PHARMACOKINETICS, PHARMACODYNAMICS, AND TOXICOLOGY OF SZN-043, A HEPATOCYTE-TARGETED WNT-POTENTIATOR, IN NONHUMAN PRIMATES (3525578)

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Background

Wnt signaling is critical for hepatocyte development and for regeneration after liver injury, and it contributes to the region-specific expression of metabolic genes. When Wnt signaling is blocked or absent, liver regeneration is impaired, and there is a delay and reduction in hepatocyte proliferation and tissue regeneration (Planas-Paz 2016). Wnt signaling is a major regulator of liver zone-specific metabolic gene expression, and active signaling promotes expression of many genes enriched in or specific to the central zone (Benhamouche 2006; Planas-Paz 2016). R-spondins enhance Wnt signaling via stabilization of Wnt receptors, and regulation of R-spondin activity robustly affects liver zonation (Planas-Paz 2016, Rocha 2015). We have built a hepatocyte-targeted R-spondin mimetic, SZN-043, that binds the E3 ubiquitin ligases, ZNRF3, RNF43, and the hepatocyte-specific receptor, ASGR1 (Zhang 2020). SZN-043 shows robust hepatocyte proliferation in mouse models of liver disease and is being progressed toward the clinic for use in liver indications characterized by hepatocyte loss.

Understanding the PKPD and toxicology of a potential therapeutic is essential to its preclinical development. SZN-043 is pharmacologically active in nonhuman primates, and the similarities between NHP and humans makes this species a highly relevant system for evaluating SZN-043.

Study design for SZN-043 pilot tox study

- 2-4 y.o. F cynomolgus monkeys
- IV dosing aligned with proposed clinical dose route
- Animals dosed twice weekly for two weeks, a treatment frequency and duration consistent with proposed clinical use
- Main study animals terminated on Day 16 (one day after last dose) with recovery animals terminated on Day 44 (29 days after last dose)

Table 1. Dose groups

Group	Test Article	Dose (mg/kg)	Main Study	Recovery
1	Vehicle	0	3	2
2	SZN-043	12.5	3	0
3	SZN-043	37.5	3	0
4	SZN-043	125	3	2

Table 2. Assessments performed in this study

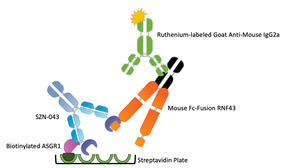
Parameter	Frequency
Mortality	At least twice daily
Cage side observations	At least twice daily
Detailed clinical observations (animals removed from cage for observation)	Once pretreatment, then weekly, and on Days 16 and 43
Clinical pathology (complete blood count, coagulation, and chemistry)	Once pretreatment, then Days 9, 16 and 43
Body weight	Predose and weekly
Food consumption	Quantitatively, once daily

Methods

PK assay measures active SZN-043

- Capture SZN-043 from serum with biotinylated ASGR1 on streptavidin coated plate
- Detect using mouse Fc-Fusion RNF43 and ruthenium-labeled goat anti-mouse IgG2a

Figure 1. Assay format



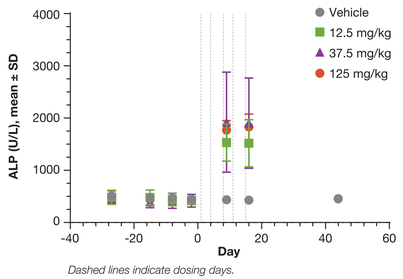
Results

- All animals survived until scheduled necropsy. There were no SZN-043-related clinical observations or changes in food consumption, body weights, or hematology, coagulation, or urinalysis parameters. In addition, there were no SZN-043-related macroscopic, organ weight, or microscopic changes.
- The no-observed-adverse-effect level was considered to be 125 mg/kg/dose, the highest dose tested in this study.

SZN-043 changes in clinical pathology limited to reversible increases in ALP

- Clinical pathology (Complete blood count, coagulation, and chemistry) evaluated pre-dose and on Days 9, 16, and 43.
- No treatment-related changes in clin path noted other than an increase in ALP which is attributed to the binding of SZN-043 to ASGR1 and was considered non-adverse. Increase in ALP is related to binding to, and removal of, the asialoglycoprotein receptor (ASGPR) from the cell surface preventing ALP clearance by ASGPR.

