SZN-043, an R-Spondin mimetic in development for the treatment of liver disease, demonstrates a strong safety profile in nonclinical toxicology studies

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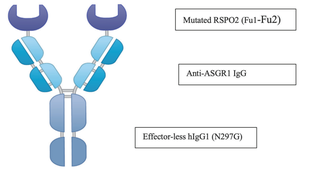
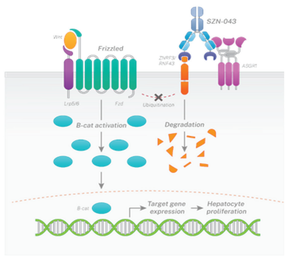
Introduction and Background

- Acute alcoholic hepatitis (AH) is a serious form of acute decompensation of alcoholic liver disease that develops in heavy drinkers.
- In the liver, the Wnt/β-catenin signaling plays an important role in tissue homeostasis, as well as in hepatocyte regeneration and maturation. Severe AH is associated with impaired hepatocyte proliferation, and increased Wnt signaling along with increased hepatocyte proliferation has been linked to increased survival.
- SZN-043 is an antibody fusion protein comprised of mutated version of RSPO fused to an antibody targeting asialoglycoprotein receptor 1 (ASGR1), a hepatocyte-specific receptor which facilitates delivery of SZN-043 to the liver (Figure 1). Binding of SZN-043 to ASGR1 results in internalization and removal from the cell surface.
- Binding of SZN-043 to ZNRF3 (zinc and ring finger 3 protein) and RNF43 (ring finger protein 43) via the RSPO moiety selectively removes these E3 ligases from the hepatocyte surface, making only hepatocytes more sensitive to the available Wnt ligands.

Figure 1. Structure and mechanism of action of SZN-043





Rationale for Toxicology Studies

- The mouse and cynomolgus monkey were shown to be pharmacologically relevant species for assessing the pharmacology, PK and toxicity of SZN-043.
- The toxicology program for SZN-043 was designed to support the planned IV dosing in humans with a frequency of every four to seven days for up to four weeks.

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One month GLP toxicology study in mice

- The objective of this GLP-compliant study was to determine the potential toxicity of SZN-043 when given by intravenous bolus injection up to twice weekly for 29 Days to mice and to evaluate the potential reversibility of any findings.

Methods

- Male and female C57BL/6J, 6–8 weeks of age, were dosed twice weekly via IV injection for a total of 9 doses.
- Dose levels were 0, 12.5, 37.5 and 125 mg/kg/dose
- A subgroup of animals was terminated one day after the last dose and a subgroup was allowed to recover for 28 days before sacrifice.
- The parameters and endpoints evaluated are shown in Table 1.

Table 1. Toxicology endpoints evaluated

Parameter	Frequency
Mortality & cage side observations	Twice daily
Clinical observations	Twice weekly
Body weight	Weekly
Food consumption	Weekly
Ophthalmic examination	Predose and end of dosing and at end of recovery
Clinical pathology	End of dosing phase and end of recovery phase
Histopathology	End of dosing phase and end of recovery phase

Clinical Pathology

- Test article-related changes in clinical chemistry parameters are described in Table 2 and Figure 2. Other than ALP, all changes were less than 15% different relative to control. No other liver enzymes were elevated.
- The increase in ALP was an expected observation due to the pharmacologic effect of SZN-043. ASGR1 is largely responsible for the elimination of ALP from serum; thus, depletion of ASGR1 by SZN-043 prevents ALP clearance.
- At the end of the recovery phase all clinical pathology changes returned to baseline with the exception of albumin levels in males and females dosed at 125 mg/kg dose.

Other Results

- There were no test article-related effects on survival.
- There were no test article-related clinical observations or effects on body weight, food consumption, hematology, or coagulation. There were no test article-related ophthalmic, macroscopic, or microscopic findings.
- Test article-related changes in organ weights noted at the terminal necropsy included higher liver to body weight ratios (Figure 3). Also observed were increases, relative to control, in adrenal gland weights in the ≥ 37.5 mg/kg group males (12–30%), higher pituitary gland weights in the 125 mg/kg group males (20–25%).
- No test article-related organ weight differences were noted at the recovery euthanasia.
- All observations in this study, including changes in clinical pathology and organ weights, were considered non-adverse due to the severity, reversibility, and or lack of histologic correlate.
- Based on the results of this study, the NOAEL was 125 mg/kg; which was the highest administered dose in the study.

