9.6	--		
Steady-State Terminal Half-Life at 800 mg in Males	8.6	--		

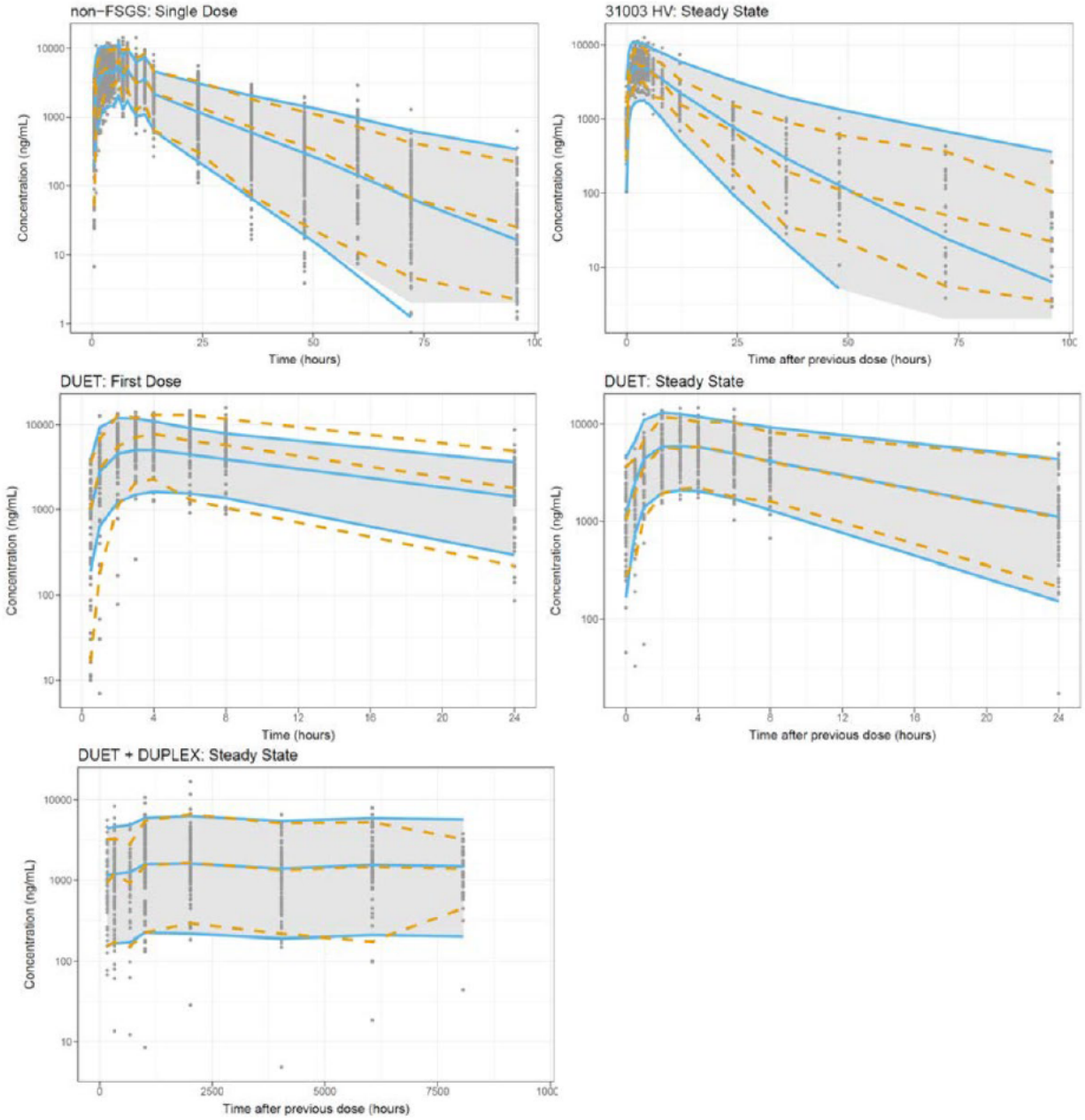
Abbreviations: ALKP = alkaline phosphatase; CL = clearance; CLss = steady-state clearance; CrCL = creatinine clearance; CYP = cytochrome P450; F = bioavailability; Frel = relative bioavailability; IIV = interindividual variance; KA = absorption rate constant; Q = distribution clearance; RSE = residual squared error; SD = standard deviation; Tlag = absorption lag time; Vc = central volume of distribution; Vp = peripheral volume of distribution.
Notes: Frel = $(Dose/400)^{-0.495}$ if Dose ≥200 mg, F = $(200/400)^{-0.495}$ if Dose <200 mg. Steady-state CL is the sum of CL/F and induction change in CL; the RSE is not estimated for this parameter as well as other derived parameters. Induction half-life and SD of additive error are fixed. The reference subject is a white female receiving a 400-mg capsule, no CYP3A4 inhibitor, with CrCL of 112 mL/min, and with ALKP of 68 U/L.
Source: Applicant's PopPK report. Table 8 on Page 41.

Figure 24. Goodness of Fit Plots for Final PopPK Model



Source: Applicant's PopPK report. Figure 10 on page 42.
Abbreviations: CWRES, conditional weighted residuals; IWRES, individual weighted residuals; popPK, population pharmacokinetic

Figure 25. VPC Plots After Single Dose (Left) and at Steady State (Right)



Source: Applicant's PopPK report. Figure 12 and 13 on page 43.
Abbreviations: VPC, visual predictive checks

The final model equations are as follows:

Figure 26. Model Equations

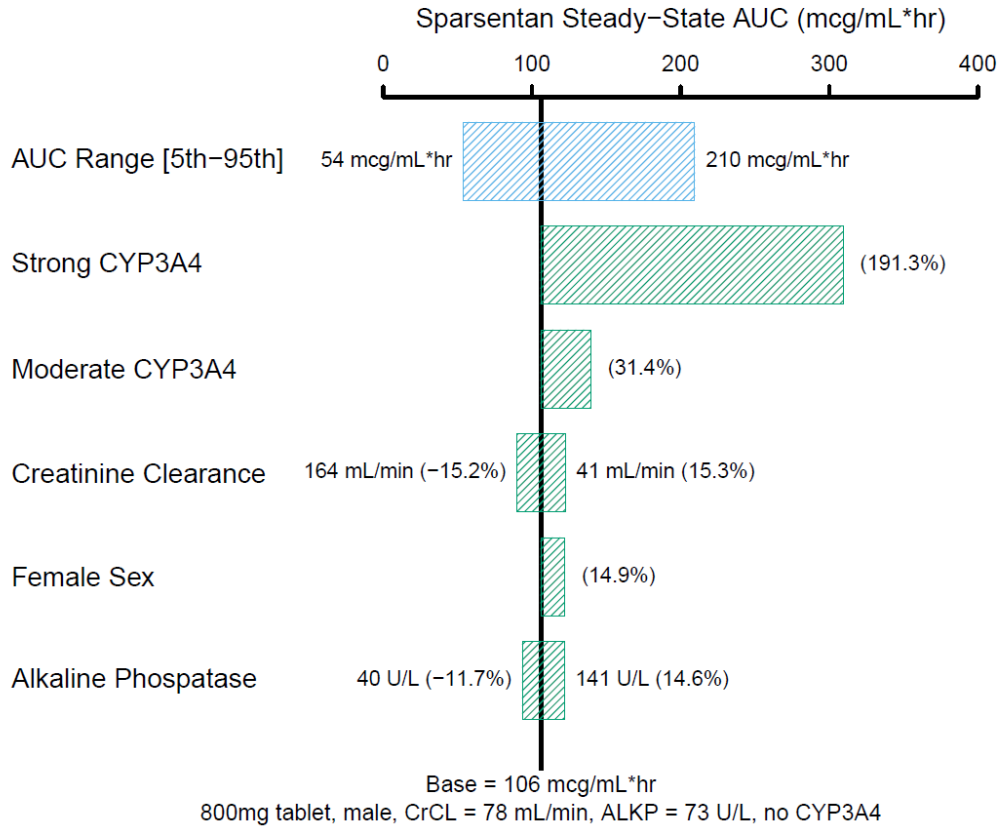
CL/F	=	$(3.88 + 1.23 \times (1\text{-}\exp(-k_{1/2,\ induction} \times Day)))$ $\times (ALKP/68)^{-0.208} \times (CrCL/112)^{0.222}$ ($\times \exp(-0.273)$ if moderate CYP3A4) ($\times \exp(-1.069)$ if strong CYP3A4) ($\times \exp(0.139)$ if Male) / F_{rel}
Vc/F	=	49.3 ($\times \exp(0.309)$ if Black or African American) ($\times \exp(0.265)$ if Asian) / F_{rel}
F_{rel}	=	$(Dose/400)^{-0.495}$ if Dose >200 mg $(200/400)^{-0.495}$ if Dose ≤200 mg
KA	=	0.740 ($\times \exp(-0.306)$ if tablet) ($\times \exp(0.080)$ if crushed tablet)
T_{lag}	=	0.32 ($\times \exp(-0.269)$ if tablet) ($\times \exp(-1.175)$ if crushed tablet)

Here, $k_{1/2,\ induction}$ is the rate constant of induction during a multiple-dose regimen; it is fixed to $\log(2)/T_{1/2,induction}$. F_{rel} is the relative bioavailability, and T_{lag} is the absorption lag time. ALKP and CrCL are baseline alkaline phosphate and CrCL values, respectively.

Source: Applicant's PopPK report. Equations on page 40.
Abbreviations: CL/F, apparent clearance; CrCL, creatinine clearance; CYP, cytochrome P450 isoenzyme; KA, constant; T_{lag}, lag time; Vc/F, apparent volume of distribution

The magnitudes of covariate effects are presented in Figure 27. The similar figures with steady-state C_{max} and C_{min} shows similar trend (not presented in this review). Other covariates, such as population (subjects with FSGS versus HV), age, weight, serum aspartate aminotransferase, serum alanine aminotransferase, total bilirubin, albumin, total protein have no significant effect on the apparent clearance or apparent volume of distribution of sparsentan. No effect of other comedications ((b) (4) P-gp inhibitor, (b) (4), mild CYP3A4 inhibitor) was detected.

Figure 27. Effects of Covariates on Steady-State PK Exposures



Source: Applicant's PopPK report. Figure 15 on page 48.
Abbreviations: ALKP, alkaline phosphatase; AUC, area under the concentration-time curve; CrCL, creatinine clearance; CYP, cytochrome P450 isoenzyme; PK, pharmacokinetic

External Validation of PopPK Model With PK Data From PROTECT

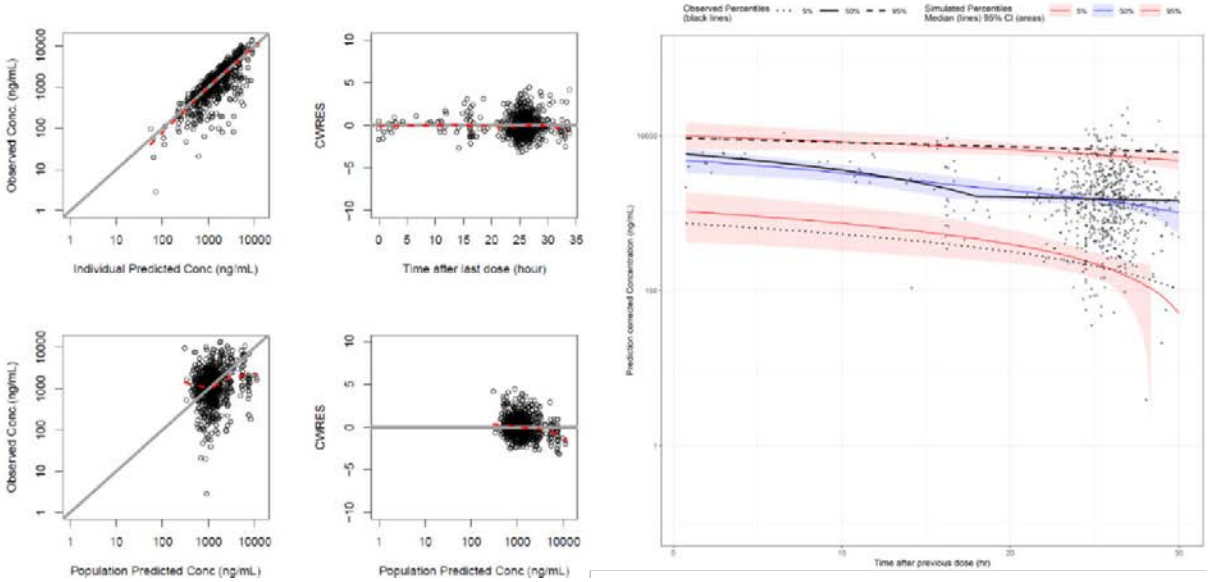
The PopPK model developed based on the PK results from subjects with HV and FSGS described above was evaluated externally using the PK data in IgAN patients in PROTECT. The PROTECT PK data are comprised of trough PK samples collected from 174 IgAN subjects at Weeks 6, 12, 48, 70, 94, and at Week 110/EOT/ET. After 19 samples were excluded from the modeling analysis (12 samples were BLQ and seven samples were with missed reference dosing time), resulting in the data consisting of 679 PK samples from 174 subjects.

A typical subject in the PK analysis dataset was a 46-year-old, white, male subject weighing 83 kg, with mildly impaired renal function. Compared to FSGS patients, there were more male subjects (69.5%) and more subjects with mild renal impairment (RI) (37.4%) or moderate RI (35.6%) in the IgAN population. There were also more Asian subjects (35.6%) in the IgAN population. The median [range] age was 46.0 [18.0, 73.0] and the median [range] weight was 83.1 [48.5, 174].

The previous PopPK model was rerun with the PROTECT PK results using the option of maximum evaluation of 0 in NONMEM to obtain post hoc estimate of the PK parameters in subjects with IgAN. The goodness of fit (GOF) plots and prediction-corrected visual predictive checks (pcVPC) of the PROTECT PK are presented in Figure 28. Numerical predictive check

results indicate 6.8% of the observations were above the 95th percentile of the model predictions. There was 52.6% of the observed PK above the 50th percentile.

Figure 28. GOF Plots (Left) and pcVPC (Right) of PK Data From PROJECT



Source: Applicant's PROTECT PopPK/E-R report. Figure 4 and 5 on page 33-34.
Abbreviations: GOF, goodness of fit; pcVPC, prediction-corrected visual predictive checks; PK, pharmacokinetic

Reviewer's Comments

Population PK analysis

The Applicant's final model developed based on PK data from HV and FGSG patients is acceptable to characterize sparsentan PK and adequately describes the observed PK data. The parameters were estimated with acceptable precisions (%RSE <20%) for the parameters for CL at single dose and at steady state, and dose-dependent bioavailability. The GOF plots by dose levels and by time do not show any obvious bias. While the visual predictive check plots indicate an underprediction in single dose PK of DUET study, the PopPK model generally captures the central tendency and variability of the observed PK data at steady-state and those with single dose PK in healthy subjects. The covariate analysis is acceptable. The summary for relevant covariate effects is following:

- **Body weight:** Body weight was not significant in covariate search. No apparent trend was noted in the ET_A for CL or Vc and body weight.
- **Renal impairment:** PK exposures are expected to increase with decreasing renal function: 15.2% decrease in AUC with CrCL of 164 mL/min (95th percentile) and a 15.3% increase with CrCL of 41 mL/min (5th percentile). Dose adjustment based on CrCL is not deemed necessary, which is further supported by the exposure-response (E-R) analysis (refer to Section 14.5.2.1). A limited number of patients with severe RI (n=3) was included in the PopPK analysis, hence the PopPK analysis cannot provide any meaningful inference on the impact of severe RI on sparsentan PK.
- **Acid reducing agents:** Applicant's PopPK model is not adequate to characterize the impact of acid reducing agents (ARAs) on sparsentan PK and to inform the need for dose adjustment

based on coadministration of ARAs. Applicant's PopPK analysis treated ARA co-administration status as a binary covariate for each patient and did not account for medication history prior to study entry and dosing records for concomitant ARAs, which are critical to capture the DDI effect on absorption phase and subsequently on relative bioavailability. Such data include classes/types of ARAs, dose level, dose frequency, formulation, timing of ARA administration relative to sparsentan administration, route of administration, start and stop date and clock time, duration, dose modification, etc.

- **CYP3A4 inhibitors:** The estimates for DDI effect with moderate and strong CYP3A4 inhibitors are predominantly coming from the inclusion of the DDI study. The PopPK modeling did not account for dosing information and time-dependent effects of co-administered CYP3A4 inhibitors and hence, the estimates for DDI effect size are considered less informative.

Adequacy of PopPK Analysis to Describe PK in IgAN Patients

The PK data from subjects with IgAN were not included in PopPK model development nor covariate analysis but were used for external validation of the previously developed model. Therefore, the reviewer conducted a sensitivity analysis by refitting the model and re-estimating parameters using the combined dataset (subjects with HV, FSGS, or IgAN). The parameter estimates are consistent with the PopPK model with subjects with HV or FGSG. No apparent bias in the GOF plots was noted. The updated ET_A-ET_A plots or ET_A-covariate relationships do not indicate obvious model misspecification. ET_A shrinkages are acceptable (<30%) for CL/F and Vc/F. Also, with Applicant's external validation, the diagnostic plots, pcVPC, and numerical predictive check results suggest that the final PopPK model adequately describes the observed PK data of IgAN patients from PROTECT. The pcVPC suggested that the model predictions are in line with the central tendency and variability of the observed PK in subjects with IgAN in PROTECT. The reviewer agrees that the model parameters can be used to derive individual predicted exposures for E-R analysis for IgAN patients in PROTECT study.

14.5.2. Summary of Applicant's Exposure-Response Analysis

14.5.2.1. Exposure-Response for Efficacy

Exposure-Efficacy Population

The E-R efficacy dataset includes data from 135 sparsentan-treated subjects from PROTECT for whom valid Week 36 efficacy endpoints and individual predicted PK parameters were available. The median [range] age and body weight in the E-R efficacy population was 48 [18, 73] years and 82.5 [48.5, 174] kg. The median [range] estimated glomerular filtration rate (eGFR) were 51 [25, 128] mL/min/1.73 m^2. The majority were male subjects (67.4%), and white (57%) or Asian (40.7%). The majority (88.1%) of the subjects had a normotensive blood pressure and 26.7% of the subjects had a urine protein to creatinine (UP/C) ratio of >1.75 g/g. Antihypertensive drugs were used by the majority of the patients, while immunosuppressant drugs, histamine-2 (H2) blockers, and nonsteroidal anti-inflammatory drugs (NSAIDs) were used by less than 15% of the subjects.

Exposure-Efficacy Analysis Variables

The E-R analysis for efficacy was conducted for the change from baseline in UP/C at Week 36. The median [range] UP/C was 1.24 [0.240, 4.22] at baseline and 0.694 [0.0560, 4.39] at Week 36. The steady-state AUC calculated using average dose up to Week 36 (AUC_{ss}) was used as the exposure metric for E-R efficacy. The median [range] AUC_{ss} was 111 [38.7, 347] µg·h/mL.

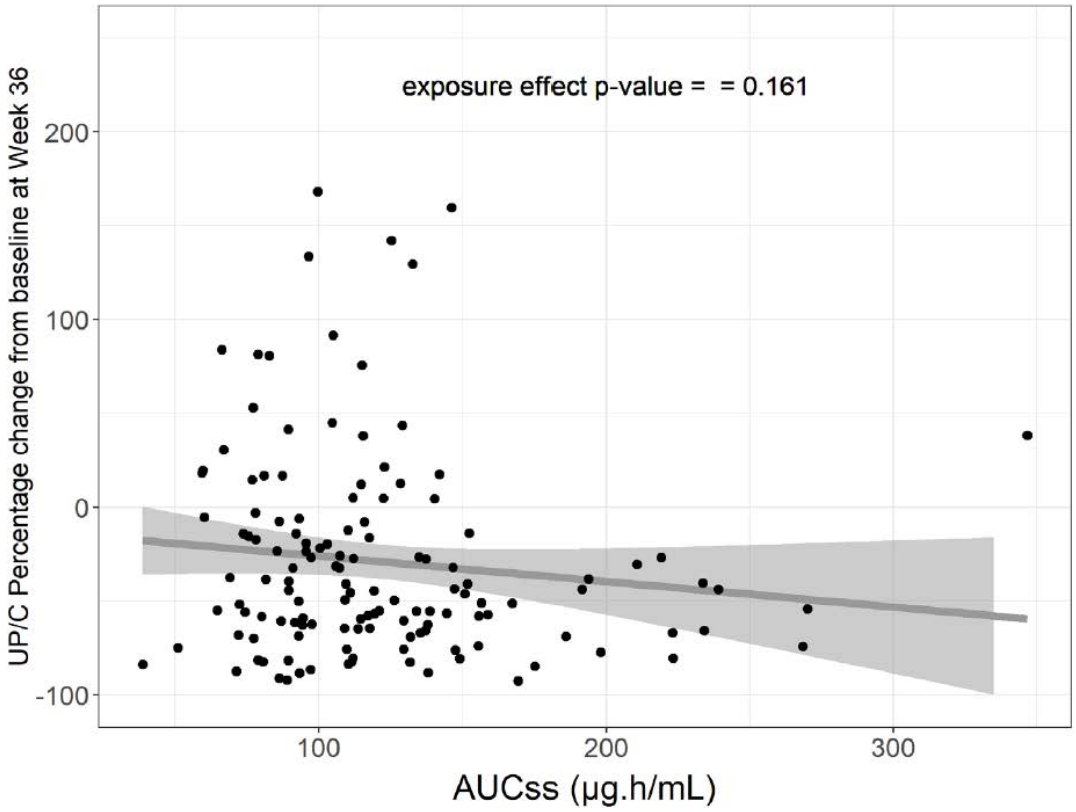
Covariates

The following covariates were evaluated in the ER analyses: body size (weight, BMI, or BSA), age, sex, race, total urine protein (categorical: >1.75 g/day versus ≤1.75 g/day), eGFR (categorical: ≥90, 60 to 89, 45 to 59, 30 to 44, or continuous), hypertensive (systolic blood pressure ≥140 mmHg and DBP ≥90 mmHg) versus normotensive, and concomitant medications (antihypertensives, immunosuppressive agents, NSAIDs, histamine-2 blockers).

Results

The univariate E-R relationship suggests a nonsignificant (p>0.05) linear trend between a greater reduction in UP/C at Week 36 and an increasing exposure (Figure 29). A backward elimination step starting from the full covariate model, keeping exposure, identified baseline eGFR as a significant covariate for the percentage change from baseline in UP/C at Week 36. Upon inclusion of baseline eGFR, the effect of exposure remained nonsignificant (p>0.05).

