RODENT TOXICOLOGY STUDIES FOR A NOVEL ANTI-CANCER AGENT, SOR-C13

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ABSTRACT

Soricidin is a proprietary, 54-mer peptide that was isolated from the sub-maxillary saliva gland of the Northern Short-tailed Shrew. SOR-C13 is a novel 13-mer synthetic peptide based on the first 13 amino acids of the C-terminus of soricidin. SOR-C13 inhibits the function of TRPV6 [the 6th member of the transient receptor potential (TRP) vanilloid cation channel group] and selectively induces apoptosis in cell lines from ovarian and breast cancers as well as a number of other tumor types. Xenograft studies in mice have confirmed the *in vivo* effect of SOR-C13 as a single agent against ovarian and breast cancer tumors, and have also provided evidence of enhanced activity in combination with carboplatin/paclitaxel (ovarian cancer model) and paclitaxel (breast cancer model). As part of the program undertaken to support entry into clinical trials, a GLP-compliant 28-day IV toxicity study of SOR-C13 was conducted in Sprague-Dawley rats (n=10/sex/group). Animals were treated with SOR-C13 at dose levels of 0, 100, 200, or 400 mg/kg/day (0, 600, 1,200, or 2,400 mg/m^2/day, respectively) for 28 consecutive days. SOR-C13 was generally well tolerated. At 400 mg/kg/day, there were adverse clinical signs of decreased activity, shallow respiration, blue or pale skin, paws, muzzle, and/or mucous membrane, with slight to moderate swelling of the paws. Overall, a NOAEL was established at 200 mg/kg/day (1,200 mg/m^2/day) and the dose Severely Toxic to 10% of the animals (STD10) was >400 mg/kg/day (>2,400 mg/m^2/day) which corresponded to a mean combined sex AUC of 8,005 µg•min/mL).

INTRODUCTION

- Soricidin is a 54-amino acid peptide found in the paralytic venom of the northern short-tailed shrew (*Blarina brevicauda*). SOR-C13 is a synthetic peptide derived from the C-terminus of soricidin.
- SOR-C13 inhibits TRPV6 [the 6th member of the transient receptor potential (TRP) vanilloid cation channel group]. TRPV6 is over-expressed in cancers of the ovary, breast, and prostate, as compared to levels of TRPV6 in normal, healthy tissues (Zhuang *et al.*, 2002; Dhennin-Duthille *et al.,* 2011). In these tumors, TRPV6 appears to initiate proliferative and pro-survival pathways. The differential expression of TRPV6 in cancerous *vs.* healthy tissue offers the potential for targeted treatment of malignant tissue by SOR-C13 while minimizing effects on healthy tissue.

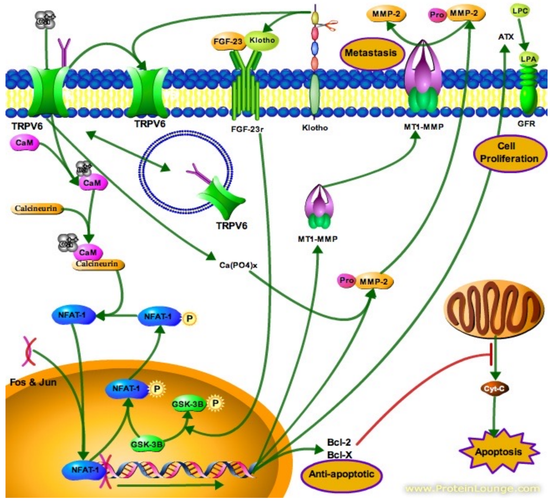

Figure 1. Diagrammatic representation of the role of TRPV6 in proliferation and prevention of apoptosis
Legend: CaM [calmodulin], FGF-23 [Fibroblast Growth Factor 23], MMP-2 [Matrix Metalloproteinase-type 2], ATX [autotaxin], LPC [lysophosphatidylcholine], LPA [lysophosphatidic acid], Ca(PO$_4$) [calcium phosphate], NFAT [nuclear factor of activated T-cell transcription factor], GSK-3B [a glycogen synthase kinase that re-phosphorylates NFAT, deactivating it and returning it from the nucleus to the cytoplasm], Fos [DNA binding domain], Jun [DNA binding domain]