Table 2. Clinical pathology changes observed

Parameter	Observation
ALP	Minimal to moderate increase
Albumin	Minimal to mild decrease
Albumin/Globulin ratio	Minimal decrease
Total protein	Minimal decrease in females only
Calcium	Minimal decrease in females only at 125 mg/kg

Figure 2. Mean (SD) serum ALP at Day 30

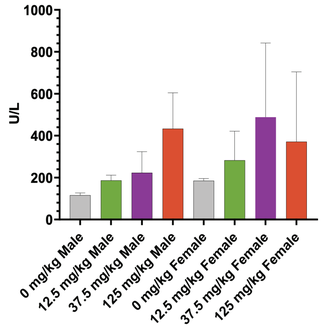
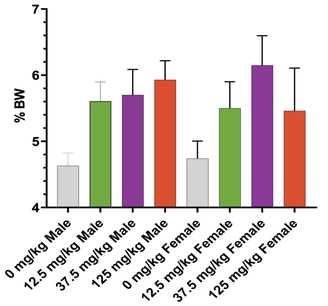


Figure 3. Mean (SD) liver to body weight ratio (%BW) on Day 30



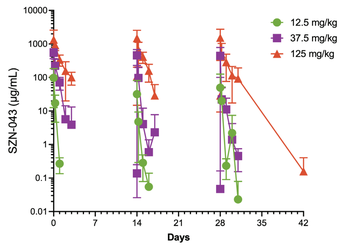
Toxicokinetics

- Exposure was similar between the sexes.
- Exposure increased with dose in a greater than dose proportional manner. This is consistent with saturation of target mediated drug disposition (TMDD) related to binding and clearance by ASGR1 and/or E3 ligases.
- Exposure was approximately 3-fold higher on Day 1 than on Days 15 and 29. Accumulation ratios were 0.309, 0.525, and 0.891 on Day 15 and were 0.768, 0.352, and 0.720 on Day 29 at 12.5, 37.5, and 125 mg/kg, respectively.
- The incidence of immunogenicity was low, ranging from 4–9% in SZN-043-treated animals.

Table 3. Mean SZN-043 TK parameters in mice

Day	Dose (mg/kg)	$AUC_{(0\text{-}Day1)}$ (hr*µg/mL)	C_{max} (µg/mL)
1	12.5	513	97.9
	37.5	4970	541
	125	18200	1350
15	12.5	159	31.7
	37.5	2610	455
	125	16300	1490
29	12.5	394	49.7
	37.5	1750	441
	125	13100	1530

Figure 4. Mean (S.D.) serum concentrations of SZN-043



One month GLP toxicology study in cynomolgus monkeys

- The objectives of this GLP study were to determine the potential toxicity and TK of SZN-043 when given by IV bolus injection twice weekly for 29 days to cynomolgus monkeys, and to evaluate the potential reversibility of any findings.

Study Design

Table 4.

Group No.	Test Material	Dose Level (mg/kg)	No. of Animals			
			Main Study		Recovery Study	
			Males	Females	Males	Females
1	Control	0	4	4	2	2
2	SZN-043	12.5	3	3	-	-
3	SZN-043	37.5	3	3	-	-
4	SZN-043	125	4	4	4	4

Methods

- Cynomolgus monkeys, aged 22–60 months, were allocated to study groups as shown in Table 4.
- Animals were dosed with SZN-043 twice weekly by IV injection until Day 29. Main Study animals were terminated on Day 30 and Recovery Animals terminated on Day 58.
- The following parameters and endpoints were evaluated: mortality, clinical signs, body weights, food consumption, ophthalmology, veterinary physical examinations, qualitative electro cardiology, neurologic examinations, blood pressure and heart rate, respiration rate, clinical pathology, TK, ADA, PD biomarkers, complement, organ weights, and macroscopic and microscopic examinations.

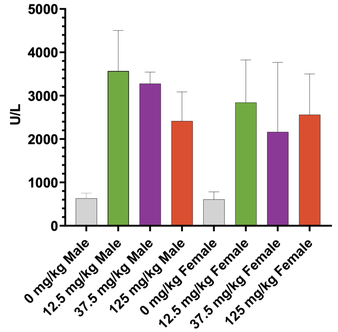
Table 5. Toxicology endpoints evaluated

Parameter	Frequency
Mortality & cage side observations	Once daily
Clinical observations	Twice weekly
Body weight	Weekly
Food consumption	Daily
Cardiovascular safety pharmacology endpoints	Predose and during Weeks 1, 4, and last week of recovery
Neurologic examination	
Ophthalmic examination	
Clinical pathology	Predose and on Days 16, 30, and end of recovery phase
Histopathology	End of dosing phase and end of recovery phase
Immunogenicity (anti-drug antibody [ADA] analysis)	Predose and on Days 15, 29, and end of recovery phase

Clinical pathology

- SZN 043-related changes in clinical chemistry parameters were limited moderate to marked increases in alkaline phosphatase (ALP) that were generally of similar magnitude on Days 16 and 30, with recovery for animals at by Day 57 (Figure 4). This increase was expected based on the binding of SZN-043 to ASGR1 resulting in depletion from the cell surface.
- No other liver enzymes were elevated.