Figure 29. Linear Regression Fit Between AUC_{ss} and %CFB in Urine Protein to Creatinine Ratio at Week 36



Source: Applicant's PROTECT PopPK/E-R report. Figure 11 on page 45.
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; UP/C, urine protein to creatinine ratio

Table 96. Final E-R Model for % CFB in Urine Protein to Creatinine Ratio at Week 36

Term	Estimate	95% CI	P Value
(Intercept)	-4.44	-28.7, 19.8	-
Slope of AUC_{ss}, (ug.h/mL)	-0.165	-0.349, 0.019	0.078
Slope of eGFR, centered to median value of 51 mL/min/1.73 m^2	-0.593	-0.94, -0.246	0.0009

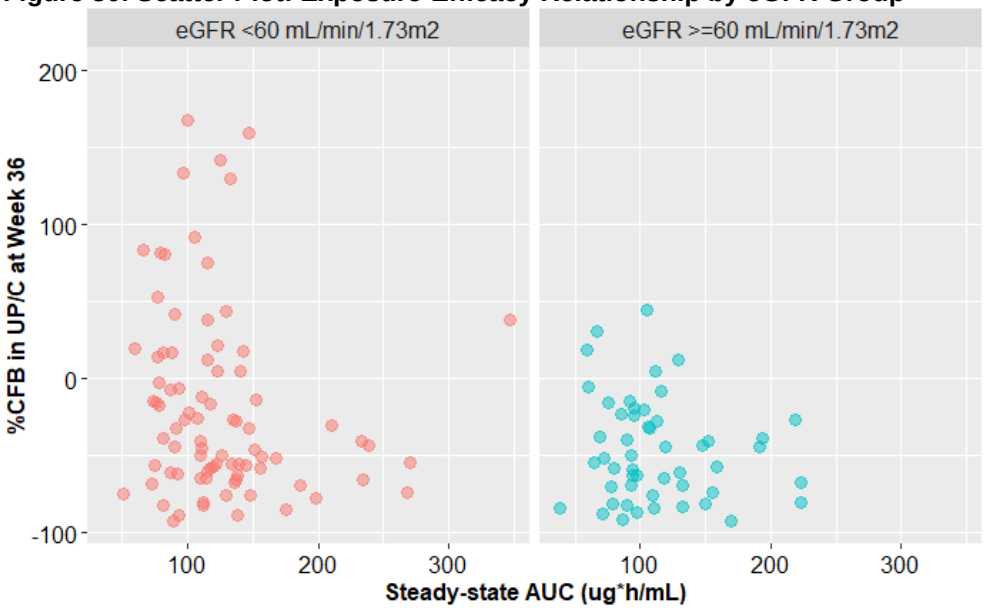
Source: Applicant's PROTECT PopPK/E-R report. Table 13 on page 46.
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; eGFR, estimated glomerular filtration rate; E-R, exposure-response; UP/C, urine protein to creatinine ratio

Reviewer's Comment

The Applicant's conclusion that there is no apparent E-R relationship seems reasonable. Using the exposure metrics derived based on the average dose during the treatment also may be reasonable. However, it should be noted that the presented E-R relationship may be confounded by titration and is largely based on a single dose level. Overall, 94.8% of subjects were titrated to the target dose and 26 subjects (12.9%) in the sparsentan treatment group had dose reductions after titration to the target dose. The mean of the averaged dose for E-R population was 363 mg (93% of the protocol dose of 200 mg × 2 weeks and 400 mg × 34 weeks).

While the univariate and the multivariate analysis does not suggest a statistically significant E-R relationship, the data suggest that lower PK exposures (e.g., AUC_{ss} <150 µg*h/mL) may be associated with a higher UP/C (undesired effect) in some patients (Figure 29). As reported in the subgroup analysis for efficacy (case study report for PROTECT, Figure 8, page 89), this trend appears to be largely driven by patients with baseline eGFR <60 mL/min/1.73 m^2 (Figure 30). Given that the PK exposures are expected to be higher in those with impaired renal function, the available data do not support a dose adjustment (increase or decrease) in patients with moderate RI.

Figure 30. Scatter Plot: Exposure-Efficacy Relationship by eGFR Group



Source: FDA reviewer's plot.
Abbreviations: AUC, area under the concentration-time curve; CFB, change from baseline; eGFR, estimated glomerular filtration rate; UP/C, urine protein to creatinine ratio

14.5.2.2. Exposure-Response for Safety

Exposure-Safety Population

The E-R dataset for safety included data from 174 out of 202 sparsentan-treated subjects with IgAN from the PROTECT study who were in the safety analysis dataset and also had post hoc PK parameters. The AE rates in the sparsentan-treated subjects were similar in subjects with or without PK measurements. The median [range] age and body weight in the exposure-safety analysis dataset was 46 [18, 73] years and 83.1 [48.5, 174] kg. The majority of patients were male (69.5%), and white (62.1%) or Asian (35.6%). The majority of the subjects had a normotensive blood pressure and 26.4% of the patients had a UP/C greater than 1.75 g/g. Most subjects had mild (24.1%) or moderate (55.2%) renal impairment based on eGFR. Antihypertensive drugs were used by 66.1% of the patients, and immunosuppressant drugs were used in 10.9% of the patients, while H2 blockers and NSAIDs were used by less than 10% of the subjects.

Exposure-Safety Variables

The E-R analyses for safety was conducted for (1) hypotension (any grade), (2) hyperkalemia (any grade), and (3) peripheral edema (grade 3+ as binary endpoint, and worst grade with multiple grades based on edema assessment scores described in PROTECT). Hypotension and hyperkalemia were analyzed as binary endpoints. Event time was the first occurrence of the adverse event. Peripheral edema was analyzed as a categorical endpoint (i.e., Grade 0 to 4). The worst AE grade of peripheral edema was captured for each subject. Event time was the first occurrence of the worst AE grade.

The AUC calculated using average dose during the double-blind period (AUC_{AD}) was used as the primary exposure metric for E-R for safety. The AUC using maximum dose (AUC_{MD}) during the double-blind period was used as a secondary exposure metric. The mean AUC_{AD} was 118 μg*h/mL and ranged between 36.1 and 346 μg*h/mL. The mean AUC_{MD} was 124 μg·h/mL and the range was 35.9 to 350 μg·h/mL.

Covariates

The covariates included in the E-R analyses were body size, age, sex, race, total urine protein, eGFR, hypertensive versus normotensive, baseline K+, concomitant medications (antihypertensives, immunosuppressive agents, NSAIDs, H2-blockers).

Results

- **Hypotension of any grade** occurred in 41 of 174 subjects (23.6%) in the E-R safety dataset. Univariate logistic regression analysis shows a numerical trend of decreasing incidence with increasing exposure which was not statistically significant across the observed exposure range. A multivariate logistic regression shows a similar trend and identified concomitant immunosuppressant drugs as a statistically significant covariate, where subjects with concomitant immunosuppressant drugs in the DB period had a higher event rate of hypotension. Sensitivity analysis using AUC_{MD} as an exposure metric showed similar results.
- **Hyperkalemia of any grade** was observed in 18 of the 174 subjects (10.3%) in the E-R safety dataset. Univariate logistic regression analysis shows a statistically significant increase

of event rate with increasing exposure across the observed exposure range (p =0.0283). A backward elimination step starting from the full covariate model, keeping exposure, identified potassium at baseline, high baseline UP/C, and concomitant use of immunosuppressant drugs in the double-blind period as being significant covariates. The exposure effect remained significant (p=0.0472). Based on the final model, the incidence rate of hyperkalemia any grade was predicted to be 7.2%, 8.0%, 9.7%, and 13.4% at the median AUC_{AD} quartiles of Q1, Q2, Q3, and Q4, respectively. Similar results were found for the analysis using AUC_{MD} as an exposure metric.

- **Peripheral edema of Grade 3+** was observed in 3 of 174 subjects in the E-R safety dataset. Univariate logistic regression analysis shows that the ER relationship was not statistically significant (p>0.05). No covariate analysis was conducted due to the very low event rate and nonsignificant E-R relationship.
- **Peripheral edema worst grade** was analyzed as an ordered categorical parameter. Of the 174 subjects in the safety analysis dataset, no events were reported in 116 of the subjects. The number of subjects with a Grade 1, Grade 2, Grade 3, or Grade 4 worst event was 42 (24.1%), 12 (6.9%), 3 (1.7%), and 1 (0.6%), respectively. Univariate ordinal logistic regression shows a statistically significant (p=0.031) increase of event rate with the AUC_{AD} effect across the observed exposure range. Multivariate analysis identified antihypertensive drugs in the double-blind period use as being significant for peripheral edema. The relationship between peripheral edema and AUC_{AD} became nonsignificant (p>0.05) after including this covariate in the final model. Similar results were found in the analysis using AUC_{MD} as an exposure metric.

Reviewer's Comment

The exposure-safety relationship was assessed based on the PK data from largely one dose level. Within the observed exposure range in PROTECT, no clinically meaningful E-R relationships are expected for hypotension of any grade and peripheral edema worst grade. No meaningful inference can be made for peripheral edema of Grade 3+ because of the small event rate (n=3). A significant relationship was observed between sparsentan exposure and the incidence of hyperkalemia of any grade. Monitoring for hyperkalemia is recommended.

14.6. Pharmacogenetics

Not applicable.

14.7. Physiologically Based Pharmacokinetic Analyses Review

Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's physiologically based pharmacokinetic (PBPK) analyses to evaluate the DDI potential as:

- A victim of moderate CYP3A inhibitors, strong and moderate CYP3A inducers, and P-gp inhibition

- An inducer of CYP2B6, CYP2C9, and CYP2C19

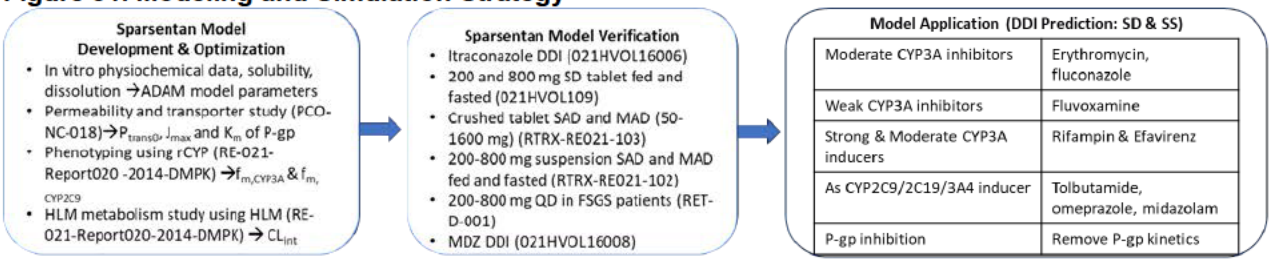
The Division of Pharmacometrics has reviewed the PBPK analyses report (RE-021-0023), the response to FDA's information requests submitted on May 31st (seq 0015, 10-response-clin-pharm), and the modeling supporting files, and concluded that:

- The PBPK analyses are adequate to evaluate the effects of weak and moderate CYP3A inhibitors and moderate CYP3A inducers on the PK of sparsentan. Drug interactions of sparsentan are expected to be
  - Weak with the moderate CYP3A inhibitor fluconazole and erythromycin
  - Minimal with the weak CYP3A inhibitor fluvoxamine
  - Weak with the moderate CYP3A inducer efavirenz
- The PBPK analysis may underpredict the induction effect of rifampin on sparsentan due to a potential increase in biliary excretion via an increase in P-gp expression. However, the potential underprediction has minimal impact on prescribing information since the Applicant proposes avoiding concomitant use of sparsentan with strong CYP3A inducers based on the current prediction result.
- The PBPK analyses are inadequate to evaluate the effects of sparsentan on midazolam due to lack of verification of the CYP3A induction and inhibition parameters of sparsentan. However, minimal effects on midazolam are expected when midazolam is co-administered with 200-mg or 400-mg sparsentan because no effect was observed when midazolam was co-administered with 800-mg sparsentan.
- The analyses were inadequate to evaluate the effects of sparsentan on the exposure of the substrates of CYP2C9 and CYP2C19 due to lack of in vitro to in vivo correlation of CYP2C induction and demonstrated predictive performance using the PBPK modeling approach to predict CYP2C induction.

Methods

Simulations related to evaluation of sparsentan as a victim of CYP3A inhibitors and inducers or as a perpetrator of CYP enzymes were performed using the PK/pharmacodynamic profiles mode in the Simcyp® Simulator (Version 19 Certara, Sheffield, UK). Schemes of the PBPK simulation strategy are shown in Figure 31, which summarizes the studies used for sparsentan model development and verification, and model applications in DDI predictions. The final model input parameters are summarized in Table 97. The sparsentan PBPK model consists of an ADAM absorption model including a MechPeff model, a full PBPK model for distribution and an enzyme kinetics model for elimination. The Simcyp library files itraconazole_Fasted soln, SV-rifampin-MD, SV-Efavirenz, SV-Erythromycin_EC, SV-Fluvoxamine, SV-Fluconazole, Sim-midazolam, SV-Tolbutamide, and SV-Omeprazole were used for DDI simulations without any modification.

Figure 31. Modeling and Simulation Strategy



Source: This flow chart was generated by the reviewer based on the PBPK report re-021-0023
Abbreviations: CYP, cytochrome P450 isoenzyme; DDI, drug-drug interactions; FSGS, focal segmental glomerulosclerosis; SD, single dose; MAD, multiple ascending dose; P-gp, p-glycoprotein; QD, once daily

Table 97. Final Input Parameters in the Sparsentan Model

PARAMETER	Sparsentan	Reference
Physchem and Blood Binding		
Molecular Weight	592.76	Report RE-021 Characterisation
log P	4.26	Report RE-021 Characterisation
Compound type	Ampholyte	Report RE-021 Characterisation
pKa	5.31(acid), 4.09 (base)	Report RE-021 Characterisation
B:P	0.612	RE-021-Report064-2016-DMPK
Fu_p	0.009	RE-021-Report064-2016-DMPK
Main binding protein		RE-021-Report064-2016-DMPK
Distribution		Full body 14-organ distribution model
V_{SS} (L/kg)	0.16	Predicted (Method 2) with PerL model
Absorption		ADAM model
Formulation type	Solid IR formulation	
Dissolution model	Mass Balance Only	
GI transit model	Segregated transit	
Mean Particle Size (um)	[redacted]	Measured
First order disintegration/particle release rate constant (h^{-1})		Estimated by modelling *in vitro* dissolution data for the corresponding formulation
Intrinsic solubility (mg/mL)		SIVA solubility model
$LogK_{m:w,unionised}$	4.61	SIVA solubility model
$LogK_{m:w,\ ionised}$	3.39	SIVA solubility model
fu_{gut}	0.022	Predicted by Simcyp Simulator
Critical Supersaturation Ratio	10	Simcyp default
Precipitation rate constant (h^{-1})	4	Simcyp default
Permeability model	MechPeff model	
P_{trans0} (x10^{-4} cm/s)	54.4	
$P_{eff,max}$ (jej) (x10^{-4} cm/s)	6.29	
P-gp J_{max} intestine and canalicular membrane (pmol/min/pmol)	[redacted]	(b) (4)
P-gp K_m intestine and canalicular membrane (µM)		

Elimination		
CYP3A4 CL_{int} (µl/min/pmol)	0.497	CYP CL_{int} calculated by assigning HLM CLu_{int} based on enzyme contribution and abundance
CYP2C9 CL_{int} (µl/min/pmol)	0.212	
CYP2E1 CL_{int} (µl/min/pmol)	0.00275	
Additional HLM CL_{int} (µL/min/mg)	NA	
Interaction		(b) (4)
CYP3A4 K_i (µM)	[redacted]	
CYP3A4 $K_{app,u}$ (µM)		
CYP3A4 k_{inact} (h^{-1})		
CYP3A4 Ind_{max} (fold-change)		
CYP3A4 $IndC_{50}$ (µM)		
CYP3A4 gamma		
CYP2C9 Ind_{max} (fold-change)		
CYP2C9 $IndC_{50}$ (µM)		
CYP2C9 gamma		
CYP2C19 Ind_{max} (fold-change)		
CYP2C19 $IndC_{50}$ (µM)		
CYP2C19 gamma		

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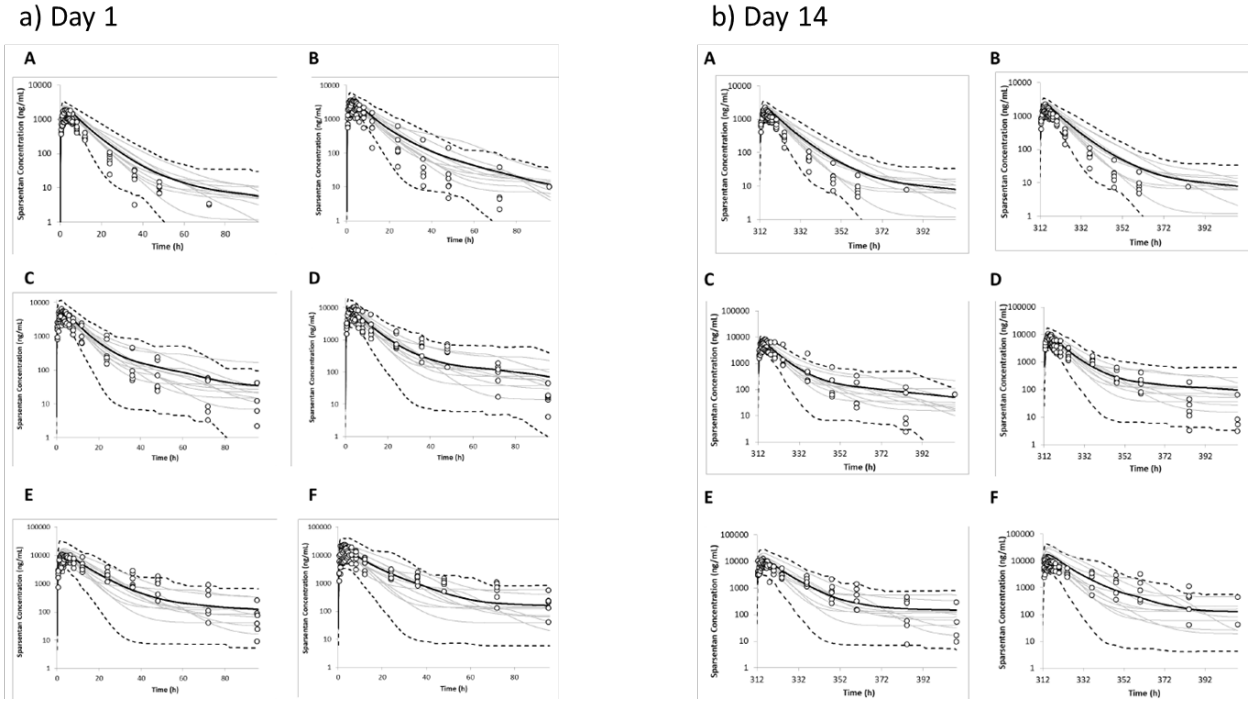
Source:

Results

Can the PBPK Model Adequately Describe the PK Profiles of Sparsentan?

Yes. The sparsentan PBPK models could reasonably well describe sparsentan PK following administration of single and multiple doses of sparsentan in healthy subjects in the fasted state, but there is a trend towards overprediction at lower doses and underprediction at higher doses (Figure 32 and Table 98, Table 99, Table 100). The model largely captured the effects of food on the AUC of sparsentan but underestimated the food effects on C_{max} at 800-mg dose and above, which have minimal effect on the evaluation of sparsentan interaction since the therapeutic doses are 200-mg and 400-mg.