- The inhibition of TRPV6 channels by SOR-C13 offers a novel therapeutic strategy for the treatment of TRPV6 over-expressing tumors. SOR-C13 selectively inhibits the proliferation of several cancer cell types *in vitro*. The anti-tumor activity of SOR-C13 has been demonstrated *in vivo* in mouse xenograft cancer models (see Figure 2).

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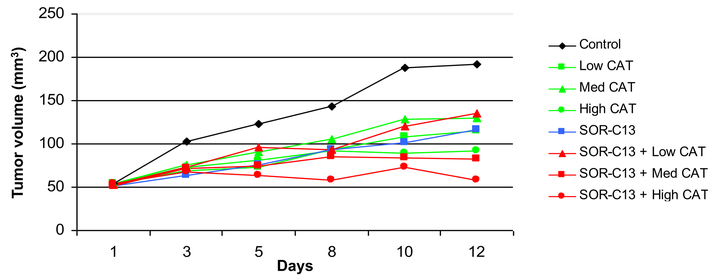

Figure 2. Anti-tumor activity of SOR-C13 alone and in combination with carboplatin/paclitaxel (CAT) in a mouse ovarian cancer xenograft model. Female NOD-SCID mice were subcutaneously implanted with SKOV-3 ovarian carcinoma cells and administered 0 or 300 mg/kg of SOR-C13 [daily x 12, intraperitoneal (IP)], or low dose CAT (carboplatin 20 mg/kg + paclitaxel 6 mg/kg), medium dose CAT (carboplatin 40 mg/kg + paclitaxel 12 mg/kg), high dose CAT (carboplatin 60 mg/kg + paclitaxel 18 mg/kg) (Day 1 and Day 8, IP), or SOR-C13 in combination with each of the low, medium and high doses of CAT (daily x 12, IP). Statistical comparisons at Day 12: All treatments were significantly different ($p < 0.01$) *vs.* control. High dose CAT was significantly different ($p < 0.05$) *vs.* medium and low dose CAT. All combination treatments were statistically significant ($p < 0.01$) *vs.* control.

- *In vivo* efficacy studies were also conducted to compare daily and intermittent dosing of SOR-C13. The results (not shown) demonstrated comparable anti-tumor activity of SOR-C13 with the different treatment schedules.

METHODS

- A non-GLP, dose-range finding toxicity study of SOR-C13 was conducted in Sprague-Dawley rats. Rats (1 male, 2 female/group) were administered intravenous (IV) injections of SOR-C13 at dose levels of 0, 100, 225 or 450 mg/kg/day (0, 600, 1,350, 2,700 mg/m^2/day) for 8 consecutive days. Reversible, non-adverse effects on clinical chemistry were observed at ≥ 225 mg/kg/day, and an enlarged spleen was observed in one animal in each of the 225 and 450 mg/kg/day groups. The MTD for 8 repeated doses of SOR-C13 in rats was determined to be ≥ 450 mg/kg/day (≥ 2,700 mg/m^2/day)
- In a pivotal GLP study, Sprague-Dawley rats were dosed daily with SOR-C13 (in phosphate buffer saline, pH 7.2) for 28 consecutive days via an IV slow bolus push injection (~0.5 min) at a dose volume of 5 mL/kg.