Figure 2.

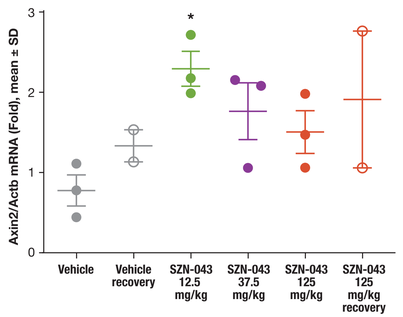


Dashed lines indicate dosing days.

SZN-043 shows evidence of Wnt signaling in the liver

- Axin2, a Wnt target gene. was elevated in the livers of animals treated with SZN-043, as measured by qPCR

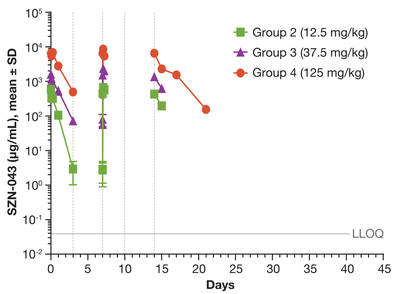
Figure 3.



* p < 0.05, One way ANOVA, Holm-Sidak. Please add this as a footnote.

Exposure to SZN-043 confirmed and consistent with binding to target

Figure 4.



Mean, SD; Dashed lines indicate dosing days

- Dose-normalized AUC increases with dose consistent with target-mediated drug disposition (TMDD) related to binding to ASGR1
- No evidence of accumulation with repeated dosing

Table 3.

Dose (mg/kg)	Dosing Day	$AUC_{0\text{-}tlast}$ (µg-day/mL), mean ± (SD)	$AUC_{0\text{-}tlast}$/D (µg-day/mL/mg/kg), mean ± (SD)	$AUC_{0\text{-}tlast}$ Accum Ratio, mean ± (SD)	C_{max} (µg/mL), mean ± (SD)	C_{max} (µg/mL/mg/kg), mean ± (SD)	C_{max} Accum Ratio, mean ± (SD)
12.5	0	292 (45.3)	23.3 (3.62)	NA	590 (75.1)	47.1 (6.01)	NA
12.5	14	367 (53.0)	29.4 (4.24)	1.26 (1.17)	669 (67.4)	53.5 (5.39)	1.14 (0.079)
37.5	0	1290 (349)	34.2 (9.30)	NA	1620 (94.9)	43.2 (2.53)	NA
37.5	14	1240 (141)	33.1 (3.77)	0.964 (0.405)	2360 (237)	63.1 (6.31)	1.45 (0.0615)
125	0	7510 (1310)	60.1 (10.5)	NA	7410 (1090)	59.3 (8.69)	NA
125	14	6310 (1840)	50.5 (14.7)	0.866 (0.327)	9310 (1990)	74.5 (15.9)	1.17 (0.306)

Low incidence and impact of immunogenicity

- ADA analysis conducted in all animals predose and Study Day 15, and in recovery animals at Day 44
- Incidence of immunogenicity is low with no apparent effects on TK or toxicity
- No apparent impact on TK
  - Low interindividual variability
  - No change in PK between first/last dose

Table 4.

Dose	Positive/Total	Day of positive test
0 mg/kg	1/5	15
12.5 mg/kg	0/3	NA
37.5 mg/kg	1/3	15
125 mg/kg	2/5	44, 44

Study design for PKPD study

- The PKPD of SZN-043 in NHP was evaluated at doses at or below those tested in the toxicology study and in a range suitable for describing the concentration dependency of PKPD. Serum was collected at selected timepoints up to 25 days after initiation of dosing for analysis of SZN-043 and ALP, a biomarker of ASGR1 target occupancy. Non-compartmental PK parameters were estimated
- 2–4 y.o. cynomolgus monkeys, 2M/2F group
- Assays:
  - SZN-043 concentrations
  - ADA

Table 5.