Figure 32. Simulated and Observed PK Profiles Following Oral Administration of Once Daily Doses of Sparsentan in the Fasted State in Healthy Subjects



Source: Figures 21 and 25 in the PBPK report (re-021-0023)
Semilog of simulated (lines) and observed (empty circles) sparsentan plasma concentrations following oral administration of (A) 50, (B) 100, (C) 200, (D) 400, (E) 800, or (F) 1600 mg sparsentan in the fasted state. Solid lines (dark black line represents population mean across all virtual studies, grey continuous lines represent each virtual study) are simulated means and dashed lines are corresponding 5th and 95th percentiles.
Abbreviations: PK, pharmacokinetics

Table 98. Simulated and Observed PK Parameters Following Oral Administration of Single Doses of Sparsentan in Healthy Subjects in the Fasted and Fed States, Study RTRX-RE021-103 (Crushed Tablet)

Dose (mg)	Trial	N	fasted			fed			fed/fasted		
			AUC_{inf} (ng*h/mL)	t_{max} (h)	C_{max} (ng/mL)	AUC_{inf} (ng*h/mL)	t_{max} (h)	C_{max} (ng/mL)	AUC_{inf}	t_{max}	C_{max}
50	Predicted	6	22256	2 39	2181	23655	3.71	2255	1.06	1.55	1.03
	Observed	6 (× 10)	12050	3 28	1391	10490	4.24	1351	0.87	1.29	0.97
	pred/obs		1.8	0.7	1.6	2.3	0.9	1.7	1.2	1.2	1.1
100	Predicted	6	37649	2.75	3350	41825	4.02	3738	1.11	1.46	1.12
	Observed	6 (× 10)	25090	3 25	2846	19960	4.26	2314	0.80	1.31	0.81
	pred/obs		1 5	0.8	1.2	2.1	0.9	1.6	1.4	1.1	1.4
200	Predicted	6	58658	2 37	5521	68501	4.11	6340	1.17	1.73	1.15
	Observed	6 (× 10)	45010	2 51	4634	39510	4.26	4951	0.88	1.70	1.07
	pred/obs		1 3	0.9	1.2	1.7	1.0	1.3	1.3	1.0	1.1
400	Predicted	6	83978	2.00	7424	101003	4.38	8388	1.20	2.19	1.13
	Observed	6 (× 10)	83000	3 50	6966	71590	5.61	8083	0.86	1.60	1.16
	pred/obs		1.0	0.6	1.1	1.4	0.8	1.0	1.4	1.4	1.0
800	Predicted	6	128578	1.81	10919	156836	4.16	12133	1.22	2.30	1.11
	Observed	6 (× 10)	161100	4 25	8623	176600	6.20	17300	1.10	1.46	2.01
	pred/obs		0.8	0.4	1.3	0.9	0.7	0.7	1.1	1.6	0.6
1600	Predicted	6	150473	1 99	12334	208270	4.35	15594	1.38	2.19	1.26
	Observed	6 (× 10)	206300	5.00	12260	310700	6.00	30820	1.51	1.20	2.51
	pred/obs		0.7	0.4	1.0	0.7	0.7	0.5	0.9	1.8	0.5

Source: Tables 10 and 11 in the PBPK report (re-021-0023)
Abbreviations: AUC_{inf}, area under the curve to infinity; C_{max}, maximum plasma concentration; PK, pharmacokinetic; t_{max}, time to maximum plasma concentration

Table 99. Simulated and Observed PK Parameters Following Oral Administration of Single Doses of Sparsentan in Healthy Subjects in the Fasted and Fed States, From the Food Effect Study 021HVOL109 (Tablet)

200 mg single dose

Prandial State	PK Parameter	Observed (n=15)		Simulated (n=10x15=150)		Predicted To Observed Ratio	
		C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	C_{max} Pred/Obs	AUC_{last} Pred/Obs
Fasted	geometric mean	5150	58100	4660.22	51805.79	0.90	0.89
	arithmetic mean	5380	69400	5246.55	59962	0.98	0.86
	N	15	15	150	150		
Fed	geometric mean	6280	50500	5482.38	61672.26	0.87	1.22
	arithmetic mean	6520	62100	5919.09	69314.13	0.91	1.12
	median	6210	43100	5717.15	58733.02	0.92	1.36
Fed /Fasted Ratio	geometric mean	1.22	0.87	1.18	1.19	0.96	1.37
	arithmetic mean	1.21	0.89	1.13	1.16	0.93	1.29

800 mg single dose

Prandial State	PK Parameter	Observed (n=15)		Simulated (n=10x15=150)		Predicted to Observed Ratio	
		C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	C_{max} (ng/mL)	AUC_{last} (h*ng/mL)	C_{max} Pred/Obs	AUC_{last} Pred/Obs
Fasted	geometric mean	7650	138000	8754.60	98447.71	1.14	0.71
	arithmetic mean	8350	160000	10839.45	126027.56	1.30	0.79
	N	15	15	150	150		
Fed	geometric mean	16200	169000	10097.28	127691.65	0.62	0.76
	arithmetic mean	16800	185000	12060.32	164099.09	0.72	0.89
	Median	16400	154000	9542.94	114662.91	0.58	0.74
Fed /Fasted Ratio	geometric mean	2.12	1.22	1.15	1.30	0.54	1.06
	arithmetic mean	2.01	1.16	1.11	1.30	0.55	1.13

Source: Tables 8 and 9 in the PBPK report (re-021-0023)
Abbreviations: AUC_{last}, area under the curve to the last quantifiable time point; C_{max}, maximum plasma concentration; PK, pharmacokinetic

Table 100. Simulated and Observed PK Parameters Following Oral Administration of Single Doses of Sparsentan in Healthy Subjects in the Fasted and Fed States, Study Protocol RTRX-RE021-102 (Suspension)

Dose (mg)	Trial (Number of Subje cts)	Faste d		Fe d		Fed/Fasted	
		AUC_{last} (ng*h/mL)	C_{max} (ng/mL)	AUC_{last} (ng*h/mL)	C_{max} (ng/mL)	AUC_{last}	C_{max}
200	Sim (15x10)	39588	3433	53253	4823	1.35	1.40
	Obs (15)	23700	2200	38200	4600	1.61	2.09
	Sim/Obs	1.67	1.56	1.39	1.05	0.83	0.67
400	Sim (16x10)	50957	4336	78602	6857	1.54	1.58
	Obs (16)	45900	3870	63000	6920	1.37	1.79
	Sim/Obs	1.11	1.12	1.25	0.99	1.12	0.88
800	Sim (15x10)	64669	5271	109789	8850	1.70	1.68
	Obs (15)	64300	4970	126000	11900	1.96	2.39
	Sim/Obs	1.01	1.06	0.87	0.74	0.87	0.70

Source: Table 14 in the PBPK report (re-021-0023)
Abbreviations: AUC_{inf}, area under the curve to infinity; C_{max}, maximum plasma concentration; PK, pharmacokinetic;

Can PBPK Analyses Predict the Effects of Strong and Moderate CYP3A Inhibitors and Inducers on the PK of Sparsentan?

Yes. The sparsentan PBPK model could be used to predict the effects of CYP3A inhibitors and inducers. The predicted effects of fluconazole and erythromycin on sparsentan exposure were similar to the observed effect of cyclosporine, a moderate CYP33A inhibitor (Table 101). Because efavirenz is not expected to induce intestinal P-gp (Mouly et al. 2002), the sparsentan PBPK model could also be used to predict the induction effect of efavirenz, and efavirenz was predicted to have minimal induction effect on sparsentan PK (Table 101). The effect of rifampin on sparsentan exposure may be underpredicted, which has minimal impact on the product labeling for the reasons detailed below.

Reviewer's Comments

- To predict the effects of moderate CYP3A inhibitors and inducers on sparsentan PK, the fraction metabolized by CYP3A ($f_{m,CYP3A}$) is one of the key parameters that needs to be verified in the sparsentan PBPK model. The relative contribution of CYP3A4, CYP2C9, and CYP2E1 in hepatic metabolism was estimated to be 81.3%, 18.5%, and 0.2%, respectively, based on the metabolites identified in HLMs and their formation by recombinant CYP3A4, CYP2C9 and CYP2E1 (RE-021-Report020-2014-DMPK). Thus, the $f_{m,CYP3A}$ value of 0.81 was applied to the sparsentan PBPK mode. This model could reproduce the inhibitory effect of itraconazole on sparsentan which was not affected by the inhibition or induction parameters incorporated in the sparsentan PBPK model (Table 101). Therefore, the $f_{m,CYP3A}$ value is considered verified.
- Itraconazole is an inhibitor of CYP3A and P-gp. Sparsentan is a substrate of CYP3A and P-gp. The observed effect of itraconazole on sparsentan PK could be due to inhibition of both CYP3A and P-gp. (b) (4), the kinetic parameters of the P-gp could not be verified. Sparsentan has a high permeability with a passive permeability value ranging from 13 to 20× 10^{-6} cm/s in the presence of the P-gp inhibitor verapamil (Study PCO-NC-018). The human ADME study showed that the fraction absorbed of sparsentan was close to 90%. These data suggest that P-gp may play a minimal role in sparsentan absorption following oral administration of a single 400-mg dose of sparsentan. Therefore, the observed effect of itraconazole on sparsentan PK is most likely due to CYP3A inhibition. In addition, the reviewer conducted a PBPK simulation to evaluate the effects of itraconazole on sparsentan following multiple doses of itraconazole and sparsentan using the submitted sparsentan PBPK model. The result showed that the inhibitory effects of itraconazole on sparsentan at steady state are similar to those observed following a single dose of itraconazole (Table 101).
- To assess the potential of a P-gp inhibitor on sparsentan exposure, the Applicant (b) (4) . This approach is not acceptable (b) (4) . However, a P-gp inhibitor is expected to have little effect on sparsentan PK because, as mentioned above, P-gp may play a minimal role in sparsentan absorption.
- Predicted effect of rifampin on sparsentan: Rifampin is known to decrease the exposure of P-gp substrates by P-gp induction. Even though P-gp plays a minimal role in sparsentan

absorption due to its high permeability, increase in P-gp expression by rifampin could potentially increase hepatic biliary clearance of sparsentan. Therefore, the effect of rifampin on sparsentan exposure may be underpredicted (b) (4) . This potential underprediction will have no impact on dosing recommendation since the Applicant proposes avoiding concomitant use of sparsentan with strong CYP3A inducers.

Table 101. Predicted and Observed Effects of CYP3A Perpetrators on Sparsentan PK Following Co-Administration of Multiple-Dose CYP3A Perpetrators With 200-mg Sparsentan in Healthy Subjects

CYP3A Perpetrators	Perpetrator Dosing Regimens	Sparsentan Dosing Day	C_{max} Ratio	AUC_{0-inf} Ratio	Trials
Strong CYP3A inhibitors	Itraconazole 200 mg capsule BID on D1 then QD 9d	D6	1.25	2.74	Observed
	200 mg capsule BID on D1 then QD 9d	D6	1.37	3.28	Simulated with full model
			1.37	3.01	Simulated without CYP3A TDI
			1.35	3.12	Simulated without CYP3A induction
	200 mg capsule BID on D1 then QD 9d	10d	1.55	2.37	Simulated*
Moderate CYP3A inhibitors	Fluconazole 200 mg QD 17d	D15	1.24	2.18	Predicted
		15d	1.39	2.07	Predicted
	Erythromycin 500 mg QID 17d	D15	1.22	1.93	Predicted
	Erythromycin 500 mg QID 15d	15d	1.25	1.60	Predicted
Weak CYP3A inhibitors	Fluvoxamine 36.65 mg QD 17d	D15	1.04	1.09	Predicted
	Fluvoxamine 36.65 mg QD 15d	15d	1.05	1.07	Predicted
Strong CYP3A inducer	Rifampin 600 mg QD 17d	D15	0.73	0.42	Predicted
	Rifampin 600 mg QD 15d	15d	0.77	0.53	Predicted
Moderate CYP3A inducer	Efavirenz 600 mg QD 17d	D15	0.81	0.58	Predicted
	Efavirenz 600 mg QD 15d	15d	0.88	0.73	Predicted

Source: Tables 18 - 27 in the PBPK report, Table 3 in the 10-response-clin-pharm.pdf and reviewer's analysis
Values are geometric mean; * reviewer's analysis
Abbreviations: AUC_{0-inf}, area under the curve from zero to infinity; BID, twice daily; C_{max}, maximum plasma concentration; CYP, cytochrome P450 isoenzyme; QD, once daily; QID, four times daily; PK, pharmacokinetics; TDI, time dependent inh bition

Can PBPK Analyses Be Used To Estimate the Effects of Sparsentan on Substrates of CYP2Cs and CYP3A?

The Applicant's analyses of CYP2C induction potential can only be considered as a risk assessment and should not be used as labeling information for the reasons detailed below.

Effects of Sparsentan on CYP3A Substrates

In vitro, sparsentan is a time-dependent inhibitor and inducer of CYP3A. Following oral administration of single and multiple doses of sparsentan, sparsentan exposure increased dose proportionally. The clinical DDI study of sparsentan (800 mg) with midazolam showed that sparsentan had no effect on midazolam exposure (Study 021HVOL16008). To confirm that the observed linear PK and no effect of sparsentan on midazolam were due to the net effect of inhibition and induction but not due to lack of DDI potential of sparsentan, the Applicant was requested to simulation sparsentan PK and its interaction with midazolam without the CYP3A induction or inhibition parameters of sparsentan. The predicted results showed that sparsentan was an inhibitor and inducer of CYP3A (Table 102), suggesting that the absence of an effect of sparsentan on midazolam exposure was likely due to the net effect of inhibition and induction of CYP3A.

Successful simulations of clinical PK and midazolam DDI studies demonstrate the utility of the set of interaction parameters used in the sparsentan model including CYP3A induction parameters (Ind_{max}, $IndC_{50}$), CYP3A inhibition parameters (K_i, k_{inact}, K_I) and nonspecific binding parameters (f_{umic} and f_{uinc}) as a whole. However, the available clinical data are insufficient to differentiate the CYP3A inhibition or induction of sparsentan thus the CYP3A inhibition and induction parameters of sparsentan cannot be independently verified, which limits the utility of the sparsentan model to predict the effect of sparsentan on midazolam at different doses of sparsentan. However, since no effect on midazolam exposure was observed following administration of 800-mg of sparsentan, at which dose sparsentan exposure was 2 and 4 times higher than that achieved at 200-mg and 400-mg, respectively, minimal effects on midazolam are expected when midazolam is co-administered with 200-mg or 400-mg sparsentan.

Table 102. Comparison of Simulated Midazolam Interaction Study With Sparsentan in the Presence and/or Absence of CYP3A Induction or Time-Dependent Inhibition Parameters in the Sparsentan PBPK Model

Simulation Type	PK Parameter	Observed			Simulated			Sim/Obs
		Mean	% CI Lower	% CI Higher	Mean	% CI Lower	% CI Higher	
With CYP3A Induction and TDI	AUC Ratio	0.98	0.9	1.08	0.97	0.89	1.06	0.99
	C_{max} Ratio	1.05	0.98	1.14	0.95	0.92	0.99	0.91
Without CYP3A Induction	AUC Ratio	0.98	0.9	1.08	3.19	2.87	3.54	3.25
	C_{max} Ratio	1.05	0.98	1.14	1.57	1.51	1.63	1.49
Without CYP3A4 TDI	AUC Ratio	0.98	0.9	1.08	0.49	0.46	0.51	0.50
	C_{max} Ratio	1.05	0.98	1.14	0.65	0.63	0.67	0.62

CI: 90% confidence interval.

Source: Table 4 in 10-response-clin-pharm.pdf
Abbreviations: CYP, cytochrome P450 isoenzyme; PK, pharmacokinetics; TDI, time dependent inhibition

Effects of Sparsentan on CYP2C Substrates

Currently, there are limited data that show that CYP2C induction could be predicted using induction parameters generated from the in vitro hepatocyte induction studies. PBPK simulations

using the approach described below are only considered as a risk assessment because the in vitro-in vivo correlation for CYP2C induction and the predictive performance of CYP2C induction have not been demonstrated.

In vitro induction parameters are affected by variability in induction response across hepatocyte donors thus often need to be calibrated to the response of the positive control, such as rifampin (RIF), produced in the hepatocytes from the same donor. The calibration is done based on the relationship between rifampin induction of the corresponding CYP enzyme in vitro ($Ind_{max,RIF}$ and $IndC_{50,RIF}$) and in vivo ($Ind_{max,RIF\ in\ vivo}$ and $IndC_{50,RIF\ in\ vivo}$) by using the following equations:

$$\text{Calibrated } Ind_{max,sparsentan} = 1+[(Ind_{max,sparsentan} -1)/(Ind_{max,RIF}-1)]*(Ind_{max,\ RIF,\ in\ vivo} -1)$$

$$\text{Calibrated } IndC_{50,sparsentan} = IndC_{50,sparsentan}/IndC_{50,\ RIF}*IndC_{50,RIF,in\ vivo}$$

In the in vitro CYP2C induction study of sparsentan (Study RE-021-0027), the effects of the positive control rifampin were only investigated at one concentration (20mM) thus its induction parameters ($Ind_{max,RIF}$ and $IndC_{50,RIF}$) in the hepatocytes from the same donor cannot be generated. The fold induction of 20mM rifampin on CYP2C observed in the study RE-021-0027 was assumed to be the maximal response of rifampin ($Ind_{max,RIF}$) and was used in the calibration of the Ind_{max} of sparsentan (re-021-0023). The $IndC_{50}$ of sparsentan was not calibrated (re-021-0023).

The simulated effects of sparsentan on CYP2C substrates are summarized in Table 103. It should be noted that the $Ind_{max,RIF\ in\ vivo}$ for CYP2C19 (SV-Rifampin MD model summary) used by the Applicant to calibrate $Ind_{max,sparsentan}$ underpredicted the effect of rifampin on omeprazole C_{max} and AUC by 5- and 3.7-fold, respectively (SV-Omeprazole model summary), therefore the simulated effect of sparsentan on omeprazole exposure was likely underpredicted. The reviewer simulated the effects of sparsentan on omeprazole using induction parameters of sparsentan calibrated to the induction parameters of rifampin that could reproduced the clinical interaction between rifampin and omeprazole, and the results showed that sparsentan had a potential to reduce omeprazole exposure by half (Table 103). Since sparsentan has potential to induce both CYP2C9 and CYP2C19, clinical DDI studies with substrates of CYP2C9 and CYP2C19 are warranted.

Table 103. Simulated Effects of Sparsentan on the Exposure of CYP2C9 or CYP2C19 Substrates Following Oral Administration of Sparsentan Once Daily

Affected CYP	CYP substrate	sparsentan dose (mg)	AUC Ratio	C_{max} Ratio	Performed by
CYP2C9	tolbutamide	200	0.66	0.88	Applicant
		800	0.58	0.84	Applicant
CYP2C19	Omeprazole	200	0.8	0.85	Applicant
		800	0.82	0.88	Applicant
		200	0.54	0.65	Reviewer
		400	0.5	0.6	Reviewer

Source: Tables 32 and 34 in the PBPK report (re-021-0023) and reviewer's analyses
Sim-Healthy Subject population aged 20 -50 with female ratio of 0.5 was used in these simulations. CYP substrates were given on Day 15 following once daily dosing of sparsentan for 17 days. Geometric mean ratios were reported. Simulations were performed using Simcyp V19.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum plasma concentration; CYP, cytochrome P450 isoenzyme

Conclusions

- The PBPK analyses are adequate to evaluate the effects of weak and moderate CYP3A inhibitors and moderate CYP3A inducers on the PK of sparsentan. Drug interactions of sparsentan are expected to be
  - Weak with the moderate CYP3A inhibitor fluconazole and erythromycin
  - Minimal with the weak CYP3A inhibitor fluvoxamine
  - Weak with the moderate CYP3A inducer efavirenz
- The PBPK analysis may underpredict the induction effect of rifampin on sparsentan due to potential increase in biliary excretion via increase in P-gp expression. However, the potential underprediction has minimal impact on prescription information since the Applicant proposed avoiding concomitant use of sparsentan with strong CYP3A inducers based on the current prediction result.
- The PBPK analyses are inadequate to evaluate the effects of sparsentan on midazolam due to lack of verification of the CYP3A induction and inhibition parameters of sparsentan. However, minimal effects on midazolam are expected when midazolam is co-administered with 200-mg or 400-mg sparsentan because no effect was observed when midazolam was co-administered with 800-mg sparsentan.

The analyses were inadequate to evaluate the effects of sparsentan on the exposure of the substrates of CYP2C9 and CYP2C19 due to lack of in vitro to in vivo correlation of CYP2C induction and demonstrated predictive performance using the PBPK modeling approach to predict CYP2C induction.

15. Trial Design

15.1. Important Study Dates

The PROTECT study is currently on-going; the first patient first visit occurred on December 11, 2018. The preplanned interim data lock, which was scheduled to occur after approximately 280 subjects completed Week 36, occurred on July 30, 2021. Unblinding for the interim analysis (i.e.,

analysis to evaluate for the primary endpoint) occurred on August 6, 2021. On May 26, 2021, the study was fully enrolled (406 subjects).

15.2. Protocol Amendments

The clinical protocol was amended globally five times. An overview of these amendments is provided in the table below.

Table 104. Overview of Integrated Protocol Amendments, Study PROTECT

	Patients Randomized n (%)	Summary of Significant Changes
Amendment 1, 3/7/2019	8 (2)	The Amendment addressed comments from the Agency's Advice Letters dated 1/7/2019 and 1/25/2019, and included the following significant changes: • The protocol was revised to indicate that patients should resume the same treatment regimen that they were on at study entry (i.e., same ACE inhibitor or ARB at the same doses) after completion of the Maintenance phase during Weeks 111 to 114, unless in the investigator's opinion, an alternative treatment approach would be warranted • The protocol was amended to recommend that systemic corticosteroids and/or immunosuppressive therapy for the treatment of IgAN be avoided for the duration of participation in the study. If, in the investigator's opinion, systemic corticosteroid and/or immunosuppressive therapy is warranted, such intervention may be provided in addition to study medication at the discretion of the investigator • The protocol clarified that for sensitivity analyses of the primary endpoint, the multiple imputation approach would be expanded to allow for varying impact of missing data by incorporating a shift parameter in the imputation model and including control-based imputation • The protocol clarified "baseline" proteinuria and eGFR would be defined as the last pretreatment value available prior to the first dose of study medication • The secondary endpoint of rate of change of eGFR from Week 6 to Week 58 was removed from the testing procedure intended to control type I error • The enrollment criteria were modified to remove the exclusion of patients with type 1 diabetes mellitus, uncontrolled type 2 diabetes mellitus, and nonfasting blood glucose >180 mg/dL at Screening • RAAS inhibitors and ERAs were added to the list of prohibited medications during the study
Amendment 2, 5/7/2019	39 (10)	Based on findings from nonclinical and clinical toxicology data, the contraception requirement for male patients was removed
Amendment 3, 3/10/2019	222 (55)	Per recommendation from the DMC, assessments of orthostatic hypotension were added

	Patients Randomized n (%)	Summary of Significant Changes
Amendment 4, 7/13/2020	258 (64)	• An open-label extension period was added • The previously prespecified sample size reassessment after 80% of patients were randomized was removed and instead, the sample size was increased from 280 to 380 patients • Guidance related to COVID-19 was added
Amendment 5, 4/6/2021	360 (89)	The amendment implemented changes that would not be expected to have a major impact on study conduct (e.g., updated information for physical exam and specified measurement of heart rate for orthostatic hypotension detection)

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; AESI, adverse event of special interest; RAAS, renin-angiotensin-aldosterone system; ERA, endothelin receptor antagonist; COVID-19, coronavirus disease 2019; IgAN, immunoglobulin A nephropathy

15.3. Trial Administrative Structure

Data Monitoring Committee

The study has an external independent data monitoring committee (DMC). The DMC membership and responsibilities are defined by a written charter. The responsibilities of the DMC include interim monitoring for safety, monitoring of study conduct, including the recruitment rate, dropouts, and rescue treatment, and making recommendations to the Applicant for future conduct of the study.

Each meeting begins with an open session that Applicant representatives and coordinating investigators could attend. Data presented in the open session could include enrollment data, baseline characteristics, important protocol deviations, and other administrative data. This is followed by a closed session that included only individuals from the DMC and the unblinded DMC support team (Contract Research Organization [(b) (4)]). The unblinded DMC support team includes an independent statistician, programmer, quality control programmer, and senior biostatistics reviewer. Data that could compromise the integrity of the study (e.g., comparative data and/or any unblinded data) are analyzed and discussed only in the closed session.

The committee has met approximately bi-annually and the Applicant has submitted the open meeting minutes[7] for the seven completed DMC meetings. See Table 109 for a summary of the meeting minutes.

See Section 15.5 for information on the administrative structure as relates to preparing the NDA submission and/or blinding related issues.

Independent Statistician

An independent statistician from (b) (4) was responsible for preparation of DMC reports (in the format of tables, figures, listings). This independent statistician was a nonvoting member of the DMC.

[7] In a pre-NDA communication with the Agency, the Applicant stated that given the on-going nature of the study, they would provide the DMC materials and minutes that they have access to at the time of the NDA submission (i.e., the open DMC meeting minutes). The Agency agreed that the Applicant's proposal was reasonable.