Figure 4. Mean (SD) serum ALP concentrations on Day 30

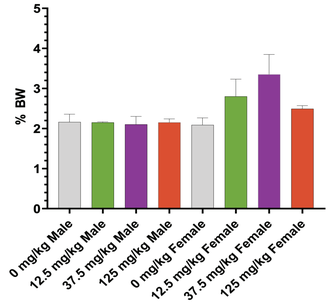


Other results

- All animals survived to scheduled necropsy. One animal at 37.5 mg/kg experienced signs of an anaphylactoid-type reaction following dosing on Day 29, which upon further evaluation was attributed to ADA-mediated hypersensitivity.
- There were no SZN-043-related effects on body weights, qualitative food consumption, ophthalmology, veterinary physical examinations, qualitative electrocardiology, neurologic examinations, blood pressure and heart rate, respiration rate, hematology, coagulation, and urinalysis, or macroscopic and microscopic examinations.
- SZN-043-related organ weight (mean absolute and/or relative) differences were observed in the liver in female animals (Figure 5). The increase in liver weight was non-dose dependent and had no microscopic correlate.
- No changes were observed in any other measured parameter, including macroscopic and microscopic evaluation.

- All observations in this study, including changes in clinical pathology and organ weights, were considered non-adverse due to the severity, reversibility, and or lack of histologic correlate.
- Based on the results under the conditions of this study, the NOAEL was 125 mg/kg which was the highest administered dose in the study.

Figure 5. Mean (SD) liver to body weight ratio (%BW) on Day 30



Toxicokinetics

- Serum concentrations of SZN-043 increased with dose with some evidence of greater than proportional increase between 12.5 and 37.5 mg/kg that is consistent with TMDD associated with binding of SZN-043 to its cell surface targets (Figure 6 and Table 6).
- There were no substantial differences in exposure between males and females.
- There was no evidence of substantial accumulation with repeated dosing.
- The incidence of immunogenicity was moderate to high, ranging from 33% at 12.5 mg/kg, 83% at 37.5 mg/kg to 87.5% at 125 mg/kg. Presence of ADA did not substantively affect the observed TK in this study.

Figure 6. Mean (SD) serum SZN-043 concentrations

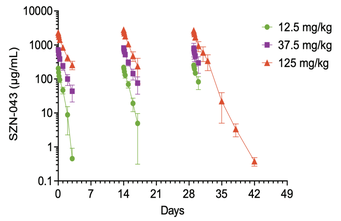


Table 6. Mean (SD) serum TK parameters

Day	Dose (mg/kg)	C_{max} (µg/mL)	C_{max}/Dose (µg/mL/ mg/kg)	AUC_{0-1} (µg*day/ mL)	AUC_{0-1}/Dose (µg*day/mL/ mg/kg)	AUC_{last} (µg*day/ mL)	AR AUC
1	12.5	204 (25.8)	16.3 (2.06)	91.7 (14.6)	7.33 (1.17)	124 (25.5)	NA
	37.5	713 (106)	19 (2.83)	382 (52.4)	10.2 (1.4)	621 (120)	NA
	125	2310 (337)	18.5 (2.7)	1360 (198)	10.9 (1.59)	2330 (370)	NA
15	12.5	219 (20.6)	17.5 (1.65)	123 (20.7)	9.81 (1.66)	179 (37)	1.35 (0.229)
	37.5	810 (73.3)	21.6 (1.96)	491 (54.2)	13.1 (1.45)	823 (139)	1.29 (0.0959)
	125	2820 (384)	22.5 (3.07)	1700 (228)	13.6 (1.82)	2830 (452)	1.26 (0.106)
29	12.5	252 (10.5)	20.2 (0.884)	143 (25.3)	11.4 (2.02)	NA	1.61 (0.426)
	37.5	779 (333)	20.8 (8.88)	456 (216)	12.2 (5.77)	NA	1.18 (0.561)
	125	2690 (301)	21.5 (2.41)	1600 (302)	12.8 (2.42)	2200 (880)	1.19 (0.240)

Conclusions

- **SZN-043 was evaluated in GLP-compliant toxicology studies in mice and cynomolgus monkeys with twice weekly administration for 28 days followed by a one-month recovery period**
- **SZN-043 was well tolerated with no drug-related mortalities**
- **SZN-043-related observations were limited to minimal to moderate changes in serum chemistry parameters, notably increases in ALP that were attributed to binding of SZN-043 to ASGR1 resulting in depletion of the receptor from the cell surface. In mice, minimal to mild changes in serum albumin were observed.**
- **Increases in some organ weights were noted in some SZN-043 treated groups, with increases in liver weight consistently increased in both mouse and cynomolgus monkey, and pituitary and adrenal gland in mice**
- **All SZN-043 related effects were considered non-adverse, and all (with the exception of serum albumin in mice) returned to baseline after recovery**
- **Serum exposure to SZN-043 was consistent with the expectations, with greater than dose proportional increase in AUC which was attributed to TMDD related to binding to ASGR1.**
- **The incidence of immunogenicity was low in mice, and moderate to high in cynomolgus monkeys, but did not substantively affect serum SZN-043 exposures or the interpretation of these studies**
- **Based on the results of these studies, the NOAEL was 125 mg/kg which was the highest administered dose tested**