Table 1. Study treatment groups

Treatment Group	Dose Level		# of Animals					
			Main		Recovery[c]		Toxicokinetics	
	mg/kg/day	mg/m^2/day[b]	M	F	M	F	M	F
Vehicle control[a]	0	0	10	10	5	5	3	3
Low dose	100	600	10	10	-	-	9	9
Mid dose	200	1,200	10	10	-	-	9	9
High dose	400	2,400	10	10	5	5	9	9

[a]Control animals received the vehicle item (phosphate buffered saline, pH 7.2) alone
[b]Dose level in mg/kg/day was converted to mg/m^2/day based on a conversion factor of 6 (FDA, 2005)
[c]14-day recovery period following 28 days of treatment
F: female; M: male

- Toxicity parameters evaluated included: mortality, clinical signs, food consumption, body weight, ophthalmological examinations, hematology, clinical chemistry, coagulation, urinalysis, organ weights, and macroscopic/microscopic examinations of tissues and organs.
- Blood samples were collected from satellite animals (control group= 3/sex; treatment groups= 9/sex) on Days 1 and 28 for toxicokinetic profiling.

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RESULTS

Main Toxicity Assessment

- There was no mortality observed in this study and no significant changes in ophthalmology, coagulation, hematology and urinalysis parameters or organ weights. There were no macroscopic or microscopic findings that were attributed to the administration of SOR-C13. Significant clinical signs were only observed in the high dose group (400 mg/kg/day; 2,400 mg/m^2/day)

Table 2. Summary of clinical signs observed in the high dose group[a]

Clinical sign	% of high-dose animals affected	Frequency of occurrence[b]	Time course[c]
Decreased activity	100%	2-7 occurrences/animal (average of 3 occurrences/animal)	Occurring predominantly during Days 1-17
Shallow respiration	53%	1 occurrence/animal	Occurring almost exclusively on Day 1
Blue skin, paws and/or mucous membranes	100%	1-7 occurrences/animal (average of 4 occurrences/animal)	Occurring predominantly during Days 1-14
Swelling of paws	100%	2-3 occurrences/animal	Occurring predominantly on Days 1-18

[a]High dose animals administered SOR-C13 at 400 mg/kg/day (2,400 mg/m^2/day).
[b]Clinical signs generally occurred at a greater frequency in males compared to females.
[c]All clinical signs were observed immediately post-dose and were transient with recovery by 30 minutes post-dose.

- Based on the clinical signs observed in the high dose group (400 mg/kg/day), the NOAEL was established at 200 mg/kg/day (1,200 mg/m^2/day) and the Severely Toxic Dose to 10% of the animals (STD_{10}) was >400 mg/kg/day (>2,400 mg/m^2/day).

Toxicokinetics

- Toxicokinetic analysis revealed that the area under the plasma concentration *vs.* time curve ($AUC_{(0-t)}$) values were similar for male and female rats on Days 1 and 28. In general, $AUC_{(0-t)}$ values increased in a less than proportional manner on Day 1 and greater than proportional manner on Day 28, due primarily to Day 28 exposure levels for the high-dose (400 mg/kg/day; 2,400 mg/m^2/day) animals.

Table 3. Mean SOR-C13 AUC_{0-t} values on Day 1 and Day 28

Dose Level		AUC_{0-t} (µg•min/mL)			
		Day 1		Day 28	
mg/kg/day	mg/m^2/day[a]	M	F	M	F
100	600	2,455	2,038	580	927
200	1,200	2,813	2,556	1,739	1,396
400	2,400	6,581	9,185	8,108	7,901

[a]Dose level in mg/kg/day was converted to mg/m^2/day based on a conversion factor of 6 (FDA, 2005).
N= 9 rats/sex/group
F: female; M: male

- Based on the lack of increase in $AUC_{(0-t)}$ values on Day 28 compared to Day 1, there was no accumulation of SOR-C13.

SUMMARY AND CONCLUSIONS

- SOR-C13 was generally well tolerated; some adverse clinical signs were observed in the high dose group (400 mg/kg/day; 2,400 mg/m^2/day)
- No primary target organs for SOR-C13 toxicity were identified
- SOR-C13 has a favourable nonclinical toxicology profile in rats, supporting the entry of SOR-C13 into clinical trials

References available upon request.