Steering Committee

A Steering Committee, including external healthcare providers or other "relevant" individuals, statisticians, and epidemiologists with clinical and/or research expertise relevant to the design and conduct of the PROTECT study, has been responsible for the general oversight of the study, providing scientific advice regarding all aspects of study design, protocol development, conduct, and data collection. The Steering Committee is responsible for scientific decisions impacting study conduct and study delivery. The Applicant submitted minutes for meetings of the Steering Committee.

Institutional Review Board/Independent Ethics Committee

The study is only being conducted at sites where Institutional Review Board/Independent Ethics Committee (IRB/IEC) approval has been obtained. The protocol, investigator's brochure, informed consent forms, proposed advertising material, written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents are provided to the IRB/IEC by the investigator. No drug is released to the site for dosing until written IRB/IEC authorization has been received by Travere (the Applicant) or their designee (i.e., (b) (4)).

15.4. Study Assessments

Baseline Values

All baseline measurements were collected during the screening period and calculated by the central laboratory. The last pretreatment value available prior to the first dose of study medication was used for the baseline measurements for the primary and key secondary endpoints.

Schedule of Assessments

Screening

The screening period lasted up to 28 days prior to randomization. The following assessments were obtained during the screening period: full physical examination, peripheral edema assessment, vital signs, Panel A clinical laboratory assessments (see table below for details), lipid panel, coagulation tests, 24-hour urine collection, pregnancy test, and 12-lead ECG.

Treatment

The treatment period starts on the first day of study drug administration and extends until Week 110. Study visits are conducted at 2, 4, 6, and 12 weeks after randomization and at approximately 12-week intervals thereafter. The following key assessments are obtained during the treatment period (at every visit unless otherwise noted):

- Full physical exam (Day 1, Week 58, EOT), targeted physical examination (except when full physical exam performed)
- Peripheral edema assessment
- Vital signs

- Panel A clinical laboratory assessments (see table below for details) (except Weeks 2 and 4), Panel B clinical laboratory assessments (see table below for details) (Weeks 2 and 4 only)
- Lipid panel (starting at Week 6)
- NT-proBNP (except Weeks 2, 4, 36)
- Renin, aldosterone, and endothelin (Day 1, Weeks 6, 12, 24, 48, 70, 94, and EOT)
- Coagulation tests (Weeks 6, 24, 48, 70, and EOT)
- 24-hour urine collection (except Week 2)
- Pregnancy test

In addition, eGFR (2009 CKD-EPI) is calculated at every visit.

Table 105. Laboratory Parameters, Study PROTECT

	Components	
Parameter	**Panel A**	**Panel B**
Serum Chemistry	Sodium, potassium, chloride, bicarbonate, total protein, albumin, calcium, phosphate, glucose, hemoglobin A1c, cystatin, uric acid, BUN, creatinine, bilirubin (total, direct, indirect), ALT, AST, alkaline phosphatase, gamma glutamyltransferase, creatinine kinase, amylase, lipase	Same as Panel A except for phosphate, hemoglobin A1c, cystatin, and uric acid
Hematology	Red blood cells, hemoglobin, hematocrit, MCV, MCH, MCHC, platelets, white blood cells, white blood cell differential (neutrophils, eosinophils, basophils, lymphocytes, monocytes)	Same as Panel A except for white blood cell differential
Urinalysis	Color, appearance, dipstick (pH, specific gravity, protein, glucose, ketones, bilirubin, blood, urobilinogen, leukocyte esterase)	Same as Panel A

Source: Clinical Study Protocol, Amendment 5
Panel A assessments are conducted at all visits except Weeks 2 and 4. Panel B assessments are conducted at Weeks 2 and 4 only.
Abbreviations: BUN, blood urea nitrogen; ALT, alanine transaminase; AST, aspartate transaminase; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

Follow-up

Following completion of the treatment period, subjects will enter the 4-week follow-up period where study medication will be discontinued and standard-of-care treatment, including the same angiotensin-converting enzyme (ACE) inhibitor and/or ARB treatment regimen the subjects was on at study entry will be used. The following assessments will be obtained at Week 114: targeted physical examination, peripheral edema assessment, vital signs, Panel A clinical laboratory assessments (see table above), lipid panel, NT-proBNP, aldosterone, renin, endothelin, coagulation tests, and 24-hour urinalysis. Estimated glomerular filtration rate will also be calculated at this visit.

15.5. Study Procedures

Randomization

Eligible subjects were randomized within 28 days of the first screening visit in a 1:1 ratio to sparsentan or irbesartan. Randomization was performed through an interactive response technology. Randomization was stratified by eGFR value (30 mL/min/1.73 m^2 to <60 mL/min/1.73 m^2 and ≥60 mL/min/1.73 m^2) and urine protein excretion (≤1.75 g/day and >1.75 g/day).

Blinding and Unblinding

Blinding

The study is double-blinded. investigators, site clinical teams, and clinical research organization teams involved in the routine conduct of the study will remain blinded to initial randomized treatment assignment throughout the study.

Both sparsentan and irbesartan have been provided to study participants as over-encapsulated tablets that are matched and indistinguishable at the level of the (b) (4) capsule shell (Figure 33). Each capsule shell is (b) (4)

Figure 33. Sparsentan and Irbesartan Samples, Study PROTECT

(b) (4)

Unblinding

Details regarding unblinding were specified by the Applicant in the Data Access and Dissemination Plan (dated July 23, 2021). Blinding access levels are defined based on which unblinded content an individual/entity receives and when. The following access levels are defined in the Data Access and Dissemination Plan:

- Full access: Individuals and entities with full access to unblinded data from the interim analysis for Subpart H are defined as those who have access to unblinded data (i.e., summarized and individual patient study data) prior to the interim result press release and through the confirmatory analysis. Individuals with knowledge of this information are in possession of confidential information prior to public release of data and exceeding that which is planned to be in the public domain.
- Knowledge-level access: Individuals and entities informed at this level will have access to data (generally in the form of unblinded summary tables and figures containing no patient-level information) prior to public release of information. Individuals with knowledge of this information prior to public release are in possession of confidential information up to the point of the public release that exceeds that which is planned to be in the public domain.
- Public domain level: Individuals and entities informed at this level will only see information available in the public domain and will receive such information in concert with public release.
- Inform-level access: Individuals and entities informed at this level will be advised of study results (data summaries only) shortly prior to and in conjunction with the timing of the public release of information. Individuals with knowledge of this information will be in possession of confidential information exceeding that which is in the public domain.
- Final access: These individuals and entities are defined as those that have access to unblinded data (i.e., summarized and individual patient-level study data) after the public release. Individuals with knowledge of this information are in possession of confidential information after public release of information and exceeding that which is planned to be in the public domain. These individuals are precluded from participating in subsequent study conduct activities and will not have access to incoming patient-level data from the study following the database lock for the interim analysis for Subpart H.

The table below describes the levels of access for each team involved in the study.

Table 106. Level of Access for Each Team Involved, Study PROTECT

Team	Composition	Level of Access	Responsibilities
Blinded Study Team	Internal: Medical Monitor, Clinical Operations, Data Manager, Programmers, Statistician, Regulatory External: (b) (4) (separate from staff to be unblinded), (b) (4)	Public Domain	Upon unblinding, these team members will remain blinded and will handle the day-to-day responsibilities of the study. These team members will not have access to unblinded data, except what is in the public domain.
Unblinded Biometrics Team (Up to 5 internal individuals)	Internal: Statisticians, Programmers External: Statistical programming vendor	Full	The internal team will provide oversight of the unblinded vendor's work, manage access to unblinded data, and reproduce key results independently.
Unblinded Medical Team (2-3 internal individuals)	Internal: Lead MD, CMO, VP Pharmacovigilance External: DMC, (b) (4) (independent statistical center)	Full	The internal medical team will provide medical interpretation of unblinded data. The DMC will review all unblinded safety and efficacy results. (b) (4) will facilitate the DMC's review.
Unblinded Decision-making Team (9 additional internal individuals, up to 3 KOLs)	Internal: CEO, SVP R&D, CCO, CMO, General Counsel, SVP Investor Relations, VP Pharmacovigilance, VP Regulatory, VP Biometrics, Global Product Strategy, EU Nephrology Lead External: KOL(s)	Knowledge	The Unblinded Decision-Making Team will review available information and decide next steps for business decision making.
Executive Team (3 additional internal individuals)	Travere's Executive Team	Knowledge	The Executive Team will initiate public and financial disclosure preparation.
Board of Directors (9 additional individuals)	Travere's Board	Inform	The Board of Directors will review available information and discuss business decision making and required disclosures.
Public Disclosure Team (3 additional internal individuals)	Internal: SVP Investor Relations, CEO, General Counsel, Regulatory Lead, Biostatistics, VP Biometrics, Medical Lead, CMO, Global Product Strategy, Legal (IP) External: Outside counsel and advisors, small number of designated KOL(s)	Knowledge	The Public Disclosure Team will prepare and approve public disclosure of information.

Expanded Unblinding Team Limited	Internal: Unblinded Biometrics Team, Unblinded Medical Team, SVP R&D, CMO, VP Pharmacovigilance, VP Regulatory, Regulatory Lead, Regulatory Operations, Clinical Pharmacology (as needed) External: Regulatory consultants	Full	The Expanded Unblinding Team Limited will support data presentation and interpretation; and assist with FDA, EMA, and other health authority interactions.
Expanded Unblinding Team Complete (up to 9 additional internal individuals)	Internal: Unblinded Biometrics Team, Unblinded Medical Team, Medical Writing, SVP R&D, CMO, VP Pharmacovigilance, VP Regulatory, Regulatory Lead, EU Regulatory Lead, Regulatory EU, Label Team Lead, Regulatory Operations, Clinical Pharmacology, Technical Operations External: (b) (4) (separate from the blinded team), Medical Writing ((b) (4) , statistical consultants, regulatory consultants, clinical pharmacology consultants, PopPK/E-R analysis vendor	Final	Data presentation and interpretation, support NDA/MAA submission
Label Team (3 additional internal individuals)	Internal: Cross-functional working group with members from regulatory, biostatistics, commercial, legal, nonclinical. program management External: Outside counsel, Market Access and HEOR consultant, Regulatory consultants	Knowledge	Prepare draft label
Vendors • ISS/ISE • PK/PD • Regulatory strategy	External	Final	Data delivery to these entities to facilitate subsequent analyses
Vendors (Market Access and HEOR) Potential Corporate Partners	External	Knowledge (post-press release)	Aggregated data delivery to facilitate subsequent analyses Due diligence and/or partnership activities

Source: Data Access and Dissemination Plan, Version 1.0 (dated July 23, 2021), Study PROTECT
Abbreviations: CEO, chief executive officer; CMO, chief marketing officer; E-R, exposure-response; HEOR, health economics and outcomes research; ISE, integrated summary of effectiveness; ISS, integrated summary of safety; ; KOL, key opinion leaders; MD, medical doctor; PD, pharmacodynamics; PK, pharmacokinetics; R&D, research and development; SVP, senior vice president; VP, vice president

Dosing

The target dose of sparsentan is 400 mg daily and irbesartan is 300 mg daily. For the first 2 weeks of the double-blind period, patients will initially receive half of the target dose (defined as

the initial dose) of either sparsentan or the active control, irbesartan. Patients who display asymptomatic systolic blood pressure values ≤100 mmHg, diastolic blood pressure values ≤60 mmHg, or present with clinical symptoms of orthostatic hypotension but otherwise tolerate the initial dose will continue after the Week 2 visit without titrating up to the target dose. Patients whose dose is not titrated up to the target dose at Week 2 may titrate up to that dose at any time based on evaluation by the investigator and in consultation with the medical monitor, as needed.

At the discretion of the investigator, patients may reduce their dose in the double-blind period from the target dose to half the target dose for safety or tolerability reasons.

Study drug will be temporarily discontinued for any patient who fulfills the criteria for the adverse event of special interest (AESI) of abnormal liver function test result. The protocol also includes criteria for permanent discontinuation of study drug due to abnormal liver function tests (see below for details).

Study drug will be permanently discontinued for any of the following: (1) receipt of a kidney transplant or initiation of chronic dialysis, (2) any serious adverse event (SAE), AESI, clinically significant laboratory abnormality, intercurrent illness or other medical condition that indicates to the investigator that continuation on study medication is not in the best interest of the patient, (3) significant protocol deviation, (4) investigator discretion, (5) patient decision, (6) pregnancy, (7) diagnosis of class II-IV CHF, (8) hyperkalemia resistant to treatment (defined as a serum potassium >5.5 mEq/L that persists or recurs despite standard-of-care or protocol-recommended treatment), (9) termination of the study, or (10) lost to follow-up.

Compliance

The investigator or designee will assess the patient's compliance with study medication dosing at each visit after Day 1. Study medication compliance is defined as the ratio of the number of actual capsules or tablets taken over the number of capsules or tablets that should have been taken during the dosing period multiplied by 100. Patients will be asked to return all unused study medication and used and unused packaging at each visit. If the investigator has concerns about a patient's dosing compliance, he/she will reiterate the dosing requirements to the patient, and the discussion will be documented in the source documents.

Concomitant Medications

It is recommended that systemic corticosteroid and/or immunosuppressive therapy for the treatment of IgAN be avoided for the duration of participation in the study. If, in the investigator's opinion, systemic corticosteroid and/or immunosuppressive therapy is warranted, such intervention may be provided in addition to study medication at the discretion of the investigator. Consultation with the Medical Monitor is recommended before starting interventional therapy, when possible.

Antihypertensive therapy is recommended to achieve a target blood pressure <125 mm/75 mm Hg.[8] Treatment with additional antihypertensive agents is allowed during the study, with the exception of those that inhibit the renin-angiotensin-aldosterone system (e.g., ACE inhibitors, aldosterone blockers, aliskiren, ARBs) and endothelin systems.

[8] Consistent with the 2012 KDIGO Clinical Practice Guideline blood pressure target for patients with IgAN and proteinuria >1 g/day.

The following medications are prohibited during the treatment period (i.e., Day 1 through Week 110): inhibitors of the renin-angiotensin-aldosterone system, inhibitors of the endothelin system, potassium-sparing diuretics, thiazolidinediones, sodium-glucose cotransporter-2 inhibitors, digoxin, amiodarone, amphetamines and derivatives, prescribed weight loss medications (e.g., orlistat), St. John's Wort, strong CYP3A inhibitors.

The following medications are prohibited for 7 days prior to study visits and should be used with caution at other times during the study: sulfamethoxazole/trimethoprim, cimetidine, pyrimethamine, cetirizine, cobicistat, probenecid, vandetanib, dolutegravir, ranolazine, dronedarone, ritonavir, telaprevir, and fibrates.

Adverse Events of Special Interest

Abnormal liver function test results and COVID-19 adverse events were prespecified as AESIs.

Abnormal Liver Function Test Results

Abnormal liver function test results that meet at least one of the following criteria will be reported to the Medical Monitor within 24 hours of awareness:

- The abnormality represents a new elevation in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) >3 times the upper limit of normal (ULN), with or without an elevation of total serum bilirubin >2 times ULN
- The abnormality represents a 2-fold increase in ALT or AST above the baseline value in patients who had elevated values prior to starting study medication

If a patient meets either of the above criteria, the following steps will be taken:

1. Temporary discontinuation of study medication
2. Repeat testing of ALT, AST, liver-specific alkaline phosphatase, and total bilirubin within 48 to 72 hours to confirm the abnormalities
3. If the abnormality is confirmed by repeat results, the following will be done:
    a. Completion of an AESI Report Form that documents both the liver function test findings and any associated signs or symptoms
    b. Monitoring of liver enzymes and serum bilirubin 2 or 3 times weekly. The frequency of retesting can decrease to once weekly or less if the abnormalities stabilize and the patient is asymptomatic
    c. Additional testing to evaluate liver function, as appropriate (e.g., INR, direct bilirubin)

Study drug will not be resumed until monitoring indicates that the abnormalities have resolved or stabilized.

Study drug will be permanently discontinued for any of the following: (1) ALT or AST >8 times ULN, (2) ALT or AST >5 times ULN for more than 2 weeks, (3) ALT or AST >3 times ULN and total bilirubin >2 times ULN or INR >1.5, or (4) ALT or AST >3 times ULN with symptoms of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5% eosinophils). For these patients, in addition to monitoring of liver tests, the investigator will also perform other relevant clinical and laboratory measurements to identify potential causes of the abnormalities.

Cases of increased liver function tests will always be considered serious if they meet both the following criteria: (1) study medication is suspected to have caused hepatocellular injury, generally shown by a confirmed elevation of 3-fold or greater above ULN in ALT or AST, and (2) the ALT or AST elevations are accompanied by a total bilirubin >2 times the ULN or INR of >1.5 without initial findings of cholestasis (i.e., elevated serum liver-specific alkaline phosphatase).

COVID-19 Adverse Events

If a patient is diagnosed with COVID-19 by a positive test result and becomes symptomatic, the event will be reported as an SAE. If a patient has a positive test result for COVID-19 and is asymptomatic, it will be reported as an AE.

Measures to Prevent Missing Data

A distinction is made between subjects who prematurely discontinue study drug treatment and those who withdraw consent to any follow-up in the study. If a subject is withdrawn from study drug treatment, they are to continue their participation in the study. The reason for premature discontinuation of study drug or subject withdrawal for any follow-up in the study must be documented in the eCRF. A subject who permanently discontinues from the study during the double-blind period will, if possible, complete the end-of-treatment assessments as close to possible to the subject's last dose of study medication. Alternatively, if the last dose of study medication is on a scheduled study visit, that visit will be considered an end-of-treatment visit, and end-of-treatment assessments will be performed.

The investigator will make reasonable efforts to contact subjects who fail to return for scheduled study visits. These actions might include, but are not limited to, the following (as applicable): (1) contacting all telephone numbers for the subject and his/her listed contacts; (2) contacting the subject's primary care physician, referring specialist, or other healthcare professional; (3) sending email, text, and postal mail with certified letters to all the subject's addresses and contacts; (4) reviewing available medical records/notes for details of hospitalizations, clinic visits, or other procedures that may indicate the status of the subject; (5) utilizing the internet to search for additional contact information; and (6) checking local, regional, and national public records to locate the subject or search for mortality status as allowed by law.

16. Efficacy

16.1. SAP Amendments

SAP Amendments

The second amendment was submitted after all subjects were randomized (Table 107). Most of the changes that were implemented in versions 2 and 3 of the SAP were made to align with protocol amendments and to clarify analysis methods. Notable changes are highlighted below:

SAP 2.0

- Added sentence to clarify that slope and the difference will be annualized for ease of presentation and interpretation (Provide more clarity on the analysis Method).

- Added analysis of key secondary efficacy endpoints to include eGFR 2-year total slope (Align with Protocol Amendment 4 per FDA recommendation).

SAP 3.0

- Prespecified how lack of model convergence would be managed at the primary analysis prior to unblinding.
- Changed age category cut off point from 40 to 45 (Change in cut-off point to reflect the median age at study entry).
- Updated language to indicate the following, "Subgroup analyses will use models analogous to the primary analyses of respective endpoint (e.g., MMRM, eGFR slope, etc.) without imputation and based on the MAR assumption."

No changes were made to primary analyses at the interim look.

Table 107. SAP Changes

SAP Version	Document Effective Date	Subjects Randomized Prior to Effective Date (n, %)[b] (N=406)
SAP 1.0	4/11/2019	28 (6.9%)
SAP 2.0	1/5/2021	323 (79.6%)
SAP 3.0	7/21/2021	406 (100.0%)

Source: Applicant's Appendix 16.1.9
eCTD Links to submission:
Clinical study report: \\CDSESUB1\EVSPROD\nda216403\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\igan\5351-stud-rep-contr\021igan17001
Analysis data sets: \\CDSESUB1\EVSPROD\nda216403\0001\m5\datasets\021igan17001\analysis\adam\programs
Conditional Power analysis: \\CDSESUB1\EVSPROD\nda216403\0001\m5\53-clin-stud-rep\535-rep-effic-safety-stud\igan\5354-other-stud-rep\tvtx-re021-tr009

(b) (4)

2 Page(s) have been Withheld in Full as B4 (CCI/TS) immediately following this page

16.3. DUET Study (Phase 2)

The DUET study is a randomized, double-blind, multicenter, phase 2 study in subjects ages 8 to 75 years with biopsy-verified primary FSGS (or documentation of a genetic mutation in a podocyte protein associated with the disease), eGFR ≥30 mL/min/1.73 m^2, and UP/C ≥1.0 g/g. Subjects were randomized 3:1 to one of the following three dose level cohorts:

- Dose Level One (Cohort 1): 200 mg sparsentan or 300 mg irbesartan
- Dose Level Two (Cohort 2): 400 mg sparsentan or 300 mg irbesartan
- Dose Level Three (Cohort 3): 800 mg sparsentan or 300 mg irbesartan

After completing the 8-week double-blind treatment period, eligible subjects had the option to enter a 496-week open-label period. The primary efficacy endpoint is the change from baseline to the Week 8 visit of the natural log (ln) of the UP/C. The study is being conducted at 45 sites in three countries. As of the data cut-off date of February 5, 2021, the study completed the double-blind period, and 73 patients have been exposed to at least one dose of sparsentan and 36 patients have been exposed to at least one dose of irbesartan.

The efficacy evaluable set was used for the analysis of the primary efficacy endpoint and was defined as all subjects who received at least one dose of double-blind investigational product and had both baseline and Week 8 UP/C values. Per the Applicant's analysis, the mean (SD) baseline UP/C for the efficacy evaluable set was slightly higher for the sparsentan (pooled) group (4.7 (3.8) g/g) compared to the irbesartan group (4.0 (2.7) g/g).

Per the Applicant's analysis, there was a statistically significant greater reduction in UP/C among the pooled (all doses) sparsentan group compared with the irbesartan group at Week 8. The percentage change from baseline to Week 8 in UP/C was -44.8% (95% CI -52.7%, -35.7%) for the sparsentan group compared to -18.5% (95% CI -34.6%, 1.7%) for the irbesartan group. The ratio of sparsentan/irbesartan for the percentage change from baseline to Week 8 in UP/C was 0.7 (95% CI 0.5, 0.9) (p=0.006). Information on the percentage change in UP/C from baseline to Week 8 at each dose level of sparsentan are provided in Table 6.

17. Clinical Safety

17.1. Imbalances of Broad FMQs That Are Not Adverse Events of Special Interest

Imbalances of broad FDA Medical Dictionary for Regulatory Activities queries (FMQs) that were not AESIs include fatigue, nausea, malignancy, diabetic ketoacidosis, and hypersensitivity (Table 108). There was no clear mechanistic basis for these findings. Overall, the findings were not concerning.