Dose Group	Day of Dosing	Dose level (mg/kg)
1	1	0.5
2	1	2
3	1	5
4	1, 4	12.5

Dose dependent change in PK indicative of TMDD

- The PK of SZN-043 in NHP was consistent with an IgG-like molecule with evidence of target-mediated drug disposition, whereby exposure increased more than proportional to dose, providing further evidence of occupancy of ASGR1.
- Modest change in PK with second dose on Day 3 suggesting that ASGR1 expression and turnover are not affected by SZN-043.

Figure 5.

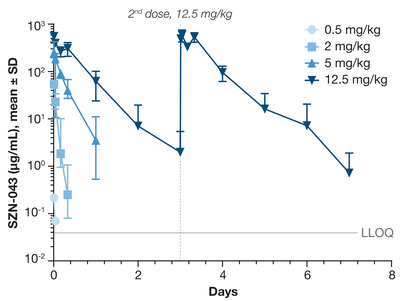


Table 5.

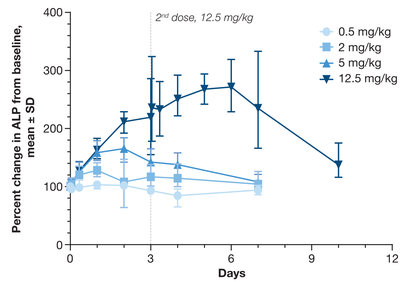
Dose (mg/kg)	CL (mL/day/kg), mean ± (SD)	Terminal $t_{1/2}$ (Days), mean ± (SD)	MRT (Days), mean ± (SD)	V_c (mL/kg), mean ± (SD)
0.5	74500 (6700)	NC	0.016 (0.001)	1620 (264)
2	554 (234)	0.072 (0.061)	0.820 (1.17)	28.1 (3.86)
5	112 (25.7)	0.170 (0.029)	0.245 (0.064)	18.1 (6.73)
12.5 (Day 0)	56.6 (16.7)	0.316 (0.127)	0.465 (0.152)	20.2 (3.79)
12.5 (Day 3)	35.7 (10.9)	0.360 (0.105)	0.550 (0.141)	23.6 (3.41)

CL = Clearance, $T_{1/2}$ = half-life, MRT = mean residence time, V_c = central compartment volume of distribution, NC = not calculated

ALP increase demonstrates occupancy of ASGR1

- ALP increase consistent with binding and internalization of ASGR1
- Doses ≥ 2 mg/kg cause increase in ALP
- Effect is sustained with second dose at 12.5 mg/kg

Figure 6.



Incidence of ADA is low with no evidence of effect on PK

- Immunogenicity evaluated pre-dose and Day 22 in Groups 1–3 and Day 25 in Group 4

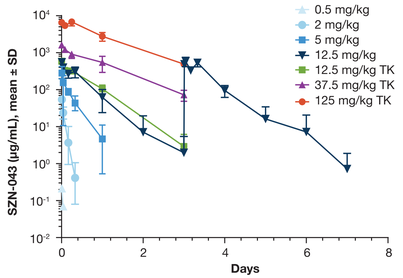
Table 6.

Dose	Positive/Total
0.5 mg/kg	1/4
2 mg/kg	0/4
5 mg/kg	2/4
12.5 mg/kg	3/4*

*One animal tested positive pre-dose

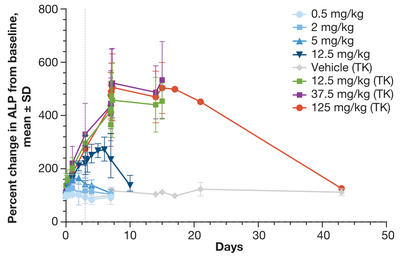
Consolidated PK and TK data illustrate dose-dependent saturation of ASGR1

Figure 7.



Doses at or above 12.5 mg/g appear to provide maximal target occupancy of ASGR1

Figure 8.



Conclusions

- **SZN-043 can be administered to NHPs at doses up to 125 mg/kg twice weekly without evidence of adverse effects.**
- **SZN-043 shows target-mediated pharmacology as evidenced by increases in serum ALP and Wnt-signaling in the liver.**
- **The PK of SZN-043 is consistent with that of an IgG-based molecule binding to the hepatocyte membrane protein ASGR1**

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