Fatigue

More patients had the preferred term (PT) of fatigue in the sparsentan (7.9%) group than the irbesartan (3.0%) group (Table 108). There were no fatigue SAEs, severe AEs, or AEs leading to treatment discontinuation. Five patients (2.5%) in sparsentan versus zero patients in irbesartan had unrecovered events, all were mild in severity. Among four patients in sparsentan with moderate fatigue, two experienced fatigue on the same day as other AEs: one patient had cough and abdominal discomfort and the other had dizziness, malaise, and nausea.

Nausea

More patients had AEs associated with the broad FMQ of nausea in the sparsentan (6.4%) group than the irbesartan (3.0%) group. The risk difference was mainly driven by the PT of nausea (risk difference [RD]: 2.0%). Two patients had SAEs associated with the broad FMQ of nausea in the sparsentan group.

Small Intestinal Obstruction

Subject (b) (6) was a 39-year-old Caucasian male who was hospitalized for a moderate small intestinal obstruction (PT) from study Day 346 to Day 354 that led to drug interruption (sparsentan 200 mg daily). The patient experienced stomach pain (3/10), nonbloody, nonbilious emesis and vomiting, abdominal distension, and nausea. Abnormal laboratory results included: sodium depressed at 132 mmol/l (normal range [NR]: 135 to 145); potassium elevated at 5.8 mmol/L (NR: 3.5 to 5.0); creatinine 4.31 mg/dL (baseline 2.12 mg/dL). On study Day 352, the patient had a diagnostic laparoscopy with no findings of a small bowel obstruction. The event required hospitalization and was considered resolved and not related to study drug by the investigator.

Vomiting

Subject (b) (6) was a 68-year-old Asian female who had a severe vomiting (PT) event along with other symptoms, such as low blood pressure, abdominal pain, and perirectal bleeding from study Day 1 to Day 4 that led to drug withdrawal. The event required hospitalization and was considered resolved and related to study drug by the investigator. Also see the “Symptomatic Hypotension” section (Section 7.6.6.3) for a brief narrative.

Malignancy

More patients had AEs associated with the broad FMQ of malignancy in sparsentan (3.0%) than irbesartan (0%). The risk difference was mainly driven by the PT basal cell carcinoma (RD: 1.5%). No patient on sparsentan had a severe malignancy event. A clear mechanistic basis for the observed events has not been identified. One patient in the sparsentan group had a malignancy-related SAE.

Diffuse Large B-Cell Lymphoma

Subject (b) (6) was a 48-year-old Asian male who experienced an SAE of diffuse large B-cell lymphoma (PT) that led to drug withdrawal (sparsentan 400 mg daily) and was considered resolved (complete remission) after six rounds of chemotherapy. The event was considered unlikely related to the study drug by the investigator. This patient also experienced a severe SAE of mechanical ileus that was considered resolved 6 days prior to the diagnosis of B-cell lymphoma, and the lymphoma was a suspected cause of mechanical ileus.

Diabetic Ketoacidosis

More patients had AEs associated with the broad FMQ of diabetic ketoacidosis in the sparsentan (3.5%) than the irbesartan (1.0%) group. The PTs making up this broad FMQ included nonspecific AEs, such as blood bicarbonate decreased, metabolic acidosis, and acidosis hyperchloraemic. Each PT in the broad FMQ had a RD <1%. No AEs led to permanent discontinuation of study drug. One patient in the sparsentan group had an SAE of diabetic ketoacidosis.

Subject (b) (6) was a 37-year-old white male who developed a severe SAE of diabetic ketoacidosis and new onset diabetes 130 days after receiving the first dose of the study product. These events led to study drug interruption (sparsentan 400 mg daily). The patient's glucose was elevated at 971 mg/dL (NR: 74-106) and sodium was decreased at 124 mEq/L (NR: 135-145). The patient was admitted to the hospital with a diagnosis of new onset type 1 diabetes for further medical management. The patient was put on a diabetic ketoacidosis protocol with an insulin drip. The patient was discharged home with home health to assist with management of diabetes on Day 135 and study medication was resumed the next month. The investigator assessed the life-threatening events of new onset type 1 diabetes and diabetic ketoacidosis as severe in intensity and unlikely related to the study drug.

Hypersensitivity

More patients had AEs associated with the broad FMQ of hypersensitivity in the sparsentan (10.9%) group than the irbesartan (8.4%) group. The risk difference was mainly driven by the PTs of pruritus (RD: 2.5%) and asthma (RD: 2.0%). There were no associated severe AEs. In the sparsentan group, one patient had an SAE of asthma and one patient discontinued treatment due to the treatment-emergent adverse event of rash.

Asthma

Subject (b) (6) was a 64-year-old Asian male who had a moderate SAE of asthma (PT) from Study Day 647 to 648. The patient was admitted to the hospital with a diagnosis of asthma after having experienced wheezing and dyspnea at rest for about 1 week. The diagnostic ECG showed atrial fibrillation. The chest X-ray showed prominent right paratracheal stripe, prominent

azygous vein, atherosclerotic and tortuous aorta and cardiomegaly, pulmonary congestion, right lower lobe opacity, and right pleural fluid. Laboratory testing was significant for elevated NT-proBNP of 3606 pg/ml (N: 0-125), BUN of 44 mg/dl (NR: 7-25), creatinine of 2.12 mg/dL (1.73 mg/dL) and eGFR of 33.49 mL/min (baseline 41 mL/min). The patient recovered after treatment and the event was considered not related to the study drug. There was no action taken with study medication. After the asthma was resolved, on study Day 650, the patient developed a moderate SAE of pleural effusion with volume overload that was resolved on study Day 682. No action was taken. The event was considered not related to study drug by the investigator.

Rash

Subject (b) (6) is a 60-year-old Asian female who discontinued treatment due to a moderate AE of rash on Study Day 3. On the same day, the patient also experienced AEs of moderate nausea and mild dizziness. The events were considered resolved. Study treatment was withdrawn on Day 9. The investigator assessed the event of rash as possibly related to study treatment (Case Study Report, p2566).

Table 108. Broad FMQ With Risk Difference >2%, Safety Population, Trial 021IGAN17001 (PROTECT)

System Organ Class *FDA Medical Query (Broad)*[4] Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)[5]
General disorders and administration site conditions			
Fall	54 (26.7%)	26 (12.9%)	13.9 (6.2, 21.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Dizziness	31 (15.3%)	14 (6.9%)	8.4 (2.3, 14.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Peripheral edema	26 (12.9%)	13 (6.4%)	6.4 (0.7, 12.2)
Oedema peripheral	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Peripheral swelling	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Fatigue	22 (10.9%)	12 (5.9%)	5.0 (-0.4, 10.3)
Fatigue	16 (7.9%)	6 (3.0%)	5.0 (0.6, 9.3)
Nervous system disorders			
Syncope	47 (23.3%)	20 (9.9%)	13.4 (6.2, 20.5)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Somnolence	18 (8.9%)	6 (3.0%)	5.9 (1.4, 10.5)
Fatigue	16 (7.9%)	6 (3.0%)	5.0 (0.6, 9.3)
Cardiac disorders			
Arrhythmia	32 (15.8%)	22 (10.9%)	5.0 (-1.7, 11.6)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Heart failure	32 (15.8%)	19 (9.4%)	6.4 (-0.0, 12.9)
Edema peripheral	25 (12.4%)	13 (6.4%)	5.9 (0.3, 11.6)
Peripheral swelling	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Ear and labyrinth disorders			
Vertigo	28 (13.9%)	13 (6.4%)	7.4 (1.6, 13.3)
Dizziness	25 (12.4%)	9 (4.5%)	7.9 (2.6, 13.3)
Vascular disorders			
Hypotension	26 (12.9%)	14 (6.9%)	5.9 (0.1, 11.7)
Hypotension	20 (9.9%)	6 (3.0%)	6.9 (2.2, 11.7)
Gastrointestinal Disorders			

System Organ Class *FDA Medical Query (Broad)*[4] Preferred Term	Sparsentan N=202 n (%)	Irbesartan N=202 n (%)	Absolute Risk Difference (95% CI)[5]
Pancreatitis	14 (6.9%)	7 (3.5%)	3.5 (-0.9, 7.8)
Lipase increased	10 (5.0%)	4 (2.0%)	3.0 (-0.6, 6.5)
Vomiting	14 (6.9%)	7 (3.5%)	3.5 (-0.9, 7.8)
Nausea	8 (4.0%)	4 (2.0%)	2.0 (-1.3, 5.3)
Renal and urinary disorders			
Acute kidney injury	24 (11.9%)	14 (6.9%)	5.0 (-0.7, 10.6)
Acute kidney injury	8 (4.0%)	2 (1.0%)	3.0 (-0.0, 6.0)
Gastrointestinal disorders			
Nausea	13 (6.4%)	6 (3.0%)	3.5 (-0.6, 7.6)
Nausea	8 (4.0%)	4 (2.0%)	2.0 (-1.3, 5.3)
Hepatobiliary disorders			
Hepatic Injury	13 (6.4%)	7 (3.5%)	3.0 (-1.3, 7.2)
Gamma-glutamyltransferase increased	6 (3.0%)	3 (1.5%)	1.5 (-1.4, 4.4)
Neoplasms benign, malignant, and unspecified (incl cysts and polyps)			
Malignancy	6 (3.0%)	0 (0%)	3.0 (0.6, 5.3)
Basal cell carcinoma	3 (1.5%)	0 (0%)	1.5 (-0.2, 3.2)
Blood and lymphatic system disorders			
Anemia	11 (5.4%)	6 (3.0%)	2.5 (-1.4, 6.4)
Anemia	8 (4.0%)	5 (2.5%)	1.5 (-2.0, 4.9)
Endocrine disorders			
Diabetic ketoacidosis	7 (3.5%)	2 (1.0%)	2.5 (-0.4, 5.3)
Immune system disorders			
Hypersensitivity	22 (10.9%)	17 (8.4%)	2.5 (-3.3, 8.2)
Pruritus	9 (4.5%)	4 (2.0%)	2.5 (-1.0, 5.9)
Asthma	4 (2.0%)	0 (0%)	2.0 (0.1, 3.9)

Source: adslir8, adaeir8; Software: R
[1] FMQ RD >2%
[2] PT RD >1%
[3] TEAE defined as any AE that newly appears, increases in frequency, or worsens in severity following initiation of study medication, but within 30 days after the last dose
[4] Version v2
[5] Difference is shown between sparsentan and Irbesartan
Abbreviations: AE, adverse event; CI, confidence interval; FMQ, FDA medical query; N, number of patients in treatment arm; n, Number of patients with an event; TEAE, treatment-emergent adverse event

18. Clinical Virology

Not applicable.

19. Clinical Microbiology

Not applicable.

20. Mechanism of Action / Drug Resistance

Not applicable.

21. Other Drug Development Considerations

None.

22. Data Integrity–Related Consults (Office of Scientific Investigations, Other Inspections)

22.1. Independent Data Monitoring Committee Meeting Discussions

A high-level summary of key DMC open meeting discussions is provided in the table below.

Table 109. Data Monitoring Committee Open Meeting Discussions, Study PROTECT

Date	Number of Patients Randomized	Issue/Discussion
12/4/2018	0	DMC Kick-off Meeting for the PROTECT study
6/20/2019	52	The DMC recommended that the Applicant capture and report values of BP measurements (both sitting and standing) at the time of events such as dizziness, syncope, and lightheadedness, if possible. The DMC also recommended that the Applicant consider capturing orthostatic blood pressure values, rather than just sitting blood pressure.
10/24/2019	133	The Applicant agreed to re-assess the protocol to determine how to incorporate additional BP measurements relatively soon after events such as dizziness, syncope, and lightheadedness. The Applicant also indicated that it would also encourage sites to collect orthostatic blood pressures, which along with all other available data, would help to inform decisions on dosing.
4/23/2020	230	The group discussed the incidence of asymptomatic elevations of amylase or lipase (>55% of patients) at any time during the study. The DMC noted “no clear indication of a drug-related effect over time.” The DMC recommended the Applicant investigate the relationship between eGFR and amylase/lipase and also reach out to an expert in pancreatitis for guidance on the topic. The DMC agreed with the Applicant’s proposal to provide guidance to sites on the management of these patients. The DMC noted that the overall trends in ALT, AST and bilirubin lab data had been unremarkable thus far.
9/2/2020	276	The Applicant notified the DMC that it had consulted experts in the field of pancreatitis who noted that “not a single factor is solely responsible for AMS/lipase elevations…but a possible synergy of the following factors: low eGFR [and] concomitant medications (commonly used in the [study] population): steroids, loop diuretics, thiazide diuretics, oral contraceptives” may have played a role[1].

Date	Number of Patients Randomized	Issue/Discussion
1/21/2021	323	There was further discussion of the amylase and lipase findings. Blinded analysis of the data suggested that the increase in amylase was more prevalent in patients taking "relevant" concomitant medications, such as loop diuretics; however, the data was inconclusive regarding elevations in lipase.[1] The overall trends in ALT, AST and bilirubin lab data remained unremarkable.

Source: Applicant, Data Monitoring Committee Meeting Minutes
Abbreviations: AE, adverse event; ALT, alanine transaminase; AMS, total serum amylase (blood test); AST, aspartate aminotransferase; BP, blood pressure, DMC, Data Monitoring Committee; IP, investigational product; eGFR, estimated glomerular filtration rate
[1] Per the Applicant the "DMC meetings referenced here were blinded DMC discussions during the open sessions that took place on 02 September 2020 and 21 January 2021," and that the meetings occurred before the PROTECT study was unblinded for the interim analysis. The Applicant also noted that "At the time, none of the Travere attendees had been unblinded to data from either study. Consistent with the blinded presentation of the data, the open session meeting minutes do not reflect any assessment by treatment group."

23. Labeling: Key Changes and Considerations

This Prescribing Information (PI) review includes a high-level summary of the rationale for major changes to the finalized PI as compared to the Applicant's draft PI (Table 110). The PI was reviewed to ensure that PI meets regulatory/statutory requirements, is consistent (if appropriate) with labeling guidance, conveys clinically meaningful and scientifically accurate information needed for the safe and effective use of the drug, and provides clear and concise information for the healthcare practitioner.

Table 110. Key Labeling Changes and Considerations

Full PI Sections[1]	Rationale for Major Changes to Finalized PI[2] Compared to Applicant's Initial Draft PI)
BOXED WARNING	The boxed warning was modified to include language related to the hepatotoxicity risk, in addition to the embryo-fetal toxicity risks (see Section 3.1.2.1.
1 INDICATIONS AND USAGE	The indication has been revised define the indicated population as those at risk of rapid disease progression, generally a UPCR ≥1.5 g/g. (See Section 6.2.1.6) The accelerated approval language has been edited to note that it has not been established whether FILSPARI slows kidney function decline in patients with IgAN.
2 DOSAGE AND ADMINISTRATION	Additional language regarding monitoring for pregnancy and aminotransferase and total bilirubin was added. Instructions for dosage adjustment for aminotransferase elevations was added. (See Section 7.7.1)
4 CONTRAINDICATIONS	No major revisions.
5 WARNINGS AND PRECAUTIONS	A warning for Hepatotoxicity was added. (See Section 7.7.1) A proposed warning for [redacted] (b) (4) was removed.
6 ADVERSE REACTIONS	Incidences of adverse reactions was revised to reflect the review findings. (See Section 7.6.5) [redacted] (b) (4), were removed and cross-references inserted.

Full PI Sections[1]	Rationale for Major Changes to Finalized PI[2] Compared to Applicant's Initial Draft PI)
7 DRUG INTERACTIONS	New subsections were added for: • ANTACIDS AND ACID REDUCING AGENTS • CYP2B6, 2C9 AND 2C19 SUBSTRATES • P-GP AND BCRP SUBSTRATES (See Section 8.5)
8 USE IN SPECIFIC POPULATIONS (e.g., Pregnancy, Lactation, Females and Males of Reproductive Potential, Pediatric Use, Geriatric Use, Renal Impairment, Hepatic Impairment)	The Hepatic Impairment subsection was revised for consistency with descriptions of the risk of hepatotoxicity elsewhere in the prescribing information.
9 DRUG ABUSE AND DEPENDENCE	Not applicable.
10 OVERDOSAGE	This section was revised to note that overdosages of FILSPARI may result in decreased blood pressure.
12 CLINICAL PHARMACOLOGY	The mechanism of action has been revised for clarity. The pharmacodynamics section has been revised to note that dose-response information is not available. (See Section 6.1.3) Additional language regarding the effect of sparsentan on other drugs was added. (See Section 8.5)
13 NONCLINICAL TOXICOLOGY	Additional language regarding carcinogenesis was added. Human multiples of described doses was also added. (See Section 7.1)
14 CLINICAL STUDIES	The clinical studies section was revised to reflect the review findings. (See Section 6)
17 PATIENT COUNSELING INFORMATION	This section was updated with language consistent with the REMS.
Product quality sections (i.e., DOSAGE FORMS AND STRENGTHS, DESCRIPTION, HOW SUPPLIED/STORAGE AND HANDLING)	The Description section was updated to note that sparsentan has pH-dependent solubility.

Source: FDA reviewer
[1] Product quality sections (Sections 3, 11, and 16) are pooled under the last row in this table; Section 15 of the label is not included in this table.
[2] For the purposes of this document, the finalized PI is the PI that will be approved or is close to being approved.
Abbreviations: IGAN, immunoglobulin A nephropathy; PI, Prescribing Information, REMS, risk evaluation and mitigation strategies); UPCR, urine protein to creatinine ratio

23.1. Approved Labeling Types

Upon approval of this application, the following labeling documents will be FDA-approved:

- Prescribing Information
- Medication Guide
- Carton and Container Labeling

24. Postmarketing Requirements and Commitments

The following postmarketing requirement (PMR) will be issued at the time of approval:

4330-1 Conduct a randomized, double-blind, placebo-controlled trial to describe and verify the clinical benefit of sparsentan for the treatment of IgA nephropathy. The trial should be adequately powered and of sufficient duration to detect a treatment effect on the endpoint that will be used to describe and verify the clinical benefit.

Draft protocol submission:	Completed
Final protocol submission:	Completed
Study/trial completion:	10/2023
Final report submission:	02/2024

4330-2 Conduct a pharmacokinetic drug-drug interaction trial to evaluate the effect of sparsentan, once-daily, dosed to steady state on substrates for CYP2C9 and CYP2C19 in adult healthy volunteers

Draft protocol submission:	06/2023
Final protocol submission:	09/2023
Study completion:	05/2024
Final report submission:	09/2024

4330-3 Conduct a pharmacokinetic drug-drug interaction trial to evaluate the effect of acid reducing agents on the exposure of sparsentan in adult healthy volunteers.

Draft protocol submission:	06/2023
Final protocol submission:	09/2023
Study/trial completion:	05/2024
Final report submission:	09/2024

4330-4 Conduct a pharmacokinetic drug-drug interaction trial to evaluate the effect of sparsentan once-daily dosed to steady state on substrates for P-gp and BCRP in adult healthy volunteers.

Draft protocol submission:	06/2023
Final protocol submission:	09/2023
Study completion:	05/2024
Final report submission:	09/2024

4330-5 Conduct a prospective, single-arm safety study of patients exposed to sparsentan, with two years of follow-up to assess and characterize the risk of drug-induced liver injury (DILI). This study should analyze the clinical features of DILI cases with sparsentan, such as the injury's severity, type, latency, and specifically evaluate the incidence of Hy's law cases. Information for liver injury cases should be captured with structured follow up (e.g., monthly monitoring of serum liver tests) including dechallenge and rechallenge results. A hepatic adjudication committee (HAC) should assess both the severity of the liver injury and sparsentan's role in its development

(i.e., causality). This study should aim to enroll enough patients such that if 0 events of Hy's law are observed, then the upper bound of the 95% confidence interval for the rate of Hy's law will be 1/1000.

Draft protocol submission:	06/2023
Final protocol submission:	09/2023
Study completion:	12/2027
Final report submission:	04/2028

25. Financial Disclosure

Table 111. Covered Clinical Studies: PROTECT

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 190 principals and 561 subinvestigators		
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): 4		
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: 4 Proprietary interest in the product tested held by investigator: 1 Significant equity interest held by investigator: 0 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): 0		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

Abbreviation: FDA, Food and Drug Administration

The PROTECT study is a randomized, double-blind, placebo-controlled, multicenter study, and each individual site contributed a relatively small proportion of the 280 patients evaluated at the time of the interim analysis. There were four investigators with disclosable financial interests: (b) (6) . Per the Applicant, fees paid to these investigators were for consulting on the design of the protocol and advice on the future development of sparsentan. The investigators were not paid for enrollment of patients into the study and study payments were made to the institution and not to the investigator directly. No one study site was found to drive the efficacy results. The risk to study integrity from the above financial interests is thought to be low.

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27. Review Team

Table 112. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory project manager	Anna Park
Nonclinical reviewer	Xi Yang/Srinivasa Raju Datla
Nonclinical team leader	Jean Wu
OCP reviewer(s)	Hebing Liu
OCP team leader(s)	Sudharshan Hariharan
OCP Pharmacometrics	Jihye Ahn
OCP Pharmacometrics team leader	Liang Li
OCP Pharmacometrics Assoc. Director	Jiang Liu
PBPK reviewer	Ying-Hong Wang
PBPK team leader	Yuching Yang
Clinical reviewer	Rekha Kambhampati Yanyan (Claire) Ji/Christopher Jay/ Qunshu Zhang
Clinical team leader	Aliza Thompson
Biometrics reviewer	Dali Zhou
Biometrics team leader	Jialu Zhang
Cross-disciplinary team leader	Aliza Thompson
Division director (pharm/tox)	Todd Bourcier
Division director (OCP)	Shirley Seo
Division director (OB)	Mark Rothman
Deputy director safety (clinical)	Mary Ross Southworth
Division director (clinical)	Norman Stockbridge
Office director (or designated signatory authority)	Lisa Yanoff

Abbreviations: OCP, Office of Clinical Pharmacology; OB, Office of Biostatistics

Table 113. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Theodore Carver (ATL); Sithamalli Chandramouli; Dan Berger; Nancy Waites; Lixia Cai; Debasis Ghosh; Haritha Mandula
Microbiology	N/A
OPDP	Charuni Shah
OSI	Suyoung ((Tina) Chang
OSE/DEPI	Margie Goulding/Benjamin Booth
OSE/DMEPA	Sarah Vee/Hina Mehta
OSE/DRISK	Theresa Ng/Katherine Hyatt Hawkins/Yasmeen Abou-Sayed; Laura Zendel
OSE/DPV	Heather Le/Dan Woronow
Patient labeling	Ruth Mayrosh
DPMH	Katherine Kratz/Miriam Dinatale/Leyla Sahin/Lynn Yao

Abbreviations: OPQ, Office of Pharmaceutical Quality; OPDP, Office of Prescription Drug Promotion; OSI, Office of Scientific Investigations; OSE, Office of Surveillance and Epidemiology; DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; DPV, Division of Pharmacovigilance; DPMH; Division of Pediatrics and Maternal Health

27.1. Reviewer Signatures

See next page.

Table 114. Signatures of Reviewers

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Division Director	Norman Stockbridge, MD, PhD Office of Cardiology, Hematology, Endocrinology, and Nephrology DCN	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Norman L. Stockbridge -S Digitally signed by Norman L. Stockbridge -S Date: 2023.02.16 11:40:05'00'					
Cross-Disciplinary Deputy Director	Aliza Thompson, MD, MS Office of Cardiology, Hematology, Endocrinology, and Nephrology DCN	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 1	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Aliza M. Thompson -S Digitally signed by Aliza M. Thompson -S Date: 2023.02.16 13:20:22 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Reviewer	Rekha Kambhampati, MD Office of Cardiology, Hematology, Endocrinology, and Nephrology DCN	☒ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 2, 3, 4, 6.2, 7, 10, 11, 12, 15, 16.3, 17, 22.1, 25	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.	None.	☒ Yes ☐ No
Signature/date/time stamp: Rekha Kambhampati -S Digitally signed by Rekha Kambhampati -S Date: 2023.02.15 14:06:15 -05'00'					
Biometrics Division Director	Mark Rothmann, PhD Office of Biostatistics DBII	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 6.2.1.3, 6.2.1.4, 6.3, 16.1, 16.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Mark D. Rothmann -S Digitally signed by Mark D. Rothmann -S Date: 2023.02.16 08:59:12 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Biometrics Team Leader	Jialu Zhang, PhD Office of Biostatistics DBII	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 6.2.1.3, 6.2.1.4, 6.3, 16.1, 16.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Jialu Zhang -S Digitally signed by Jialu Zhang -S Date: 2023.02.15 15:29:26 -05'00'					
Biometrics Reviewer	Dali Zhou, PhD Office of Biostatistics DBII	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 6.2.1.3, 6.2.1.4, 6.3, 16.1, 16.2	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☐ Yes ☒ No
Signature/date/time stamp: Dali Zhou -S Digitally signed by Dali Zhou -S Date: 2023.02.15 19:03:34 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Pharmacology/ Toxicology Division Director	Todd Bourcier, PhD Office of Cardiology, Hematology, Endocrinology, and Nephrology DPT	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 5.1, 5.2 (Pharmacology Activity), 7.1, 8.4, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp:	Todd M. Bourcier -S Digitally signed by Todd M. Bourcier -S Date: 2023.02.15 09:13:20 -05'00'				
Pharmacology/ Toxicology Supervisor	Jean Wu, MD, PhD Office of Cardiology, Hematology, Endocrinology, and Nephrology DPT	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 5.1, 5.2 (Pharmacology Activity), 7.1, 8.4, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp:	Jean Q. Wu -S Digitally signed by Jean Q. Wu -S Date: 2023.02.15 10:08:47 -05'00'				

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Pharmacology/Toxicology Reviewer	Srinivasa Raju Datla, PhD Office of Cardiology, Hematology, Endocrinology, and Nephrology DPT	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections 5.1, 5.2 (Pharmacology Activity), 7.1, 8.4, 13	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Srinivasa-raju Datla -S Digitally signed by Srinivasa-raju Datla -S Date: 2023.02.15 10:26:04 -05'00'					
Clinical Pharmacology Division Director	Shirley Seo, PhD Office of Clinical Pharmacology/ DCEP	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14.1, 14.2, 14.3, 14.4, 14.5	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Shirley K. Seo -S Digitally signed by Shirley K. Seo -S Date: 2023.02.16 10:54:02 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology Team Leader	Sudharshan Hariharan, PhD Office of Clinical Pharmacology/ DCEP	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14.1, 14.2, 14.3	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Sudharshan Hariharan -S Digitally signed by Sudharshan Hariharan -S Date: 2023.02.14 15:29:41 -05'00'					
Clinical Pharmacology Reviewer	Hebing Liu, PhD Office of Clinical Pharmacology/ DCEP	☒ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8.1, 8.2, 14.1, 14.2, 14.3	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Hebing Liu -S (Affiliate) Digitally signed by Hebing Liu -S (Affiliate) Date: 2023.02.14 16:21:05 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology/Pharmacometrics Associate Director	Jiang Liu, PhD Office of Clinical Pharmacology/ DPM	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 5.2, 6.1, 8, 14.1, 14.2, 14.3, 14.4	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Jiang Liu -S Digitally signed by Jiang Liu -S Date: 2023.02.15 08:06:28 -05'00'					
Clinical Pharmacology/ PBPK Team Leader	Yuching Yang, PhD Office of Clinical Pharmacology/ DPM	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 8.2, 14.5	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Yuching Yang -S Digitally signed by Yuching Yang -S Date: 2023.02.14 15:13:37 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Pharmacology/ PBPK Reviewer	Ying-Hong Wang, PhD Office of Clinical Pharmacology/ DPM	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☒ Additional Information and Analyses Sections: 8.2,14.5	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Ying-hong Wang -S Digitally signed by Ying-hong Wang -S Date: 2023.02.14 13:14:43 -05'00'					

Discipline and Role	Reviewer Name, Office/Center, and Division	Sections Authored in Full or in Part	Recommendation to Signatory	Comments on Recommendation to Signatory	Consent To Include on Public List of Reviewers
Clinical Deputy Director for Safety	Mary Ross Southworth, PharmD Office of Cardiology, Hematology, Endocrinology, and Nephrology DCN	☐ Benefit-Risk Assessment ☐ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections:	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Mary R. Southworth -S Digitally signed by Mary R. Southworth -S Date: 2023.02.16 17:00:35 -05'00'					
Clinical Reviewer	Claire Yanyan Ji, PhD Staff Fellow Office of Cardiology, Hematology, Endocrinology, and Nephrology DCN	☐ Benefit-Risk Assessment ☒ Interdisciplinary Assessment ☐ Additional Information and Analyses Sections: 7	Based on my assessment of the application: ☒ No deficiencies preclude approval. ☐ Deficiencies preclude approval. ☐ Not applicable.		☒ Yes ☐ No
Signature/date/time stamp: Yanyan Ji -S Digitally signed by Yanyan Ji -S Date: 2023.02.16 16:04:29 -05'00'					

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/s/

ALIZA M THOMPSON
02/17/2023 06:28:58 AM

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02/17/2023 08:27:02 AM

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Science
Office of Biostatistics

Statistical Review and Evaluation

CARCINOGENICITY STUDY

IND/NDA Number:	NDA 216403
Drug Name:	Sparsentan
Indication:	Sparsentan is indicated for the treatment of IgA nephropathy (IgAN)
Applicant:	Travere Therapeutics, Inc. 3611 Valley Centre Drive San Diego, CA 92130 Test Facility for Rats and Mice Study: (b) (4) [redacted]
Documents Reviewed:	Study reports (Rat Study 2223-001 and Mouse Study 2223-002) and electronic data submitted on March 13, 2022 via NDA216403/0001
Biometrics Division:	Division of Biometrics -6
Statistical Reviewer:	Zhuang Miao, Ph.D.

Concurring Reviewer: Karl Lin, Ph.D.

Reviewing Pharmacologist: Xi Yang, Ph.D.

Keywords: Carcinogenicity, Dose response

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1. Summary

In this submission the sponsor included reports of two animal carcinogenicity studies, one in CD [Crl:CD®(SD)] rats and one in CByB6F1-Tg(HRAS)2Jic mice. These studies were intended to assess the carcinogenic potential of Sparsentan, when administered via oral gavage at appropriate drug levels for 93 weeks in rats and 26 weeks in mice.

Rat Study:

Survival analyses:

1. For male rats, the comparison between the vehicle control group and low dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0109<0.05 and the p-value for log-rank test is 0.01<0.05).
2. For female rats, the survival analysis showed a statistically significant dose response relationship in mortality across the vehicle control group and the treated groups (the p-value for likelihood ratio test is 0.0079<0.05 and the p-value for log-rank test is 0.0068<0.05). The comparison between the vehicle control group and low dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0452<0.05 and the p-value for log-rank test is 0.0417<0.05).

Tumor analysis: there were no statistically significant tumor findings among males or females.

Mouse Study:

Survival analyses:

1. For male mice, the survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups or acrose the water control group and the treated groups. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups or between the water control group and each of the treated groups. The comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.001<0.05 and the p-value for log-rank test is 0.0019<0.05). The comparison between the water control group and positive control

group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.001<0.05 and the p-value for log-rank test is 0.0019<0.05).

2. For female mice, the survival analyses showed a statistically significant dose response relationship in mortality across the veihcle control group and the treated groups (the p-value for likelihood ratio test is 0.0012<0.05 and the p-value for log-rank test is 0.0072<0.05). The survival analyses didn't show any statistically significant dose response relationship in mortality across the water control group and the treated groups. The pairwise comparison between the vehicle control group and high dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0068<0.05 and the p-value for log-rank test is 0.0196<0.05). The pairwise comparisons did not show any statistically significant differences in mortality between the water control group and each of the treated groups. The comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0011<0.05 and the p-value for log-rank test is 0.0019<0.05). The comparison between the water control group and positive control group did not showed a statistically significant increase in mortality.

Tumor analysis:

1. For male mice, the pairwise comparisons between the vehicle control and the mid dose group showed a statistically significant increase in incidence of hemangiosarcoma, multicentric (p-value=0.0455<0.05).
2. For male mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.0398<0.05), squamous cell papilloma, skin (p-value<0.001), squamous cell carcinoma, stomach (p-value=0.0097) and squamous cell papilloma, stomach (p-value<0.001).
3. For male mice, the pairwise comparisons between the water control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.0398<0.05), squamous cell papilloma, skin (p-value<0.001), squamous cell carcinoma, stomach (p-value=0.0097) and squamous cell papilloma, stomach (p-value<0.001).
4. For female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of

lymphoma, multicentric (p-value=0.0035<0.05), squamous cell papilloma, multicentric (p-value<0.001), glandular polyp, uterus with cervix (p-value<0.001), squamous cell papilloma, vulva (p-value=0.0339<0.05).

5. For female mice, the pairwise comparisons between the water control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.004<0.05), squamous cell papilloma, stomach (p-value<0.001), glandular polyp, uterus with cervix (p-value<0.001), squamous cell papilloma, vulva (p-value=0.0368<0.05).

2. Background

In this submission the sponsor included reports of two animal carcinogenicity studies, one in CD [Crl:CD®(SD)] rats and one in CByB6F1-Tg(HRAS)2Jic mice. These studies were intended to assess the carcinogenic potential of Sparsentan, when administered via oral gavage at appropriate drug levels for 93 weeks in rats and 26 weeks in mice. Results of this review have been discussed with the reviewing pharmacologist Dr. Xi Yang. This review analyzed the SAS data sets of these studies received from the sponsor on March 17, 2022 via NDA216403/0001.

In this review the phrase "dose response relationship" refers to the linear component of the effect of treatment, and not necessarily to a strictly increasing or decreasing mortality or tumor incidence rate as the dose increases.

3. Rat Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups and one vehicle control group. Two hundred and forty CD rats of each sex were randomly assigned to the four groups in equal size of 60 rats per group. The dose levels for treated groups were 15, 60 and 240 mg/kg/day. The rats in the vehicle control group received the vehicle(0.5% Methylcellulose/0.25% Tween 80 (also known as polysorbate 80) in Deionized Water). The study for the rats was designed to continue for up to 93 weeks.

Table 1: Study Design in Rat Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	Vehicle	60	60
2	15	Sparsentan	60	60
3	60	Sparsentan	60	60
4	240	Sparsentan	60	60

3.1. Sponsor's analyses

3.1.1. Survival analysis

Intercurrent mortality data were analyzed using the Kaplan-Meier product-limit method. An overall test comparing all groups was conducted using a log-rank test. When this overall test was significant ($p<0.05$) and there were more than two groups, then a follow up analysis was done where each treatment group was compared to the control group using a log-rank test.

Results of all pair-wise comparisons are reported at the 0.05 and 0.01 significance levels. All endpoints were analyzed using two-tailed tests.

Sponsor's findings: Dosing of males given 60 or 240 mg/kg/day was discontinued and animals were euthanized and discarded without further evaluation during Week 29 due to the low body weights noted in these groups.

Sponsor's analysis showed the numbers (percents) of survival were 20 (33%), and 34 (57%) in vehicle control, and 15 mg/kg/day dose groups, respectively in males and 20 (33%), 30 (35%), 22 (38%), and 15 (42%) in vehicle control, 15 mg/kg/day, 60 mg/kg/day, and 240 mg/kg/day dose groups, respectively in females.

The sponsor concluded that there were no statistically significant findings among males or females for survival rates.

3.1.2. Tumor data analysis

Tumor incidence data were analyzed using both survival adjusted and unadjusted tests. The unadjusted tests were based on the incidence and number of sites examined for each tumor type. The Cochran-Armitage trend test was calculated and Fisher's exact test was used to compare each treatment group with the control group. The survival adjusted test was conducted according to the prevalence/mortality methods described by Peto et al.. Evaluation criteria (p-values of significance) were applied differently for rare tumors (background rate of 1% or less) and common tumors (background rate greater than 1%). The evaluation criteria (from the FDA) are given in the following table.

Table : Evaluation Criteria for Common and Rare Tumors	
Test for Positive Trends	**Control-High Pair-wise Comparisons**
Common and rare tumors were tested at 0.005 and 0.025 significance levels, respectively	Common and rare tumors were tested at 0.01 and 0.05 significance levels, respectively

Sponsor's findings: The sponsor concluded that there were no increases in any tumor type and all tumors were considered incidental to administration of Sparsentan.

3.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses.

3.2.1. Survival analysis

The survival distributions of animals in four groups were estimated by the Kaplan-Meier product limit method. The dose response relationship and homogeneity of survival distributions were tested for the vehicle controls, low, medium and high dose groups using the Likelihood Ratio test and the Log-Rank test. The intercurrent mortality data are given in Tables 8 and 9 in the appendix for males and females, respectively. The Kaplan-Meier curves for survival rate are given in Figures 1 and 2 in the appendix for males and females, respectively. Results of the tests for dose response relationship and homogeneity of survivals, are given in Tables 10 and 11 in the appendix for males and females, respectively.

Reviewer's findings: This reviewer's analysis showed the numbers (percents) of survival were 20 (33%), and 34 (57%) in vehicle control, and 15 mg/kg/day dose groups, respectively in males and 20 (33%), 30 (35%), 22 (38%), and 15 (42%) in vehicle control, 15 mg/kg/day, 60 mg/kg/day, and 240 mg/kg/day dose groups, respectively in females.

For male rats, the comparison between the vehicle control group and low dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0109<0.05 and the p-value for log-rank test is 0.01<0.05).

For female rats, the survival analysis showed a statistically significant dose response relationship in mortality across the vehicle control group and the treated groups (the p-value for likelihood ratio test is 0.0079<0.05 and the p-value for log-rank test is 0.0068<0.05). The comparison between the vehicle control group and low dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0452<0.05 and the p-value for log-rank test is 0.0417<0.05).

3.2.2. Tumor data analysis

The tumor data were analyzed for the positive dose response relationships and the positive pairwise comparison increases between each of the treated groups with control group. Both the dose response relationship tests and pairwise comparisons were performed using the Poly-K method described in the paper of Bailer and Portier (1988) and Bieler and Williams (1993). In this method an animal that lives the full study period ($w_{\max}$) or dies before the terminal sacrifice but develops the tumor type being tested gets a score of s_h =1. An animal that dies at week w_h without a tumor before the end of the study gets a score of $s_h = \left(\frac{w_h}{w_{\max}}\right)^k$ < 1. The adjusted group size is defined as $\sum s_h$. As an interpretation, an animal with score s_h =1 can be considered as a whole animal while an animal with score s_h < 1 can be considered as a partial animal. The adjusted group size $\sum s_h$ is equal to N (the original group size) if all animals live up to the end of the study or if each animal that dies before the terminal sacrifice develops at least one tumor, otherwise the adjusted group size is less than N. These adjusted group sizes were then used for the dose response relationship (or the pairwise) tests using the Cochran-Armitage test. One critical point for Poly-k test is the choice of the appropriate value of k, which depends on the tumor incidence pattern with the increased dose. For long term 104 week standard rat and mouse studies, a value of k=3 is suggested in the literature. Hence, this reviewer used k=3 for the analysis of this data. For the calculation of p-values the exact permutation method was used. The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons are listed in Tables 12 and 13 in the appendix for male and female rats, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study and

one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels α =0.005 for common tumors and $\alpha=0.025$ for rare tumors for the chronic rat study. For pairwise comparisonsfor the chronic rat study in the above type of submission with one chronic rat study and one transgenic mouse study, the same guidance document suggests the use of test levels α =0.01 for common tumors and α =0.05 for rare tumors for the chronic rat study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: There were no statistically significant tumor findings among males or females.

4. Mouse Study

Two separate experiments were conducted, one in males and one in females. In each of these two experiments there were three treated groups, one water control group, one vehicle control group and one positive control group. One hundred and twenty five RasH2 mice of each sex were randomly assigned to the treated and control groups in equal size of 25 mice per group. The dose levels for treated groups were 60, 200, and 600 mg/kg/day for males and females. The mice in the positive control group received 75 mg/kg N-nitroso-N-methylurea (MNU) via intraperitoneal injection on Day 1 only.

Table 2: Study Design in Mouse Study

Protocol Group No.	Dose Levels (mg/kg/day)	Identification	Number of Animals Enrolled	
			Males	Females
1	0	water	25	25
2	0	vehicle	25	25
3	60	Sparsentan	25	25
4	200	Sparsentan	25	25
5	600	Sparsentan	25	25
6	75	Positive	15	15

4.1. Sponsor's analyses

4.1.1. Survival analysis

The sponsor used the same survival analysis methods used for the rats study in this mouse study.

Sponsor’s findings: The sponsor’s analysis showed that the numbers (percents) of survival were 25 (100%), 25 (100%), 22 (88%), 23 (92%), and 24 (96%) in male mice, and 22 (88%), 25 (100%), 25 (100%), 24 (96%) and 20 (80%) in female mice in water control, vehicle control, low, medium, high and positive dose groups, respectively.

The sponsor concluded that, there were no statistically significant findings in survival rates.

4.1.2. Tumor data analysis

The sponsor used the same tumor data analysis methods used for the rat study in this mouse study

Sponsor's findings: The sponsor concluded that there were no statistically significant tumor findings in the test article groups when compared to the vehicle control group.

4.2. Reviewer's analyses

To verify sponsor's analyses and to perform additional analyses suggested by the reviewing pharmacologist, this reviewer independently performed survival and tumor data analyses.

4.2.1. Survival analysis

The survival distributions of three treated groups, one vehical control group, and one positive control group were estimated using the Kaplan-Meier product limit method. The dose response relationship in survival was tested using the likelihood ratio test and the homogeneity of survival distributions was tested using the log-rank test. The Kaplan-Meier curves for survival rates are given in Figures 3 and 4 in the appendix for male and female mice, respectively. The intercurrent mortality data are given in Tables 14 and 15 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 16 and 17 in the appendix for male and female mice, respectively. Results of the tests for dose response relationship and homogeneity of survivals among the vehicle control and three treated groups are given in Tables 18 and 19 in the appendix for male and female mice, respectively.

Reviewer's findings: This reviewer's analysis showed that the numbers (percents) of survival were 25 (100%), 25 (100%), 22 (88%), 23 (92%), 24 (96%) and 10 (66.67%) in male mice, and 22 (88%), 25 (100%), 25 (100%), 24 (96%), 20 (80%), and 10 (66.67%) in female mice in water control, vehicle control, low, medium, high and positive dose groups, respectively.

For male mice, the survival analyses didn't show any statistically significant dose response relationship in mortality across the vehicle control group and treated groups or across the

water control group and the treated groups. The pairwise comparisons did not show any statistically significant differences in mortality between the vehicle control group and each of the treated groups or between the water control group and each of the treated groups. The comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.001<0.05 and the p-value for log-rank test is 0.0019<0.05). The comparison between the water control group and positive control group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.001<0.05 and the p-value for log-rank test is 0.0019<0.05).

For female mice, the survival analyses showed a statistically significant dose response relationship in mortality across the veihcle control group and the treated groups (the p-value for likelihood ratio test is 0.0012<0.05 and the p-value for log-rank test is 0.0072<0.05). The survival analyses didn't show any statistically significant dose response relationship in mortality across the water control group and the treated groups. The pairwise comparison between the vehicle control group and high dose group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0068<0.05 and the p-value for log-rank test is 0.0196<0.05). The pairwise comparisons did not show any statistically significant differences in mortality between the water control group and each of the treated groups. The comparison between the vehicle control group and positive control group showed a statistically significant increase in mortality (the p-value for likelihood ratio test is 0.0011<0.05 and the p-value for log-rank test is 0.0019<0.05). The comparison between the water control group and positive control group did not showed a statistically significant increase in mortality.

4.2.2. Tumor data analysis

The reviewer used the same tumor data analysis methods for the rat study in this mouse study.

The tumor rates and the p-values for the positive dose response relationship tests and pairwise comparisons between vehicle control and three treated groups, and between vehicle control and positive control, between water control and three treated groups, and between water control and positive control are listed in Tables 20, 21, 22, 23, 24, 25, 26, and 27 in the

appendix for male and female mice, respectively.

Adjustment for multiple testing: For the chronic study in rats, the adjustment of multiple testing of the dose response relationship for a submission with one chronic rat study and one transgenic mouse study, the more recently revised draft (January, 2013) FDA guidance for the carcinogenicity studies suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study. For pairwise, the same guidance document suggests the use of test levels α =0.05 for both common tumors and rare tumors for the mouse study.

It should be noted that the FDA guidance for multiple testing for dose response relationship is based on a publication by Lin and Rahman (1998). In this work the authors investigated the use of this rule for Peto analysis. However, in a later work Rahman and Lin (2008) showed that this rule for multiple testing for dose response relationship is also suitable for Poly-K tests.

Reviewer's findings: The tumor types in Tables 3, 4, 5, 6 and 7 below showed p-values less than or equal to 0.05 in the tests for pairwise comparisons between water control and treated groups for males, and between vehicle/water control and positive control groups for male mice and female mice, respectively.

Table 3: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Water Control and Treated Groups -Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
MULTICENTRIC NEOPL	HEMANGIOSARCOMA	0/25 (25) 0.2801	2/25 (23) 0.2243	4/25 (23) 0.0455	2/25 (24) 0.2347

& X/ZZ (YY): X=number of tumor bearing animals; YY=mortality weighted total number of animals; ZZ=unweighted total number of animals observed;
NC = Not calculable.

Table 4: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
MULTICENTRIC NEOPL	LYMPHOMA	0/25 (25)	3/15 (14) 0.0398
SKIN	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	10/15 (14) <0.001
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (25)	4/15 (13) 0.0097
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	11/15 (14) <0.001

Table 5: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Water Control and Positive Control-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
MULTICENTRIC NEOPL	LYMPHOMA	0/25 (25)	3/15 (14) 0.0398
SKIN	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	10/15 (14) <0.001
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (25)	4/15 (13) 0.0097

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	11/15 (14) <0.001

Table 6: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Vehicle Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
MULTICENTRIC	LYMPHOMA	0/25 (25)	5/15 (14) 0.0035
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	12/15 (14) <0.001
UTERUS WITH CERVIX	POLYP, GLANDULAR	0/25 (25)	6/15 (13) <0.001
VULVA	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	3/15 (13) 0.0339

Table 7: Tumor Types with P-Values ⩽ 0.05 for Comparisons between Water Control and Positive Control-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
MULTICENTRIC NEOPL	LYMPHOMA	0/25 (24)	5/15 (14) 0.0040
	PAPILLOMA, SQUAMOUS CELL	1/25 (24)	12/15 (14) <0.001
UTERUS WITH CERVIX	POLYP, GLANDULAR	0/25 (24)	6/15 (13) <0.001
VULVA	PAPILLOMA, SQUAMOUS CELL	0/25 (24)	3/15 (13) 0.0368

Reviewer's findings: Based on the criteria of adjustment for multiple testing discussed in the mouse data analysis section, we make the following statistical conclusions:

1. For male mice, the pairwise comparisons between the vehicle control and the mid dose group showed a statistically significant increase in incidence of hemangiosarcoma, multicentric (p-value=0.0455<0.05).
2. For male mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.0398<0.05), squamous cell papilloma, skin (p-value<0.001), squamous cell carcinoma, stomach (p-value=0.0097) and squamous cell papilloma, stomach (p-value<0.001).
3. For male mice, the pairwise comparisons between the water control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.0398<0.05), squamous cell papilloma, skin (p-value<0.001), squamous cell carcinoma, stomach (p-value=0.0097) and squamous cell papilloma, stomach (p-value<0.001).
4. For female mice, the pairwise comparisons between the vehicle control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.0035<0.05), squamous cell papilloma, multicentric (p-value<0.001), glandular polyp, uterus with cervix (p-value<0.001), squamous cell papilloma, vulva (p-value=0.0339<0.05).
5. For female mice, the pairwise comparisons between the water control and the positive control group showed statistically significant increase in incidence of lymphoma, multicentric (p-value=0.004<0.05), squamous cell papilloma, stomach (p-value<0.001), glandular polyp, uterus with cervix (p-value<0.001), squamous cell papilloma, vulva (p-value=0.0368<0.05).

Zhuang Miao, Ph.D.
Mathematical
Statistician
Concur:
Karl Lin, Ph.D.
Mathematical Statistician, Team Leader, Biometrics-6
cc:
Yi Tsong, Ph.D.

5. Appendix

Table 8: Intercurrent Mortality Rate -Male Rats

	Vehicle 0 mg|kg|day (N=60)		15 mg|kg|day (N=60)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**No. of Death**
0 - 52	5	8.33	2	8.33
53 - 78	17	28.33	10	15.33
79 - 92	18	30.00	13	21.67
Ter. Sac.	20	33.33	34	56.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 9: Intercurrent Mortality Rate -Female Rats

	Vehicle 0 mg|kg|day (N=60)		15 mg|kg|day (N=60)		60 mg|kg|day (N=60)		240 mg|kg|day (N=60)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 52	4	6.67	1	1.67	2	3.33	8	13.33
53 - 78	21	41.67	16	28.33	18	33.33	26	56.67
79 - 94	15	66.67	13	50.00	18	63.33	11	75.00
Ter. Sac.	20	33.33	30	50.00	22	36.67	15	25.00

Cum. %: Cumulative percentage except for Ter. Sac.

Table 10: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Male Rats

Test	Statistic	P_Value Vehicle vs. Low
Dose-Response	Likelihood Ratio	0.0109
Homogeneity	Log-Rank	0.0100

Table 11: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control -Female Rats

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Dose-Response	Likelihood Ratio	0.0079	0.0452	0.5263	0.1215

Test	Statistic	P_Value Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Medium	P_Value Vehicle vs. High
Homogeneity	Log-Rank	0.0068	0.0417	0.5201	0.1159

Table 12: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups-Male Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60)	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low
ADRENAL GLANDS	ADENOMA, CORTICAL	0/60 (41)	3/59 (47) 0.1478
	PHEOCHROMOCYTOMA	4/60 (42)	5/59 (47) 0.5722
BRAIN	ASTROCYTOMA	1/60 (42)	0/59 (47) 1.0000
	GRANULAR CELL TUMOR	1/60 (42)	2/59 (47) 0.5426
	OLIGODENDROGLIOMA	0/60 (41)	1/59 (47) 0.5341
CAVITY, ABDOMINAL	ADENOCARCINOMA	0/60 (41)	1/59 (47) 0.5341
	OSTEOSARCOMA	1/60 (42)	0/59 (47) 1.0000
HEART	SCHWANNOMA	1/60 (42)	1/59 (47) 0.7801
KIDNEYS	ADENOCARCINOMA	0/60 (41)	1/59 (47) 0.5341
	ADENOMA, RENAL TUBULE, (AV) TYPE	0/60 (41)	1/59 (47) 0.5341
LIVER	ADENOCARCINOMA	1/60 (42)	1/59 (47) 0.7801
	ADENOMA, HEPATOCELLULAR	3/60 (42)	2/59 (47) 0.8528
LUNG	CARCINOMA, BRONCHIOLAR ALVEOL	1/60 (42)	0/59 (47) 1.0000
LYMPH NODE, MESENT	HEMANGIOSARCOMA	1/60 (42)	0/59 (47) 1.0000
MAMMARY GLAND	FIBROADENOMA	0/60 (41)	2/59 (47) 0.2824
MULTICENTRIC NEOPL	LEUKEMIA, LARGE GRANULAR LYMP	1/60 (42)	0/59 (47) 1.0000
	SARCOMA, HISTIOCYTIC	3/60 (42)	0/59 (47) 1.0000
PANCREAS	ADENOMA, ISLET CELL	3/60 (42)	5/59 (48) 0.4347
	CARCINOMA, ISLET CELL	2/60 (42)	3/59 (47) 0.5538

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60)	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low
PARATHYROID GLANDS	ADENOMA	2/60 (42)	1/59 (47) 0.8989
PITUITARY GLAND	ADENOMA, PARS DISTALIS	44/60 (52)	42/59 (55) 0.9067
PROSTATE GLAND	ADENOCARCINOMA	1/60 (42)	0/59 (47) 1.0000
Pancreas	C_islet cell adenoma+carcinoma	5/60 (42)	8/59 (48) 0.3690
SEMINAL VESICLES	ADENOCARCINOMA	0/60 (41)	1/59 (47) 0.5341
SKIN	ADENOMA, BASAL CELL	2/60 (42)	2/59 (47) 0.7332
	HAIR FOLLICLE TUMOR	0/60 (41)	1/59 (47) 0.5341
	KERATOACANTHOMA	2/60 (42)	1/59 (47) 0.8989
SKIN, SUBCUTIS	FIBROMA	3/60 (42)	2/59 (47) 0.8528
	FIBROSARCOMA	3/60 (42)	1/59 (47) 0.9542
	HEMANGIOSARCOMA	0/60 (41)	1/59 (47) 0.5341
	LIPOMA	0/60 (41)	2/59 (47) 0.2824
	LIPOSARCOMA	0/60 (41)	1/59 (47) 0.5341
SMALL INTESTINE, J	ADENOMA	0/60 (41)	1/59 (47) 0.5341
SPLEEN	LEIOMYOSARCOMA	0/60 (41)	1/59 (47) 0.5341
STOMACH, GLANDULAR	ADENOCARCINOMA	0/60 (41)	2/59 (48) 0.2880
STOMACH, NONGLANDU	ADENOCARCINOMA	0/60 (41)	1/59 (47) 0.5341
TESTES	ADENOMA, LEYDIG CELL	2/60 (42)	3/59 (47) 0.5538
	HEMANGIOMA	1/60 (42)	0/59 (47) 1.0000
THYROID GLAND	ADENOMA, C-CELL	5/60 (43)	3/59 (47) 0.8936
	ADENOMA, FOLLICULAR CELL	3/60 (42)	2/59 (47) 0.8528
	CARCINOMA, C-CELL	1/60 (42)	0/59 (47) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60)	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low
Thyroid gland	C_c-cell adenoma+carcinoma	5/60 (43)	3/59 (47) 0.8936
URINARY BLADDER	ADENOCARCINOMA	1/60 (42)	1/59 (47) 0.7801
Whold Body	C_Hemangiosarcoma	2/60 (42)	1/59 (47) 0.8989
ZYMBAL`S GLAND	CARCINOMA, ZYMBALS GLAND	1/60 (42)	0/59 (47) 1.0000

Table 13: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between the Vehicle Controls and the Treated Groups-Female Rats

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	60 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	240 mg/kg/day High (N=60) P-value - Vehicle vs. High
ADRENAL GLANDS	ADENOMA, CORTICAL	0/60 (39) 0.1484	0/60 (45) NC	1/60 (42) 0.5185	1/60 (32) 0.4507
	PHEOCHROMOCYTOMA	0/60 (39) 0.8390	4/60 (45) 0.0772	3/60 (42) 0.1346	0/60 (32) NC
BRAIN	ASTROCYTOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, PARS DISTALIS	3/60 (40) 0.9774	3/60 (45) 0.7166	1/60 (42) 0.9477	0/60 (32) 1.0000
	GRANULAR CELL TUMOR	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	MENINGIOMA	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
CAVITY, ABDOMINAL	ADENOCARCINOMA (PRIMARY SITE	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
	CARCINOMA, RENAL TUBULE, (AV)	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	LIPOSARCOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	SCHWANNOMA	0/60 (39) 0.4650	0/60 (45) NC	1/60 (41) 0.5125	0/60 (32) NC
CAVITY, ORAL	PAPILLOMA, SQUAMOUS CELL	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
Gland Thyroid	C_FOLLICULAR cell Adenoma+Carcinoma	1/60 (39) 0.4371	1/60 (45) 0.7874	1/60 (41) 0.7655	1/60 (32) 0.7018
HEART	SCHWANNOMA	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
KIDNEYS	ADENOMA, RENAL TUBULE, (AV) TYPE	2/60 (40) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, RENAL TUBULE, (AV)	1/60 (40) 0.9303	2/60 (45) 0.5446	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	LIPOSARCOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
LIVER	ADENOCARCINOMA	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	60 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	240 mg/kg/day High (N=60) P-value - Vehicle vs. High
LUNG	PHEOCHROMOCYTOMA	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
LYMPH NODE, INGUIN	ADENOCARCINOMA	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
MAMMARY GLAND	ADENOCARCINOMA	24/60 (47) 0.8741	12/60 (48) 0.9978	17/60 (47) 0.9522	11/60 (38) 0.9892
	ADENOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	FIBROADENOMA	15/60 (42) 0.9755	27/60 (51) 0.0730	24/60 (48) 0.1247	9/60 (35) 0.8834
MULTICENTRIC NEOPL	LEUKEMIA, GRANULOCYTIC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	LYMPHOMA	0/60 (39) 0.4650	0/60 (45) NC	1/60 (41) 0.5125	0/60 (32) NC
OVARIES	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	HEMANGIOSARCOMA	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
PANCREAS	ADENOMA, ISLET CELL	1/60 (39) 0.8514	2/60 (45) 0.5541	1/60 (42) 0.7713	0/60 (32) 1.0000
	CARCINOMA, ISLET CELL	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
PARATHYROID GLANDS	ADENOMA	0/60 (39) 0.4684	0/60 (45) NC	1/60 (42) 0.5185	0/60 (32) NC
PITUITARY GLAND	ADENOMA, PARS DISTALIS	48/60 (54) 0.1847	49/60 (57) 0.7725	51/60 (57) 0.5805	49/60 (53) 0.3828
	CARCINOMA, PARS DISTALIS	3/60 (40) 0.9691	2/60 (45) 0.8546	1/60 (42) 0.9477	0/60 (32) 1.0000
	C_PARS DISTALIS ADENOMA++CARCINOMA	51/60 (55) 0.4049	51/60 (57) 0.8246	52/60 (58) 0.8168	49/60 (53) 0.6627
SKIN	ADENOMA, BASAL CELL	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
	HAIR FOLLICLE TUMOR	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
	KERATOACANTHOMA	0/60 (39) 0.4345	0/60 (45) NC	2/60 (42) 0.2657	0/60 (32) NC
SKIN, SUBCUTIS	FIBROSARCOMA	1/60 (39) 0.6309	3/60 (46) 0.3730	0/60 (41) 1.0000	1/60 (32) 0.7018
	SARCOMA, UNDIFFERENTIATED	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=60) P-value - Trend	15 mg/kg/day Low (N=60) P-value - Vehicle vs. Low	60 mg/kg/day Med (N=60) P-value - Vehicle vs. Med	240 mg/kg/day High (N=60) P-value - Vehicle vs. High
SPLEEN	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
THYMUS	THYMOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
THYROID GLAND	ADENOMA, C-CELL	5/60 (40) 0.7817	4/60 (46) 0.8230	2/60 (41) 0.9494	2/60 (33) 0.9108
	ADENOMA, FOLLICULAR CELL	1/60 (39) 0.9395	1/60 (45) 0.7874	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, FOLLICULAR CELL	0/60 (39) 0.1476	0/60 (45) NC	1/60 (41) 0.5125	1/60 (32) 0.4507
URINARY BLADDER	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	PAPILLOMA, TRANSITIONAL CELL	0/60 (39) 0.7516	1/60 (45) 0.5357	0/60 (41) NC	0/60 (32) NC
	SCHWANNOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
UTERUS WITH CERVIX	ADENOCARCINOMA	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000
	CARCINOMA, YOLK SAC	0/60 (39) 0.2089	0/60 (45) NC	0/60 (41) NC	1/60 (33) 0.4583
	GRANULAR CELL TUMOR	2/60 (39) 0.8494	0/60 (45) 1.0000	1/60 (41) 0.8888	0/60 (32) 1.0000
	POLYP, ENDOMETRIAL STROMAL	4/60 (40) 0.3096	1/60 (45) 0.9799	4/60 (42) 0.6712	3/60 (33) 0.6976
	POLYP, STROMAL	0/60 (39) 0.2038	0/60 (45) NC	0/60 (41) NC	1/60 (32) 0.4507
	SCHWANNOMA	1/60 (39) 0.3215	0/60 (45) 1.0000	1/60 (41) 0.7655	1/60 (32) 0.7018
VAGINA	GRANULAR CELL TUMOR	1/60 (39) 0.3752	0/60 (45) 1.0000	0/60 (41) 1.0000	1/60 (33) 0.7101
	SCHWANNOMA	0/60 (39) 0.4650	0/60 (45) NC	1/60 (41) 0.5125	0/60 (32) NC
ZYMBAL`S GLAND	CARCINOMA, ZYMBALS GLAND	1/60 (39) 1.0000	0/60 (45) 1.0000	0/60 (41) 1.0000	0/60 (32) 1.0000

Table 14: Intercurrent Mortality Rate -Male Mice

	Water 0 mg|kg|day (N=25)		Vehicle 0 mg|kg|day (N=25)		60 mg|kg|day (N=25)		200 mg|kg|day (N=25)		600 mg|kg|day (N=25)		Positive 75 mg|kg|day (N=15)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	2	8.00	2	8.00	1	4.00	2	13.33
14 - 26	.	.	.	.	1	12.00	.	.	.	.	3	33.33
Ter. Sac.	25	100.00	25	100.00	22	88.00	23	92.00	24	96.00	10	66.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 15: Intercurrent Mortality Rate -Female Mice

	Water 0 mg|kg|day (N=25)		Vehicle 0 mg|kg|day (N=25)		60 mg|kg|day (N=25)		200 mg|kg|day (N=25)		600 mg|kg|day (N=25)		Positive 75 mg|kg|day (N=15)	
Week	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**	**No. of Death**	**Cum. %**
0 - 13	.	.	.	.	.	.	1	4.00	1	4.00	.	.
14 - 26	3	12.00	.	.	.	.	.	.	4	20.00	5	33.33
Ter. Sac.	22	88.00	25	100.00	25	100.00	24	96.00	20	80.00	10	66.67

Cum. %: Cumulative percentage except for Ter. Sac.

Table 16: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control-Male Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.8632	0.0384	0.0959	0.2390	0.0010
Homogeneity	Log-Rank	0.3342	0.0770	0.1530	0.3173	0.0019

Table 17: Intercurrent Mortality Comparison between Treated Groups and Water Control-Male Mice

Test	Statistic	P_Value Water vs Treated Groups Dose Response	P_Value Water vs. Low	P_Value Water vs. Med	P_Value Water vs. High	P_Value Water vs. Positive
Dose-Response	Likelihood Ratio	0.8632	0.0384	0.0959	0.2390	0.0010

Test	Statistic	P_Value Water vs Treated Groups Dose Response	P_Value Water vs. Low	P_Value Water vs. Med	P_Value Water vs. High	P_Value Water vs. Positive
Homogeneity	Log-Rank	0.3342	0.0770	0.1530	0.3173	0.0019

Table 18: Intercurrent Mortality Comparison between Treated Groups and Vehicle Control-Female Mice

Test	Statistic	P_Value Vehicle vs Treated Groups Dose Response	P_Value Vehicle vs. Low	P_Value Vehicle vs. Med	P_Value Vehicle vs. High	P_Value Vehicle vs. Positive
Dose-Response	Likelihood Ratio	0.0012	.	0.2390	0.0068	0.0011
Homogeneity	Log-Rank	0.0072	.	0.3173	0.0196	0.0019

Table 19: Intercurrent Mortality Comparison between Treated Groups and Water Control-Female Mice

Test	Statistic	P_Value Water vs Treated Groups Dose Response	P_Value Water vs. Low	P_Value Water vs. Med	P_Value Water vs. High	P_Value Water vs. Positive
Dose-Response	Likelihood Ratio	0.0764	0.0384	0.3061	0.4527	0.1097
Homogeneity	Log-Rank	0.0714	0.0770	0.3170	0.4535	0.0973

Table 20: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25) 0.2108	3/25 (23) 0.4592	2/25 (23) 0.6631	4/25 (24) 0.3136
MULTICENTRIC NEOPL	HEMANGIOSARCOMA	1/25 (25) 0.3993	2/25 (23) 0.4681	4/25 (23) 0.1489	2/25 (24) 0.4844
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.2526	0/25 (23) NC	0/25 (23) NC	1/25 (24) 0.4898
SKIN	CARCINOMA, SQUAMOUS CELL	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (24) 1.0000

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
	PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.7368	1/25 (23) 0.4792	0/25 (23) NC	0/25 (24) NC
THYMUS	THYMOMA	1/25 (25) 0.7474	0/25 (23) 1.0000	1/25 (23) 0.7340	0/25 (24) 1.0000
Whold Body	C_Hemangiosarcoma+Hemangioma	1/25 (25) 0.3993	2/25 (23) 0.4681	4/25 (23) 0.1489	2/25 (24) 0.4844

Table 21: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Water Control and the Treated Groups-Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
ADRENAL GLANDS	PHEOCHROMOCYTOMA	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (24) 1.0000
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25) 0.2108	3/25 (23) 0.4592	2/25 (23) 0.6631	4/25 (24) 0.3136
MULTICENTRIC NEOPL	HEMANGIOMA	2/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (24) 1.0000
	HEMANGIOSARCOMA	0/25 (25) 0.2801	2/25 (23) 0.2243	4/25 (23) 0.0455	2/25 (24) 0.2347
NOSE	ADENOCARCINOMA	1/25 (25) 1.0000	0/25 (23) 1.0000	0/25 (23) 1.0000	0/25 (24) 1.0000
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.2526	0/25 (23) NC	0/25 (23) NC	1/25 (24) 0.4898
SKIN	PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.7368	1/25 (23) 0.4792	0/25 (23) NC	0/25 (24) NC
THYMUS	THYMOMA	2/25 (25) 0.8750	0/25 (23) 1.0000	1/25 (23) 0.8670	0/25 (24) 1.0000
Whold Body	C_Hemangiosarcoma+Hemangioma	2/25 (25) 0.5169	2/25 (23) 0.6631	4/25 (23) 0.2933	2/25 (24) 0.6798

Table 22: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Vehicle Control and the Treated Groups-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
EARS	PAPILLOMA, SQUAMOUS CELL	0/25 (25) 0.4845	0/25 (25) NC	1/25 (24) 0.4898	0/25 (23) NC
HARDERIAN GLANDS	ADENOMA	1/25 (25) 0.2157	1/25 (25) 0.7551	0/25 (24) 1.0000	2/25 (23) 0.4681
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
MULTICENTRIC NEOPL	HEMANGIOMA	1/25 (25) 0.4199	0/25 (25) 1.0000	0/25 (24) 1.0000	1/25 (23) 0.7340
	HEMANGIOSARCOMA	1/25 (25) 0.1282	2/25 (25) 0.5000	0/25 (24) 1.0000	3/25 (23) 0.2730
SKIN	PAPILLOMA, SQUAMOUS CELL	1/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
THYMUS	THYMOMA	0/25 (25) 0.7423	1/25 (25) 0.5000	0/25 (24) NC	0/25 (23) NC
UTERUS WITH CERVIX	POLYP, STROMAL	1/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
Whold Body	C_Hemangiosarcoma+Hemangioma	2/25 (25) 0.2246	2/25 (25) 0.6954	0/25 (24) 1.0000	3/25 (23) 0.4592

Table 23: Tumor Rates and P-Values for Dose Response Relationship and Pairwise Comparisons between Water Control and the Treated Groups-Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
ANUS	CARCINOMA, SQUAMOUS CELL	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
EARS	PAPILLOMA, SQUAMOUS CELL	0/25 (24) 0.4896	0/25 (25) NC	1/25 (24) 0.5000	0/25 (23) NC
HARDERIAN GLANDS	ADENOMA	0/25 (24) 0.0992	1/25 (25) 0.5102	0/25 (24) NC	2/25 (23) 0.2340
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
MULTICENTRIC NEOPL	HEMANGIOMA	0/25 (24) 0.2396	0/25 (25) NC	0/25 (24) NC	1/25 (23) 0.4894

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25) P-value - Trend	60 mg/kg/day Low (N=25) P-value - Vehicle vs. Low	200 mg/kg/day Med (N=25) P-value - Vehicle vs. Med	600 mg/kg/day High (N=25) P-value - Vehicle vs. High
	HEMANGIOSARCOMA	3/25 (24) 0.3519	2/25 (25) 0.8384	0/25 (24) 1.0000	3/25 (23) 0.6460
STOMACH, NONGLANDU	PAPILLOMA, SQUAMOUS CELL	1/25 (24) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
THYMUS	THYMOMA	3/25 (24) 0.9968	1/25 (25) 0.9498	0/25 (24) 1.0000	0/25 (23) 1.0000
VAGINA	POLYP	2/25 (25) 1.0000	0/25 (25) 1.0000	0/25 (24) 1.0000	0/25 (23) 1.0000
Whold Body	C_Hemangiosarcoma+Hemangioma	3/25 (24) 0.3519	2/25 (25) 0.8384	0/25 (24) 1.0000	3/25 (23) 0.6460

Table 24: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control- Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
EPIDIDYMIDES	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
HARDERIAN GLANDS	ADENOMA	0/25 (25)	1/15 (12) 0.3243
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25)	4/15 (12) 0.0728
	CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/15 (13) 0.3421
MEDIASTINUM/PLEURA	CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/15 (13) 0.3421
MESENTERY/PERITONE	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
MULTICENTRIC NEOPL	LYMPHOMA	0/25 (25)	3/15 (14) 0.0398
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	1/15 (12) 0.3243
SKIN	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	10/15 (14) <0.001
STOMACH, GLANDULAR	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (25)	4/15 (13) 0.0097
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	11/15 (14) <0.001

Table 25: Tumor Rates and P-Values for Comparisons between Water Control and Positive Control- Male Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
EPIDIDYMIDES	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
HARDERIAN GLANDS	ADENOMA	0/25 (25)	1/15 (12) 0.3243

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25)	4/15 (12) 0.0728
	CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/15 (13) 0.3421
MEDIASTINUM/PLEURA	CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/15 (13) 0.3421
MESENTERY/PERITONE	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
MULTICENTRIC NEOPL	LYMPHOMA	0/25 (25)	3/15 (14) 0.0398
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	1/15 (12) 0.3243
SKIN	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	10/15 (14) <0.001
STOMACH, GLANDULAR	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (25)	4/15 (13) 0.0097
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	11/15 (14) <0.001

Table 26: Tumor Rates and P-Values for Comparisons between Vehicle Control and Positive Control- Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
EARS	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	1/15 (12) 0.3243
HARDERIAN GLANDS	ADENOMA	1/25 (25)	1/15 (13) 0.5733
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	2/25 (25)	3/15 (12) 0.1816
MULTICENTRIC NEOPL	HEMANGIOSARCOMA	1/25 (25)	3/15 (13) 0.1066
	LYMPHOMA	0/25 (25)	5/15 (14) 0.0035
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	1/15 (12) 0.3243
SKIN	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (12) 0.0991
	HAIR FOLLICLE TUMOR	0/25 (25)	1/15 (13) 0.3421
	PAPILLOMA, SQUAMOUS CELL	1/25 (25)	2/15 (12) 0.2407
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (25)	2/15 (13) 0.1110
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	12/15 (14) <0.001
URINARY BLADDER	PAPILLOMA, TRANSITIONAL CELL	0/25 (25)	1/15 (12) 0.3243
UTERUS WITH CERVIX	ADENOCARCINOMA	0/25 (25)	1/15 (12) 0.3243
	ADENOMA	0/25 (25)	1/15 (12) 0.3243
	POLYP, GLANDULAR	0/25 (25)	6/15 (13) <0.001
	POLYP, STROMAL	1/25 (25)	1/15 (12) 0.5495
VAGINA	HAIR FOLLICLE TUMOR	0/25 (25)	1/15 (13) 0.3421
VULVA	CARCINOMA, SQUAMOUS CELL	0/25 (25)	1/15 (13) 0.3421
	PAPILLOMA, SQUAMOUS CELL	0/25 (25)	3/15 (13) 0.0339
Whold Body	C_Hemangiosarcoma+Hemangioma	2/25 (25)	3/15 (13) 0.2091

Table 27: Tumor Rates and P-Values for Comparisons between Water Control and Positive Control- Female Mice

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
EARS	PAPILLOMA, SQUAMOUS CELL	0/25 (24)	1/15 (12) 0.3333
HARDERIAN GLANDS	ADENOMA	0/25 (24)	1/15 (13) 0.3514
LUNG	ADENOMA, BRONCHIOLAR ALVEOLAR	1/25 (24)	3/15 (12) 0.0980
MULTICENTRIC NEOPL	HEMANGIOSARCOMA	3/25 (24)	3/15 (13) 0.3479
	LYMPHOMA	0/25 (24)	5/15 (14) 0.0040
PHARYNX	PAPILLOMA, SQUAMOUS CELL	0/25 (24)	1/15 (12) 0.3333
SKIN	CARCINOMA, SQUAMOUS CELL	0/25 (24)	2/15 (12) 0.1048
	HAIR FOLLICLE TUMOR	0/25 (24)	1/15 (13) 0.3514
	PAPILLOMA, SQUAMOUS CELL	0/25 (24)	2/15 (12) 0.1048
STOMACH, NONGLANDU	CARCINOMA, SQUAMOUS CELL	0/25 (24)	2/15 (13) 0.1171
	PAPILLOMA, SQUAMOUS CELL	1/25 (24)	12/15 (14) <0.001
THYMUS	THYMOMA	3/25 (24)	0/15 (12) 1.0000
URINARY BLADDER	PAPILLOMA, TRANSITIONAL CELL	0/25 (24)	1/15 (12) 0.3333
UTERUS WITH CERVIX	ADENOCARCINOMA	0/25 (24)	1/15 (12) 0.3333
	ADENOMA	0/25 (24)	1/15 (12) 0.3333
	POLYP, GLANDULAR	0/25 (24)	6/15 (13) <0.001
	POLYP, STROMAL	0/25 (24)	1/15 (12) 0.3333
VAGINA	HAIR FOLLICLE TUMOR	0/25 (24)	1/15 (13) 0.3514

Organ Name	Tumor Name	0 mg/kg/day Vehicle (N=25)	Positive (N=15) P-value - Vehicle vs. Positive
	POLYP	2/25 (25)	0/15 (12) 1.0000
VULVA	CARCINOMA, SQUAMOUS CELL	0/25 (24)	1/15 (13) 0.3514
	PAPILLOMA, SQUAMOUS CELL	0/25 (24)	3/15 (13) 0.0368
Whold Body	C_Hemangiosarcoma+Hemangioma	3/25 (24)	3/15 (13) 0.3479

Figure 1: Kaplan-Meier Survival Functions for Male Rats

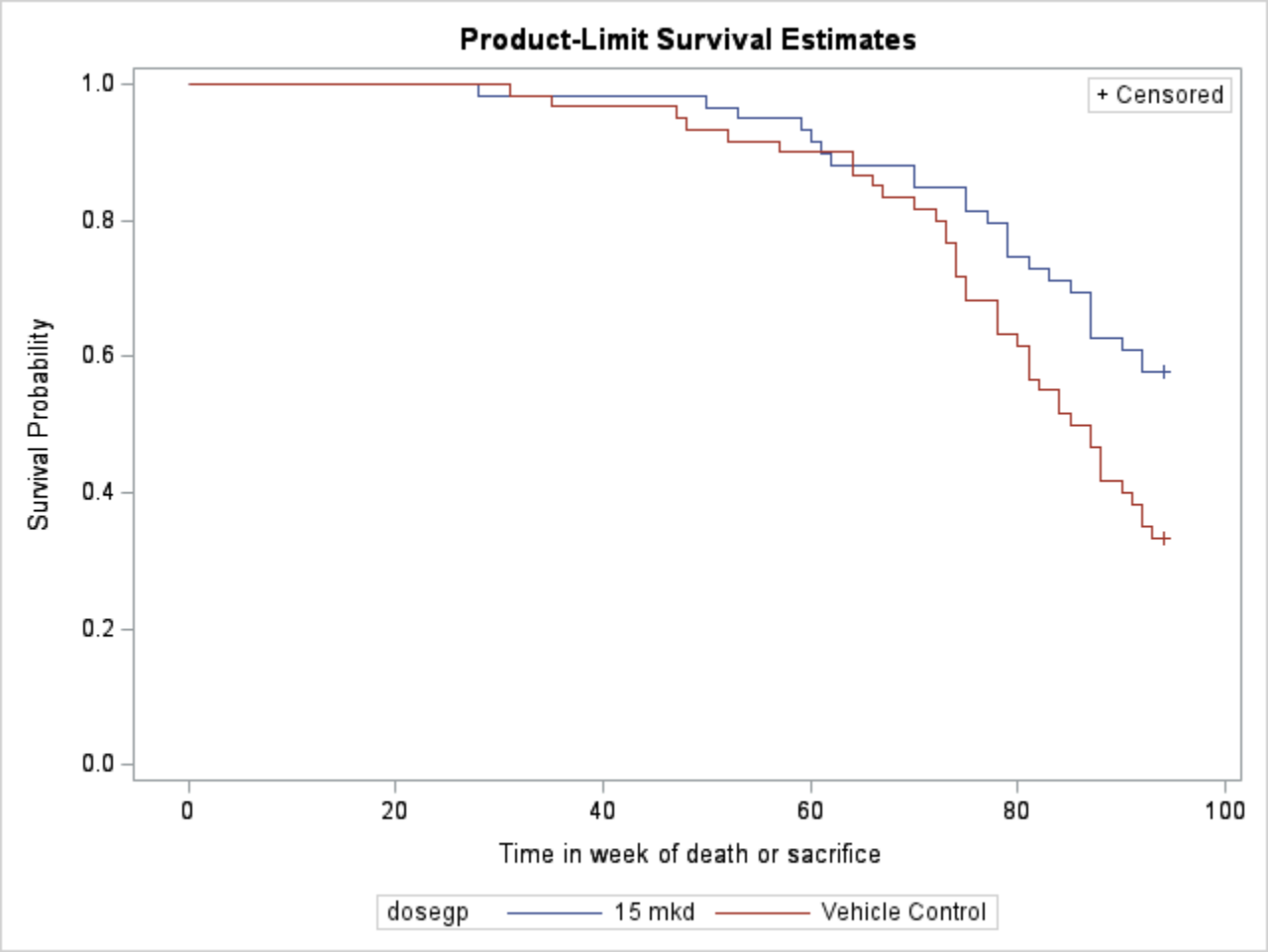


Figure 2: Kaplan-Meier Survival Functions for Female Rats

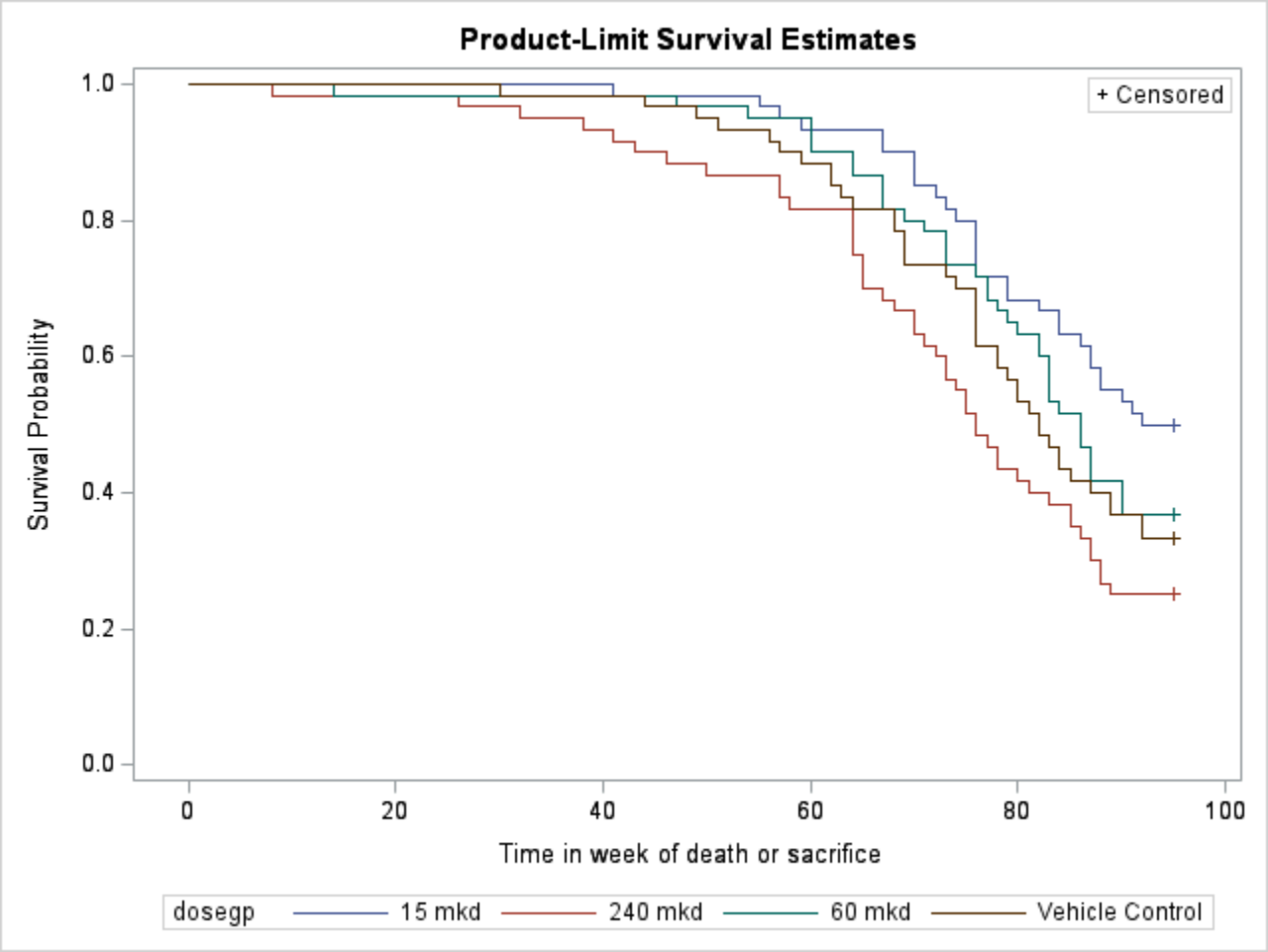


Figure 3: Kaplan-Meier Survival Functions for Male Mice

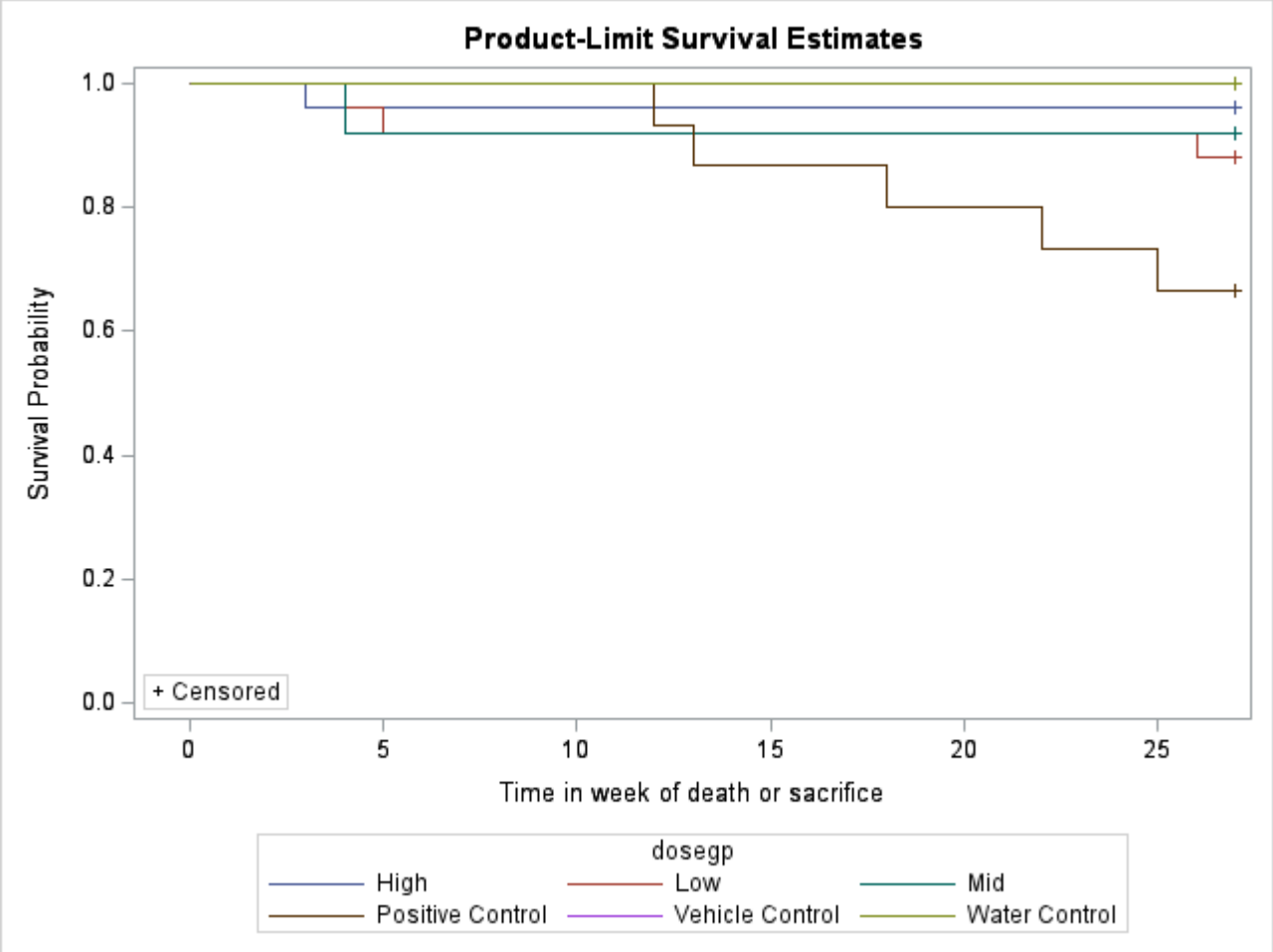
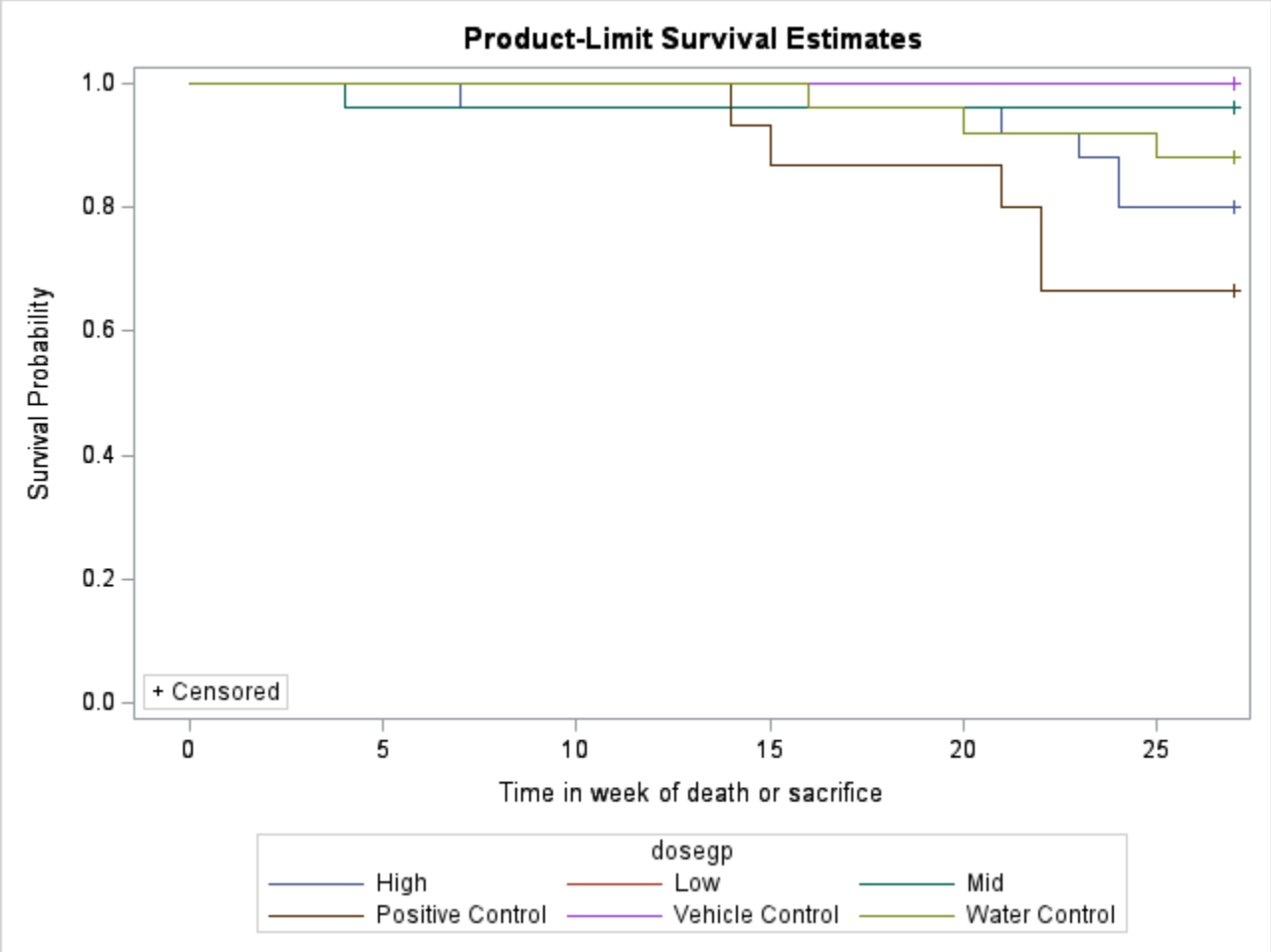


Figure 4: Kaplan-Meier Survival Functions for Female Mice



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